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Histological changes, lipid metabolism and oxidative stress in the liver of *Bufo gargarizans* exposed to cadmium concentrations

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HIGHLIGHTS

- Cadmium decreased the metamorphosis rate and inhibited the body size.
- Cadmium triggered lipid accumulation and abnormal mitochondria.
- Cadmium increased the lipid synthesis and reduced the fatty acid β-oxidation.
- Cadmium increased the mRNA expression of SOD and GPx in the liver of *Bufo gargarizans*.

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ABSTRACT:

Chinese toad (*Bufo gargarizans*) were exposed to different concentrations of cadmium (5, 50, 100, 200 and 500 μg Cd L^{-1}) from Gosner stage 3–42. Metamorphosis rate, body weight, total length and body length were measured. Histological alterations in thyroid gland and liver were examined. Changes in hepatocyte were also examined using Transmission electron microscopic. In addition, the mRNA expression of several genes involved in lipid metabolism, oxidative stress and thyroid hormones signaling pathways were also measured. Our results showed that 200 and 500 μg Cd L^{-1} decreased the metamorphosis rate and inhibited the body size of *B. gargarizans* larvae at G42. Moreover, histological examinations have clearly exhibited that cadmium caused liver damage. Ultrastructural examination revealed lipid accumulation and abnormal mitochondria. Exposure to 200 and 500 μg Cd L^{-1} significantly up-regulated mRNA expression of D2, SOD, GPx, ACC and FAE, but down-regulated mRNA expression of TRα, TRβ, PPARα, ACOX, CPT and SCP. However, low Cd concentration (5, 50 and 100) exposure did not cause any effect in genes expression. Thus, we conclude that high Cd concentrations could affect the normal processes of lipid metabolism though increasing lipid synthesis and reducing the ability of fatty acid β-oxidation, and disturb thyroid hormone pathways in liver, and induced oxidative stress. In addition, lipid metabolism might be regulated by THs. To our knowledge, the present study is the first to report the influence of cadmium on hepatic lipid metabolism in *B. gargarizans* and will greatly provide new insights into cadmium hepatotoxicity in amphibian.

1. Introduction

Amphibian populations from around the world are apparently declining or experiencing severe range reductions in their natural habitats and this decline has been happening for several decades (Houlahan et al., 2000; Stuart et al., 2004). Chemical pollution, in conjunction with habitat loss, climate change, and emerging infectious disease, are considered as one of the most likely causes of the decline of their populations (Stuart et al., 2004). Cadmium, one of the chemical contaminations, is a non-essential, ubiquitous heavy metal which can exert its toxic effects on aquatic biota even at low concentrations due to its high bioaccumulation tendency (Chandurvelan et al., 2013). The concentrations of Cd range from less than 4 μg L^{-1} to an average of 167 μg L^{-1} in the polluted waters in China (Lihong et al., 2009). However, anthropogenic activities including the production of nickel cadmium batteries, stabilizers

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and synthetic pigment as well as metals melting are responsible for the pronounced increase in the concentration of cadmium in the environment (Waisberg et al., 2003; Patar et al., 2016). Therefore, the increase of the concentration of cadmium has aroused widely public concerns in recent years.

There are numerous studies focused on the adverse effect of cadmium on amphibian during both the embryonic and larval life stages (Loumbourdis, 2005; Mouchet et al., 2007; Gross et al., 2007). For example, cadmium may result in the teratogenesis of skin, eye, and digestive system in the South African frog, *Xenopus laevis* (Sunderman et al., 1991). Our recent study also found that Cd can cause embryos malformation in *Bufo gargarizans* (Wu et al., 2017). In addition, exposure to high level of Cd can delay metamorphosis in African clawed frog (*Xenopus laevis*), and reduce metamorphic success rate in *Pleurodeles waltl* (Sharma and Patiño, 2009; Flament et al., 2003). Ranatunge et al. (2012) found that the growth was inhibited significantly in Common Asian Toad (*Duttaphrynus Melanostictus*) exposed to Cd at 0.02 mg L^{-1}. Also, many studies focused on the accumulation of cadmium, the levels of metallothioneins and the activity of detoxification enzyme in liver under cadmium treatment (Kostaropoulos et al., 2005; Loumbourdis et al., 2007; Othman et al., 2009; Capaldo et al., 2016). In contrast, there is very scarce information related to the lipid metabolism in liver of amphibian under cadmium exposure.

Lipid metabolic activity mainly occurs in the liver (Cave et al., 2016; Davies et al., 2008). The normal processes of lipid metabolism are the balance between synthesis of fatty acids (lipogenesis) and fat catabolism via β-oxidation (lipolysis). Several key enzymes including acyl-CoA oxidase (ACOX-1), Carnitine palmitoyltransferase-1 (CPT-1), Sterol carrier protein 2 (SCP-2), Acetyl CoA Carboxylase 1 (ACC-1) and Fatty acid elongase 1 (FAE-1) are involved in the lipid metabolism. ACOX-1 catalyzes the conversion of acyl-CoA into *trans*-2-enoyl-CoA (Zeng and Li, 2004). CPT- 1 controls an initiating step in the translocation of long chain fatty acids across the mitochondrial membranes for β-oxidation (Kerner and Hoppel, 2000; Gilde et al., 2003). SCP-2 contributes to the import of fatty acids and β-oxidation of branched-chain lipid (Schroeder et al., 2007). ACC-1 catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA substrate for the de novo fatty acid biosynthesis (Salie and Thelen, 2016). FAE-1 is essential for biosynthesis of highly unsaturated fatty acids (Jakobsson et al., 2006). In addition, PPAPα, a kind of transcriptional factors, are involved in the lipid metabolism via regulation of the expression of genes involved in the catabolism of fatty (Pawlak et al., 2015).

Thyroid hormones (THs) have been linked to lipid metabolism and the regulation of lipid metabolic genes. THs include L-thyroxine (T4) and Triiodothyronine (T3), and type 2 and 3 iodothyronine deiodinases (Dio2 and Dio3) controls the local THs level (Maher et al., 2016). TH binds to the thyroid hormone receptor (TRs) to regulate gene transcription. TRs exists in two isoforms: TRα and TRβ (Opitz et al., 2006; Brown and Cai, 2007). Recent finding suggests that THs can regulate fatty acid (FA) homeostasis through directly increasing the transcription of lipogenic enzymes such as ACC, malic enzyme (ME), fatty acid synthase (FAS) and mainly inducing coordinated lipid catabolism by stimulating lipophagy-mediated lipolysis and mitochondrial β-oxidation of FA (Cioffi et al., 2013; Sinha et al., 2014). Thus, these results provide supporting evidence that THs are important for lipid metabolism in liver, and there is a crosstalk between thyroid hormone pathways and lipid metabolism in liver. However, litter is known about the relationship among cadmium, THs and lipid metabolism in amphibian.

Bufo gargarizans belongs to *Bufonidae Bufo*, and is a species of toad endemic to China. *B. gargarizans* has been used as an excellent indicator organism for assessment of toxic effects of chemicals in our laboratory (Wang et al., 2016; Chai et al., 2016; Wu et al., 2017). The aim of the present work was to evaluate the adverse effects of sub-lethal concentration of cadmium exposure on growth, thyroid gland condition and hepatic lipid metabolism of *B. gargarizans*. Firstly, metamorphosis rate and size at metamorphic climax were recorded, and histopathological modifications of thyroid gland and liver were also analyzed. Besides, RT-qPCR was utilized to determine whether cadmium affects transcript levels of genes involved in hepatic lipid metabolism, oxidative stress and thyroid hormone homeostasis.

2. Materials and methods

2.1. Animals

Three mating pairs of adult *B. gargarizans* collected in February 2016 from Qinling Mountains, Shaanxi Province, China (109°06′52″E, 34°00′56″N) were held in one aquarium with shallow water (50 mm). After spawning naturally, embryos were raised in our laboratory for research. The care of animals was taken in accordance with the Animal Care Guidelines of Shaanxi Normal University and China Wildlife Conservation Association.

2.2. Chemicals and solutions

Cadmium sulfate octahydrate with a purity of approximately 99% was obtained from Sigma Corporation (Sigma-Aldrich, St. Louis, MO, USA). A stock solution was prepared weekly in dechlorinated tap water to a final concentration of 1000 μg Cd L^{-1} and experimental solutions were obtained by diluting the stock solution with dechlorinated tap water to obtain the following target concentrations: 5, 50, 100, 200 and 500 μg Cd L^{-1}. To maintain the appropriate concentrations of cadmium and water quality, test solutions were completely replaced every 48 h.

2.3. Experimental design

All naturally fertilized embryos were pooled and then embryos at Gosner stage 3 (Gosner, 1960) were selected randomly from the pool for the study. Then 80 embryos were randomly assigned to glass aquaria (50 cm × 20 cm × 20 cm) with 5 L treatment solutions with 5, 50, 100, 200 and 500 μg Cd L^{-1}, respectively. In addition, the experimental control groups exposed to 5 L dechlorinated water only. There were 3 replicates per treatment. All tanks were maintained at about 18 ± 1 °C on a 12 h light and 12 h dark regimen with continuous, gentle aeration throughout the exposure. Embryos were not fed during exposure. After hatching, larvae were offered boiled vegetables during exposure to cadmium.

At metamorphic climax (stage G42: determined as forelimbs emergence), the larvae were euthanized and then measured every parameter (body mass, total body length and total length). The exposure ended when half of larvae in control groups completed metamorphic climax. Until the end of experiment, each individual was weighted once on an analytical balance having readability of nearest 0.001 g. Lengths of the larvae were measured to the nearest 0.01 mm by Tesa-Cal Dura-Cal Digital electronic calipers. In addition, larvae at Gosner stage 42 were randomly collected for mRNA expression analysis.

2.4. Water quality

All exposures took place with dechlorinated tap water, which was insolated for 3 days at room temperature (17–19 °C). Water conditions of pH, dissolved oxygen (DO), salinity and specific