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Renal accumulation and effects of intraperitoneal injection of extracted microcystins in omnivorous crucian carp (*Carassius auratus*)

Li Li [a,*], Ping Xie [b], Hehua Lei [c], Xuezhen Zhang [a]

[a] *Key Laboratory of Freshwater Animal Breeding, Ministry of Agriculture, College of Fisheries, Huazhong Agricultural University, Wuhan 430070, People's Republic of China*
[b] *Donghu Experimental Station of Lake Ecosystems, State Key Laboratory of Freshwater Ecology and Biotechnology of China, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China*
[c] *Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430072, People's Republic of China*

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ABSTRACT

An acute toxicological experiment was designed to characterize the sequence of renal ultrastructural changes with accumulated MCs in crucian carp injected intraperitoneally (i.p.) with extracted microcystins (mainly MC-RR and -LR) at two doses, 50 and 200 μg MC-LReq. kg^{-1} body weight. Quantitative and qualitative determinations of MCs in the kidney were conducted by HPLC and LC-MS, respectively. MC-RR content in kidney of crucian carp showed a time dose-dependent increase within 48 h post-injection, followed by a sharp decline afterward, while no MC-LR in kidney was detectable throughout the experiment. Ultrastructural changes in the kidney of crucian carp progressed with increasing accumulated MCs and exposure times within 48 h post-injection, whereas renal ultrastructural recovery of crucian carp in the 50 μg MC-LReq. kg^{-1} dose group was evident at 168 h post-injection. Our ultrastructural observation suggests that the membranous structure is the main action site of MCs in the kidney, among which mitochondria damage in the tubules is clearly an early, and presumably a critically important effect of MCs. The increases in blood urea nitrogen (BUN) and creatinine (CR) in both dose groups further revealed severe impairment occurred in the kidney of crucian carp.

1. Introduction

With the increasing extent of eutrophication, contamination of cyanobacterial blooms has become one of the severe worldwide environmental problems (Welker and von Döhren, 2006; Svrcek and Smith, 2004; Paerl and Huisman, 2008). There have been many studies to document animal deaths or animal-poisoning episodes associated with the occurrence of toxic cyanobacterial blooms (Dawson, 1998; Zimba et al., 2001; Jewel et al., 2003; Qiu et al., 2007). Microcystins (MCs) are a family of cyclic hepatopeptide hepatotoxins produced by several cyanobacterial species. Among more than 80 variants of MCs, microcystin-LR (MC-LR) is the most frequent and most extensively studied variant, followed by MC-RR and MC-YR (De Figuereido et al., 2004; Svrcek and Smith, 2004). It is well known that MCs are potent inhibitors of protein phosphatases 1 and 2A (MacKintosh et al., 1990; Toivola et al., 1994; Guzman et al., 2003) with liver as target organ. The high selectivity to liver is believed to be due to toxin uptake via bile acid carriers (Suchy, 1993; Sahin et al., 1996). Histopathological studies in both fish and mammals revealed serious lesions of the liver including rounding and separation of hepatocytes, disruption of hepatic cords, hepatocyte necrosis and degeneration, and severe intrahepatic hemorrhage leading to lethal hypolovemic

* Corresponding author. Tel./fax: +86 27 87282113.
E-mail address: foreverlili78@mail.hzau.edu.cn (L. Li).

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shock or liver failure (Guzman and Solter, 2002; Li et al., 2003, 2004; Malbrouck et al., 2003; Handeland and Ostensvik, 2010).

In contrast to hepatotoxicity of MCs, there are few studies on toxic effects of MCs in the renal system. In mice, renal damages are seldom observed and this can be explained by the short survival time (1–2 h) to lethal dose (Kotak et al., 1993). In acute toxic experiment of fish, Kotak et al. (1996) reported that renal ultrastructural lesions consisted of tubular epithelial necrosis and dilation of Bowman's space of gomeruli from the two longest surviving rainbow trout (*Oncorhynchus mykiss*) i.p. at the dose of 1000 μg MC-LR kg^{-1}. When tilapia fish (*Oreochromis* sp.) were exposed for 21 days to cyanobacterial cells (60.0 mg MC-LR/fish per day), ultrastructural examination also revealed dilation of Bowman's space, increase of lysosomes and necrotic epithelial cells with pyknotic nuclei in the tubules (Molina et al., 2005). Similarly, Atencio et al. (2008) observed dilated Bowman's space, decrease of the glomerular component, tubular necrosis in tenca (*Tinca tinca*) orally exposed to cyanobacterial cells dosing 5, 11, 25 and 55 mg MC-LR/fish mixed with the food. In our previous field studies, kidney lesions were indicated by partial inosculation of foot processes of epithelial cell and proliferation of mesangial cells in glomeruli, and hydropic mitochondria in proximal tubules in fish exposed to natural cyanobacterial blooms in Meiliang Bay of Taihu (L. Li et al., 2007, 2008). Moreover, the distribution and accumulation of MCs in the kidney of fish were identified in both laboratory and field studies (Williams et al., 1995; Mohamed et al., 2003; Xie et al., 2005; Chen et al., 2006, 2007). Up to now, in vivo studies on the toxic effects of MCs on the ultrastructure of kidney are limited in fish and no information is available for the sequence of renal ultrastructural lesions associated with the kinetic of MCs.

The present study aims to establish accumulation of MCs, exposure time and the concentration of serum blood urea nitrogen (BUN) and creatinine (CR) associated with the observable toxin-induced ultrastructural changes in the kidney of crucian carp after intraperitoneal injection with extracted MCs, and to characterize the sequence of such changes, in an effort to determine the morphologic events leading to breakdown of the architecture and function of the kidney. Crucian carp (*Carassius auratus*) has been chosen as a test organism because it is a dominant freshwater species and also widely used as a food fish for Chinese people. This carp can ingest a significant portion of toxic cyanobacteria in eutrophic lakes, leading to MCs accumulation in its tissues (Xie et al., 2005).

2. Materials and methods

2.1. Toxin

Cyanobacteria (mainly composed of *Microcystis aeruginosa*) were collected from surface blooms of Lake Dianchi, Yunnan of China. Freeze-dried crude algae were extracted three times with 5% acetic acid. The extract was centrifuged (36,290 g, 4 °C, 1 h), and the supernatant was applied to a C18 reversed-phase cartridge, which had been preconditioned by washing with methanol and distilled water. The cartridge was then washed with water and eluted with methanol. The elution was evaporated to dryness and the residue was dissolved in distilled water. This solution was used for the toxic experiment. Before use, the toxin-containing solution was analyzed for MCs concentrations via HPLC (LC-20A, Shimadzu Corporation, Kyoto, Japan) equipped with and ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan) and a SPD-20A UV–vis spectrophotometer set at 238 nm. MCs concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR, MC-RR and MC-YR, Wako Pure Chemical Industries, Osaka, Japan). However, the content of MC-YR was too low that it wasn't able to be detected. The obtained microcystin was MC-RR and MC-LR with purity >80%. The MCs-containing solution was finally diluted with distilled water to 136.5 μg ml^{-1} of MC-RR and 22.7 μg ml^{-1} of MC-LR.

2.2. Fish, treatment and sample preparation

Healthy crucian carp (mean body weight 265 ± 22.6 g) were purchased from a local fish hatchery in Wuhan City, China. Fish were acclimated for 14 days in 150 l aquarium containing dechlorinated tap water and fed with commercial crucian carp food at a rate of 2% of body weight per day. Water temperature was controlled at 25 ± 1 °C, and dissolved oxygen was 6.8 ± 0.7 mg l^{-1}. Feeding was terminated 2 days before initiation of the experiment, and no food was supplied to fish during the experimental period.

After acclimation, fish were distributed randomly into three dose group (60 fish/group): fish in the low dose group and high dose group were injected intraperitoneally (i.p.) with the doses of approximately 1 ml extracted solution of MCs, amounting to equivalent of 150 and 600 μg MC-LR + MC-RR per kg body weight (bw), respectively, and the control fish were injected i.p. with equal volume of distilled water. Since LD_{50} (i.p.) in mice for MC-RR is about five times higher than for MC-LR (Gupta et al., 2003), the doses of 150 and 600 μg kg^{-1} injected with extracted toxins of MC-RR and MC-LR in the present study were equivalent to 50 and 200 μg kg^{-1} of purified MC-LR, respectively. Six sampling points were set during a period of 7 days in the experiment (1, 3, 12, 24, 48 and 168 h post injection). Ten acclimated fish without administration were expressed as 0 h and sampled 2 h prior to injection.

At each sampling point, 10 fish for each dose group and the control were anaesthetized with 0.02% MS-222 solution for 5–10 min. Blood were firstly taken via the caudal vein for determination of biochemical indices (BUN and CR). Kidney were dissected from each fish and then divided into two parts: one was immediately frozen at −70 °C for determination of toxin content, and the other one was fixed for ultrapathological study.

2.3. Extraction and determination of MCs concentration in kidney

Extraction and analysis of MCs in the kidney followed the method Xie et al. (2004) with some modification. Briefly, lyophilized sample was homogenized in a mortar