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Toxicon 49 (2007) 1150–1157

TOXICON

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Hematological and plasma biochemical responses of crucian carp (*Carassius auratus*) to intraperitoneal injection of extracted microcystins with the possible mechanisms of anemia

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Received 13 November 2006; received in revised form 7 February 2007; accepted 7 February 2007
Available online 25 February 2007

Abstract

Alterations in hematological indices such as decreases in blood cell counts (RBC), hematocrit (Ht) and hemoglobin (Hb) concentrations are key symptoms of anemia. However, few experiments were conducted to examine changes in hematological indices of fish exposed to microcystins that are believed to be fatal to circulatory systems of vertebrates. An acute toxicological experiment was designed to study hematological changes of crucian carp injected intraperitoneally (i.p.) with extracted microcystins at two doses, 50 and 200 μg MC-LReq kg^{-1} body weight. After being i.p. injected with microcystins, the fish exhibited behavioral abnormity. There were significant decreases in RBC in the high-dose group, and in Ht and Hb concentrations in both dose groups, while erythrocyte sedimentation rate (ESR) significantly increased, indicating the appearance of normocytic anemia. There were no prominent changes in the three red cell indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Increases in blood urea nitrogen (BUN) and creatinine (CR) in both dose groups suggest the occurrence of kidney impairment. Alteration in blood indices was reversible at the low dose group. Conclusively, anemia induced by kidney impairment was a key factor to cause abnormity of swimming behaviors and high mortality of crucian carp.

Keywords: Microcystins; Crucian carp; Hematological indices; Plasma biochemical parameters; Normocytic anemia; Kidney impairment

1. Introduction

Occurrence of cyanobacterial blooms and the associated cyanotoxins have been documented in many eutrophic inland waters worldwide (Paerl et al., 2001). Among cyanotoxins, the hepatotoxic microcystins (MCs) such as MC-LR and MC-RR are considered to be one of the most dangerous groups (Zimba et al., 2001). MCs are potent inhibitors of protein phosphatases 1 and 2A (Falconer and Yeung, 1992) due primarily to toxin uptake by the bile acid transport system (Runnegar et al., 1993). A variety of aquatic animals such as fish, mollusks and shrimps are able to bioaccumulate MCs

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0041-0101/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.toxicon.2007.02.009

in natural water bodies with toxic cyanobacteria (Magalhães et al., 2003; Chen and Xie, 2005; Xie et al., 2005). Crucian carp (*Carassius auratus* L), an omnivorous fish, is a dominant fresh-water species in China. This carp can ingest a significant portion of toxic cyanobacteria in eutrophic lakes, leading to MC accumulation in its tissues (Xie et al., 2004).

There have been many toxicological experiments to examine MC bioaccumulation in organs of fish (Xie et al., 2004; Soares et al., 2004; Li et al., 2005) and to evaluate toxic effect of MCs on physiological status and histopathology of fish (Råbergh et al., 1991; Kotak et al., 1996; Fischer and Dietrich, 2000). It is known that MCs accumulate mainly in the liver, but also in other organs such as the kidney, gill and intestine. In mammals, acute deaths following MC administration have been attributed to hemorrhagic shock related to liver damage and intrahepatic hemorrhage (Beasley et al., 2000), as well as cardiac failure (Le Claire et al., 1995). MCs damnify aquatic organism with the liver as main target organ, and also cause impairment of kidney (Kotak et al., 1996; Fischer and Dietrich, 2000), which is one of the most important hematogenic organs for teleost fish (Ozaki, 1982). The disruptive action of a toxicant on the erythropoietic tissue may decrease erythrocyte number and hemoglobin content as an anemic sign, and even lead to death of European catfish (Köprücü et al., 2006). In fish, blood urea nitrogen (BUN) is the second important nitrogenous excretion product after ammonia and often used as an indicator of kidney dysfunction (Bernet et al., 2001). Increased concentrations of BUN occur due to renal lesions (Burtis and Ashwood, 1996). Creatinine (CR) is a waste product of the phosphorylation of ADP at the expense of the high-energy compound creatine phosphate and increased concentrations may reflect kidney dysfunction due to structural damage (Burtis and Ashwood, 1996).

Blood parameters are useful and sensitive for the diagnosis of diseases and monitoring of the physiological status of fish exposed to toxicants, which has been shown by Adhikari et al. (2004). A large amount of studies have focused on toxic effects of heavy metals and chemical medication on fish hamatology under laboratory condition (Harikrishnan et al., 2003; Ribeiroa et al., 2006). Many studies have defined anemia as decreased RBC, Hb and Ht (Haney et al., 1992; Greenburg, 1996; Hendy et al., 2001). Hematological parameters in fish including red blood cell counts (RBC), hematocrit (Ht), hemoglobin (Hb), mean corpuscular volume (MCV) may be influenced by intrinsic and external factors (Nespolo and Rosenmann, 2002; Rios et al., 2002). Only a few reports have documented alterations in hematological parameters caused by MCs in fish (Vajcová et al., 1998; Kopp and Hetesa, 2000). Vajcová et al. (1998) reported that microcystins can lead to alterations in hematological indices of silver carp (*Hypophthalmichthys molitrix*.); however, they only measured hematological parameters at 48 h post injection without any discussion on anemia caused by MCs. Until now, there have been no information available on anemia induced by microcystins.

The purposes of this study were to evaluate the acute toxicity of MCs on anemia by determining hematological and biochemical indices, and to establish a possible relationship among alterations in hematological indices, anemia, plasma biochemical and hypofunction of hemopoiesis due to kidney impairment. Our hypothesis was that anemia caused by kidney impairment is a key factor to influence behaviors and mortality of crucian carp.

2. Materials and methods

2.1. Fish

Healthy crucian carp (mean body weight 265.0 ± 22.6 g) were purchased from a fish hatchery affiliated to College of Fisheries, Huazhong Agricultural Univeristy in Wuhan City, China, and were transported to the laboratory. The experiment was conducted with a water temperature of 25 ± 1 °C and a dissolved oxygen concentration between 6.0 and 7.1 mg l^{-1} by continuously aerating. Fish were allowed to acclimate for 14 days prior to experimentation in 150 l aquarium (95 cm L × 55 cm W × 40 cm H) containing dechlorinated tap water and were fed with commercial crucian carp food at a rate of 2.0% of body weight per day. Feeding was terminated 48 h before initiation of the experiment, and no food was supplied to fish throughout the experiment. Fish were kept in a 12 h light/dark photoperiod.

2.2. Toxin

Cyanobacteria (mainly composed of *Microcystis* spp.) were collected from surface blooms of Lake Dianchi, Yunnan of China. Crude microcystins in the cyanobacteria were extracted three times with