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Oxidative stress and inflammatory responses in the liver of swamp eel (*Monopterus albus*) exposed to carbon tetrachloride

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ABSTRACT

"Liver and gall syndrome" disease often caused dramatic economic losses in swamp eel (*Monopterus albus*) which is one of the most economically important freshwater aquaculture fish in China. Carbon tetrachloride (CCl_4)-induced liver injury model has been evidenced as a useful method to screen the hepatoprotective agents to study and prevent this disease in fish. Therefore, in order to provide a better research platform for prevention and treatment liver diseases in swamp eel, we evaluated the hepatotoxicity effects of CCl_4 on swamp eel based on the acute toxicity and biochemical responses of glutamate pyruvate transaminase (GPT), glutamate oxalate transaminase (GOT), lactate dehydrogenase (LDH), catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA), as well as mRNA expression levels of cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and 5-lipoxygenase (5-LO). The 96 h LD_{50} value of CCl_4 in swamp eel was 2.54 g/kg. Sublethal levels of CCl_4 induced time-depended liver injury in swamp eel, evidenced by the significant increases of serum GPT, GOT and LDH levels at the first 48 h injection, but restoration after 96 h injection. CCl_4 led to oxidative stress, supported by the enhanced of MDA contents, and decreased of SOD and CAT activities. Moreover, CCl_4 caused hepatic inflammatory reaction, evidenced by the significant changes of the transcription of COX and 5-LO genes in liver tissues. Overall, our results suggested that lipid peroxidation, antioxidant enzymes and COX and 5-LO pathways are important mechanisms involved in CCl_4-induced liver injury, and could be used as the effective biomarkers of CCl_4-induced liver injury model to screen the hepatoprotective agents in swamp eel.

1. Introduction

The swamp eel (*Monopterus albus*) is a bony fish (family Synbranchidae; order Synbranchiformes) lives widely in muddy ponds, swamps, canals and rice fields in the tropics and subtropics from India to southern China, Malaysia and Indonesia (Tay et al., 2003). Due to its high nutrition and medicinal value, swamp eel has become one of the most economically important freshwater aquaculture fish throughout Asia, especially in China (Zhou et al., 2002; Gao et al., 2016b). The worldwide production of swamp eel has reached 321,006 tons in 2012, while most of the production (320,966 tons) was provided by Chinese aquaculture (Liang et al., 2016). It was reported that the demand for swamp eel in Chinese market is up to 3 million tons per year in recent years (Sun et al., 2017). However, though the swamp eel farming has made rapid progress during the past decade, the annual production of swamp eel is still seriously challenged due to the infection diseases caused by pathogenic bacteria, parasites or virus (Gao et al., 2016b), and moreover, the non-infectious diseases caused by the xenobiotic challenges including aquatic environment pollution, the abuse of antibiotics and pesticides, and the use of high-protein, high-fat and moldy feed in swamp eels aquaculture industry (Qu et al., 2014; Cao et al., 2015).

Recently, a disease called "liver and gall syndrome", with the symptoms of liver enlargement, color change and even necrosis, has been frequently found in many cultured fish including swamp eel in China. It was proposed that this disease may be mainly caused by xenobiotic challenge such as drug abuse and toxic compounds exposure (Jia et al., 2012). Since swamp eels are benthonic organisms, the potential risk is high for them to expose to environmental contaminants in sediment and result in "liver and gall syndrome" disease. Dramatic economic losses in swamp eel aquaculture has been often caused by this disease due to the lack of effective methods have been found for controlling this disease in swamp eel. Thus, in order to reduce the economic loss caused by the "liver and gall syndrome" disease in swamp eel farming, it is essential to find an useful method to prevent and treat this liver disease in swamp eel.

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Nowadays, though the effective treatment methods for "liver and gall syndrome" is still limited in fish, the carbon tetrachloride (CCl_4)-induced liver injury model has been considered as a useful method to screen the hepatoprotective agents to study and prevent this disease (Yin et al., 2011; Jia et al., 2012; Jia et al., 2013). As well known, CCl_4 is widely used as a model compound to study the mechanisms of liver injury or the evaluation of anti-hepatotoxic activity of agents in mammal and fish (Zuinen et al., 2007; Aayadi et al., 2017). CCl_4 by itself does not cause cytotoxic effects on liver, but its metabolic products, such as trichloromethyl radicals ($CCl_3^{\bullet}$) and trichloromethyl peroxyl radicals ($CCl_3O_2^{\bullet}$), are suggested to be responsible for the toxicity (Makni et al., 2011). It was reported that the toxic metabolites of CCl_4 not only decreased the activities of antioxidant enzymes and initiated lipid peroxidation that leads to liver injury (Li et al., 2013),but also induced the overproduction of inflammatory mediators (Makni et al., 2011;Popović et al., 2016). For example, CCl_4 could cause hepatotoxicity by increasing the contents of lactate dehydrogenase (LDH), malondialdehyde (MDA) in rats (Rahmouni et al., 2017). Ji et al. (2018) reported that the reduction of liver antioxidative enzyme activities including superoxide dismutase (SOD) and catalase (CAT), and the increase of serum glutamate pyruvate transaminase (GPT/ALT) and glutamate oxalate transaminase (GOT/AST) levels indicated that CCl_4 could trigger hepatic injury to the rats. In addition, the toxic metabolites of CCl_4 could stimulate Kupffer cells to release more inflammatory mediators such as cyclooxygenase (COX) and 5-lipoxygenase (5-LO) in mice (Wills and Asha, 2012; Harris et al., 2015).

Previous studies have provided strong evidence that fish liver is as sensitive as mammal when exposure to CCl_4 (Chen et al., 2004; Yin et al., 2011; Jia et al., 2012). For instance, CCl_4 has been shown to significantly increase the levels of GPT, GOT and MDA, reduce the levels of SOD and CAT, and up-regulate the gene expressions of inflammatory cytokines (tumor necrosis factor-α (TNF-α)) in common carp (Jia et al., 2014). Cao et al. (2017) observed that CCl_4 induced liver damage in Jian carp by inhibiting SOD activities, promoting MDA levels, and upregulating inflammatory cytokine gene expression. However, previous studies also showed that the susceptibility of fish to CCl_4 varies with fish species. For example, the 96 h median lethal concentration (LC_{50}) of CCl_4 was obtained as 20 μL/L for rainbow trout (Krasnov et al., 2005), 14.94 μL/L for rosy barbs and 11.8 μL/L for amphioxus (Bhattacharya et al., 2008). Although CCl_4-induced liver injury model have been successfully established by studying the responses of antioxidative enzyme activities and inflammatory mediators in several fish species (Cao et al., 2015; Jia et al., 2014), the hepatotoxicity of CCl_4 on swamp eel is still unclear. Considering the dramatic economic losses caused by liver disease in swamp eel in China, it is essential to provide a liver injury model by exploring the oxidative stress and inflammatory mechanisms involving in the hepatotoxicity by CCl_4 in swamp eel.

The objectives of this study were (1) to investigate the acute toxicity of CCl_4 to swamp eel by determining the 96 h median lethal dose (LD_{50}) value. (2) to explore the hepatotoxicity mechanism of CCl_4 by studying the responses of biochemical parameters (e.g., GPT, GOT, LDH, SOD, CAT and MDA) and the gene expressions of inflammatory mediators (such as COX-1, COX-2 and 5-LO) in the swamp eel. This investigation would be beneficial to explore the action mechanisms related to the hepatotoxic of CCl_4 on the swamp eel and provide a better research platform for prevention and treatment liver diseases in swamp eel farming.

2. Materials and methods

2.1. Test organism and experimental design

Swamp eels were obtained from the College of Animal Science and Technology, Jiangxi Agricultural University in China. Fish weighed 30.00 ± 0.28 g with total length 32.17 ± 0.65 cm were brought to the laboratory and acclimatized to the laboratory conditions for two weeks. De-chlorinated, aerated tap water was adopt and maintained at 26 ± 1 °C, pH 7.5 ± 0.1 and dissolved oxygen above 5 mg/L. Natural photoperiod was in a 14 h light/10 h dark cycle. During the acclimatization period, fish were fed daily with fish food from Jiangxi Agricultural University. The fish used for toxicity tests were starved for 24 h, which gave sufficient time for the gut to be emptied of food and waste. Ethics Committee approval was obtained from the Institutional Ethics Committee of the Jiangxi Agricultural University to the commencement of the study.

Analytical grade reagent carbon tetrachloride (CCl_4, purity ≥99.5%, ρ = 1.595 g/mL (20 °C)) were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Corn oil (JiaYi Co., Ltd., Shanghai, China) was used to prepare the test solutions of CCl_4 by serially diluting to the required concentrations.

For exposure experiments, four-day acute toxicity tests were firstly performed to determine the LD_{50} value of CCl_4 in swamp eels. Based on the preliminary experiments, the doses of CCl_4 (1.16, 1.84, 2.92, 4.62 and 7.33 g/kg body weight (bw)), which could cause about 0–100% mortality, were selected for the acute toxicity study. Fish were randomly divided into control group and CCl_4 treatment groups, each containing 15 animals. Control group was intraperitoneally (i.p.) injected with 0.25 mL corn oil per 30 g of body weight, while the CCl_4 treatment groups were i.p. injected with 0.25 mL CCl_4 solution of 0.139, 0.221, 0.350, 0.554 and 0.879 g/mL per 30 g of body weight, which generated dose groups of 1.16, 1.84, 2.92, 4.62 and 7.33 g/kg bw, respectively. The fish were maintained in 90 L individual plastic aquariums (fifteen fish per aquarium) with 60 L de-chlorinated tap water. Temperature was controlled at 26 ± 1 °C, pH was 7.5 ± 0.1, dissolved oxygen was maintained above 5 mg/L and all experiments were carried out under natural photoperiod (14:10 h light:dark). Throughout the experiment, no food was provided. The mortality of fish was recorded at 24, 48, 72, and 96 h, and the LD_{50} at 96 h was calculated by the modified Kaber method (in 95% confidence interval).

Based on the LD_{50} at 96 h, i.p. injection dosages of sublethal CCl_4 with 0.254 and 0.508 g/kg bw were administrated to the fish only one time at the beginning of the sub-chronic toxicity experiments (for 144 h). Ninety fish were randomly divided into three groups: one corn oil control group (0 g/kg) and two CCl_4 treatment groups (0.254 and 0.508 g/kg). Each control and treated group was performed in three plastic aquariums (ten fish per aquarium) with 60 L de-chlorinated tap water. Other conditions during the exposure period were maintained the same as those during the acute toxicity tests. At the end of the experiment, six fish were taken randomly from each control and treated group at 12, 24, 48, 96, and 144 h post-injection. Blood samples were collected from the caudal peduncle by citrated tuberculin syringes and immediately centrifuged at 3000 rpm for 15 min at 4 °C to separate the plasma for enzymological (GOT, GPT and LDH) studies. Additionally, fish were sacrificed by decapitation and dissected for liver tissue. A partial of liver was weighed, homogenized in ice-cold physiological saline (to 0.1 g of liver was added 0.9 mL physiological saline) and centrifuged at 10000 rpm for 10 min at 4 °C, and the supernatant was conserved for assays of biochemical parameters (SOD, CAT and MDA). The other part of liver was immediately stock-frozen in liquid nitrogen, subsequently stored at −80 °C refrigerator until use for gene expression measurement (COX-1, COX-2 and 5-LO). No mortality was observed in all groups at different dosages during the whole exposure period.

2.2. Biochemical assays

Serum glutamate pyruvate transaminase (GPT), glutamate oxalate transaminase (GOT) and lactate dehydrogenase (LDH) activities were determined using spectrophotometric diagnostic kits according to the manufacturer's instructions (Jiancheng Institute of Biotechnology, Nanjing, China). GPT, GOT and LDH were expressed as international units per liter.