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

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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.

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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

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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

and extracted three times with BuOH:MeOH:H$_2$O (1:4:15), sonicated for 3 min (50% amplitude, 65 W, 20 kHz; Sonics VC130PB; Sonics and Materials, Newtown, CT, USA). The extract was centrifuged (35,290 g, 4 °C, 1 h), and the supernatant was diluted with water. This diluted extract was directly applied to a C18 reversed phase cartridge (5 g), which had been preconditioned by washing with 50 ml of 100% methanol and 50 ml of distilled water. The column was washed with 50 ml of water and 100 ml of 20% methanol. Elution from the column with 100 ml of 90% methanol yielded the MC-containing fraction. The MC-containing fraction was evaporated to dryness, and the residue was then redissolved in methanol. This solution was applied to a silica gel cartridge (2 g) that had been preconditioned by 10 ml of 100% methanol. The column containing the MC was washed with 10 ml of 100% methanol and then eluted with 20 ml of 70% methanol. The MC-containing fraction was also evaporated to dryness. This fraction was dissolved with 100 μl distilled water and used for the final detection and identification of MCs by liquid chromatography-mass spectrometry (LC-MS).

Qualitative and quantitative analysis of MCs were performed using a Finnigan LC-MS system (Thermo electron corporation, USA) comprising a thermo surveyor auto sampler, a surveyor MS pump, a surveyor PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an electrospray ionization source (ESI). The instrument control, data processing, and analysis were conducted by using Xcalibur software. Separation was carried out under the reversed phase on Agilent ZORBAX SB-C18 column (2.1 mm i.d. × 100 mm, 3.5 μm, Agilent Corporation, USA). The mobile phase consisted of solvent A (water + 0.05% formic acid) and solvent B (acetonitrile + 0.05% formic acid). The Linear gradient program: 0 min at 5% solvent B, 0.5 min at 30% solvent B, 3 min at 40% solvent B, 6 min at 70% solvent B, 14.5 min at 70% solvent B, 14.6 min at 5% solvent B, and 20 min at 5% solvent B. Sample injection volumes was typically 10 μl. The mass spectrometer was set to electrospray ionization positive-ion mode, and mass spectrometer tuning and optimization were achieved by infusing microcystin-RR with ion of $[M + 2H]^{2+}$ at m/z of 520. Quantification of MC was achieved through total signal of MS/MS. Precursor ion was $[M + 2H]^{2+}$ at m/z of 520 for MC-RR, while precursor ion was $[M + H]^+$ at m/z of 995.5 for MC-LR. Collision energy was 37% for both MC-RR and MC-LR. All the values present in the text were measured by LC-MS/MS. The limit of detection for the MCs in the tissues of fish was 0.003 μg g^{-1} DW.

2.4. Transmission electron microscopic observation

For transmission electron microscopic study, renal samples were prefixed in 2.5% glutaraldehyde solution, diced into 1 mm^3, followed by three 15 min rinses with 0.1 M phosphate buffer (pH 7.4). Post-fixation was in cold 1% aqueous osmium tetroxide for 1 h. After rinsing with phosphate buffer again, the specimens were dehydrated in a graded ethanol series of 50–100% and then embedded in Epon 812. Ultra-thin sections were sliced with glass knives on a LKB-V ultramicrotome (Nova, Sweden), stained with uranyl acetate and lead citrate and examined under a HITACHI, H-600 electron microscope.

2.5. Serum biochemical analysis

Blood samples were centrifuged at 3000 g for 15 min at 4 °C and serum was stored at −70 °C until analysis. The concentrations of serum BUN and CR were measured with commercially available reagent kits (Zhongsheng, China) based on colorimetric reaction in automatic analyzer (ACTA, Italy) according to the instruction of the manufacturers. All were measured in triplicates, and averaged for statistical use.

2.6. Statistical analysis

Statistical analysis was undertaken using SPSS 11.5 for Windows. Values were expressed as mean ± standard deviation (SD). Data of biochemical parameters were subjected to analysis of two-way ANOVA followed by a post hoc multiple comparisons test (Bonferroni's test). Normality and variance homogeneity were previously verified. Differences were considered to be significant at level $P < 0.05$.

3. Results

3.1. Mortality and gross morphology

No mortality was found in either the 50 μg MC-LReq. kg^{-1} dose group or the control group during a period of 7 days. However, in the 200 μg MC-LReq. kg^{-1} dose group, 5.0%, 8.3% and 18.3% fish mortalities were observed at 12, 24 and 48 h post-injection, respectively, and 100% fish morality was observed at 60 h post-injection.

Visual inspection at sample time points during the experiment had demonstrated a progressive degeneration in livers of treated fish. However, no increase in either kidney size or kidney weight had been observed compared to the control.

3.2. Determination of MCs concentration in kidney

In the present study, MC-RR content in the kidney of crucian carp in both dose groups showed an increasing tendency within 48 h post-injection (Fig. 1). In the 50 μg MC-LReq. kg^{-1} dose group, MC-RR in kidney varied between 0.013 and 0.088 μg g^{-1} dry weight (DW) and after
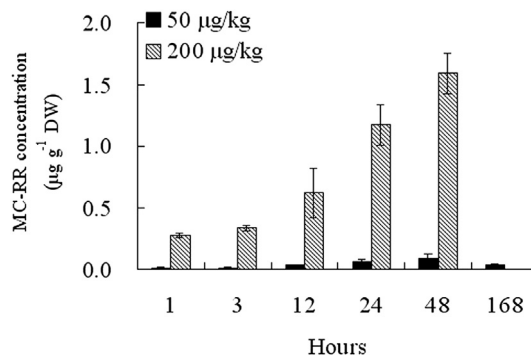

Fig. 1. The temporal changes of MCs content in the kidney of crucian carp after i.p. injection with 50 and 200 μg MC-LReq. kg^{-1} bw.

reaching a maximum at 48 h post-injection, it decreased to 0.039 μg g^{-1} DW at 168 h post-injection. In the 200 μg MC-LReq. kg^{-1} dose group, MCs increased in a time-dependent manner and varied between 0.279 to 1.592 μg g^{-1} DW. No MC-LR in the kidney was detectable in both dose groups.

### 3.3. Ultrastructural observation

3.3.1. Renal corpuscle

Control crucian carp consistently showed normal glomerular ultrastructure during the whole experiment period (Fig. 2A). After 1 h, partial fusion of the foot processes of the podocytes was firstly noted in the 200 μg kg^{-1} dose group (Fig. 2E). At 3 h, dilated Bowman's capsule and extensive fusion of foot processes in the 200 μg kg^{-1} dose group was observed with marked widening of glomerular basement membrane, resulting from insertion of mesangial cytoplasm (Fig. 2F). At 12 h, similar dilation of Bowman's space and partial fusion of podocyte foot processes occurred in the 50 μg kg^{-1} dose group (Fig. 2B). Furthermore, we observed, in the 200 μg kg^{-1} dose group, degenerative loose of glomerular basement membrane. At 24 h, ultrastructural changes of glomeruli in both dose groups were characterized with extensive fusion of podocyte foot processes, degenerative loose and disruption of glomerular basement membrane and proliferation of mesangial cells (Fig. 2G). At 48 h post-injection, glomerular

ultrastructural changes in both dose groups were basically similar to those at 24 h but more serious (Fig. 2C and H). At 168 h, the initial structure in renal corpuscle was reconstituted in the 50 μg kg^{-1} dose group (Fig. 2D). Apparently, ultrastructural alterations in renal corpuscles after i.p. injection of MCs were more rapid and severe in the 200 μg kg^{-1} dose group than in the 50 μg kg^{-1} dose group.

3.3.2. Renal tubules

Examination of the ultrastructure of renal tubules in control crucial carp revealed large, centrally located nuclei with well-delineated nuclear membranes, rich mitochondria and endoplasmic reticulum as well as rarely lysosomes (Fig. 3A). After 1 h, swollen mitochondria in proximal tubular cells were firstly observed in both dose groups and prominent increased lysosomes were also found in the 200 μg kg^{-1} dose group (Fig. 3B and E). Between 3 h and 12 h, mitochondria in tubular epithelial cells in the 50 μg kg^{-1} dose group showed mild hydropic degeneration. Similar changes as well as nuclear deformation and lymphocytic infiltration were seen in the 200 μg kg^{-1} dose group (Fig. 3F). From 24 h to 48 h, we observed, in both dose groups, hydropic degeneration of mitochondria, proliferation of lysosomes and a relative decrease in the number of other organelles, especially the rough endoplasmic reticulum (RER) (Fig. 3C and G). In the 200 μg kg^{-1} dose group, these changes were more severe: cytoplasmic
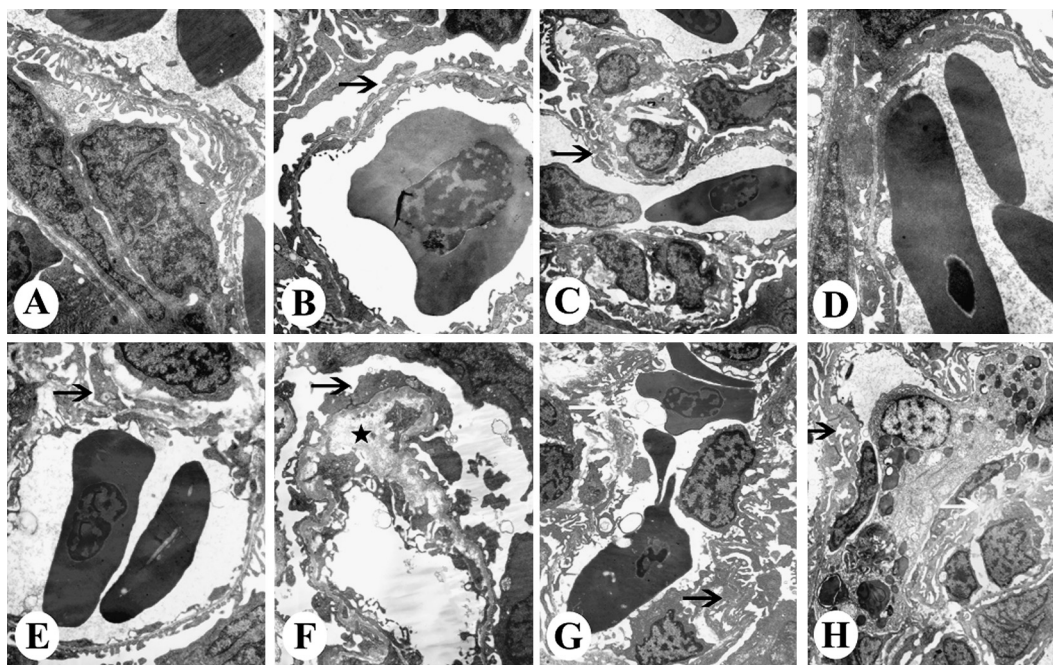
Fig. 2. Toxic effects of MCs on the renal corpuscle of crucian carp after i.p. injection with 50 and 200 μg MC-LReq. kg^{-1} bw, respectively. (A) The normal ultrastructure of renal corpuscle of control crucian carp, 6000×. (B–D) The renal corpuscle of crucian carp in the 50 μg MC-LReq. kg^{-1} dose group: (B) showing dilated Bowman's capsule and partial fusion of podocytes foot processes (black arrow) at 12 h post-injection, 6000×; (C) showing extensive fusion of the foot processes (black arrow), loose degeneration of glomerular basement membrane, insertion (black star) and proliferation of mesangial cells at 48 h post-injection, 4000×; (D) showing recovery glomerulus at 168 h post-injection, 10,000×. (E–H) The renal corpuscle of crucian carp in the 200 μg MC-LReq. kg^{-1} dose group: (E) showing partial fusion of the foot processes (black arrow) at 1 h post-injection, 6000×; (F) showing dilated Bowman's space, extensive fusion of the foot processes (black arrow), marked widening of glomerular basement membrane and mesangial insertion (black star) at 3 h post-injection, 6000×; (G) and (H) showing extensive fusion of foot processes (black arrow), loose degeneration and disruption of glomerular basement membrane (white arrow) accompanied with insertion and proliferation of mesangial cells at 24 h (4000×) and 48 h (3500×) post-injection, respectively.
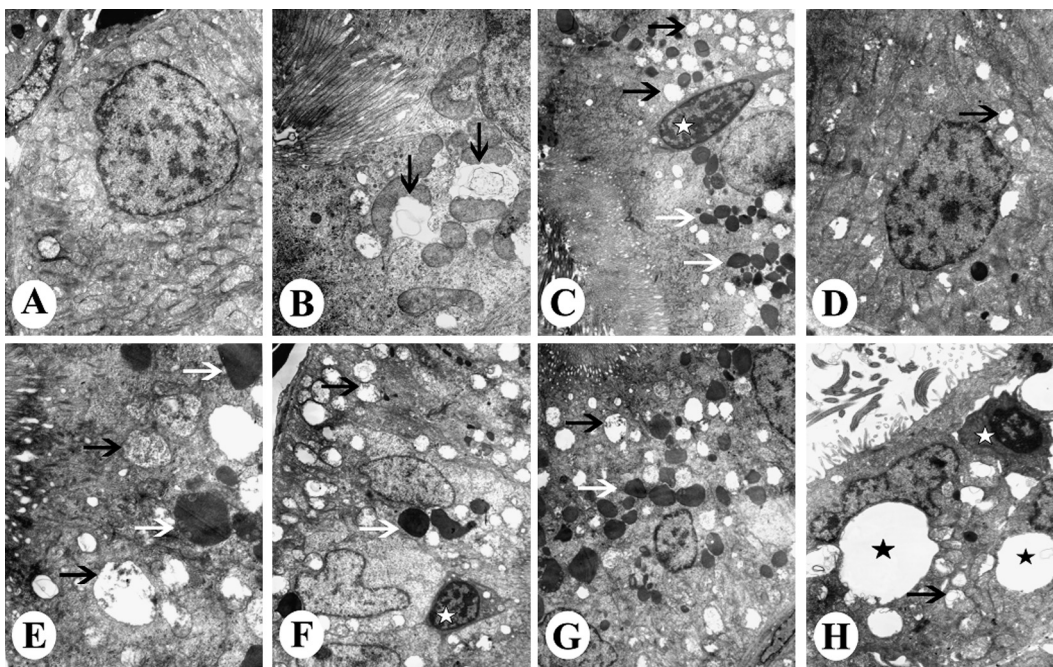
Fig. 3. Toxic effects of MCs on renal tubules of crucian carp after i.p. injection with 50 and 200 μg MC-LReq. kg^{-1} bw, respectively. (A) Normal tubular cells of control crucian carp, 6000×. (B–D) Tubular cells of crucian carp in the 50 μg MC-LReq. kg^{-1} dose group: (B) showing swollen mitochondria (black arrows) at 1 h post-injection, 10,000×; (C) showing hydropic degeneration of mitochondria (black arrows) and prominent increase of lysosomes (white arrows) as well as lymphocytic infiltration (white star) at 48 h post-injection, 4000×; (D) showing slight swelling of a few mitochondria at 168 h post-injection (black arrow), 6000×. (E–H) Tubular cells of crucian carp in the 200 μg MC-LReq. kg^{-1} dose group: (E) and (F) showing hydropic mitochondria (black arrows), increased lysosomes (white arrows) and lymphocytic infiltration (white star) at 1 h (10,000×) and 3 h (3500×) post-injection, respectively; (G) showing hydropic mitochondria (black arrow), prominent increased lysosomes (white arrow) and nuclear pycnosis at 24 h post-injection, 4000×; (H) showing extensive cytoplasmic vacuolization (black stars), hydropic mitochondria (black arrow), pycnotic nuclei and shedding microvilli in tubular lumina at 48 h post-injection, 6000×.

vacuolization, nuclear deformation and pycnosis, microvilli shedding in the proximal tubular lumina (Fig. 3H). Necrotic tubular epithelial cells were often observed adjacent to seemingly healthy tubules. At 168 h post-injection, it is noted that the recovery of tubular ultrastructure were obviously visible and only slight swollen mitochondria persisted in the 50 μg kg^{-1} dose group (Fig. 3D).

3.4. Serum biochemistry

Compared with the control, fish in both dose groups showed a significant increase in BUN ($P < 0.05$) (Fig. 4). The

significant increases in CR level ($P < 0.05$) were also observed at 3 and 24 h in the 50 μg MC-LReq. kg^{-1} dose group and at 1, 3, 12 and 24 h in the 200 μg MC-LReq. kg^{-1} dose group, respectively. It should be noted that there was no data of BUN and CR at 168 h post injection in the 200 μg kg^{-1} dose group due to fish mortality.

4. Discussion

Even though the target organ for MCs is the liver, in the present study, MC-RR content in the kidney was detected in both dose groups as early as 1 h post-injection and showed
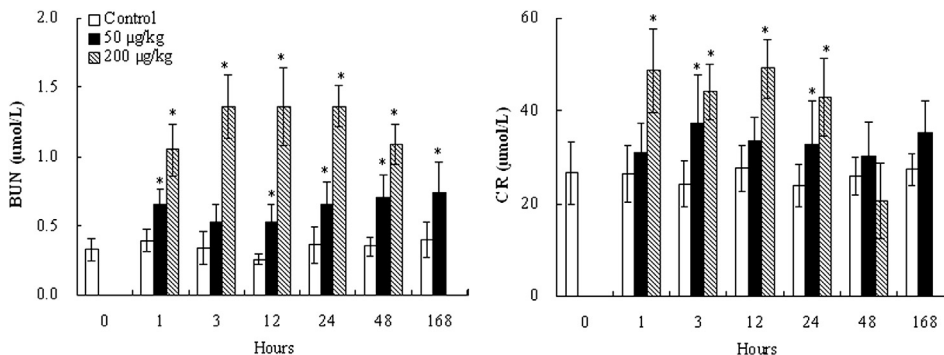
Fig. 4. The temporal changes of BUN and CR in the serum of crucian carp after i.p. injection with 50 and 200 μg MC-LReq. kg^{-1} bw. Values are expressed with mean ± SD ($n = 10$). *Indicates significant differences at $P < 0.05$ between MC-treated groups and the control group.

a time-dose dependent increase within 48 h post-injection, suggesting that kidney was involved in the elimination of MCs from the body and a possible important organ of action of MCs on crucian carp. In the 200 μg kg^{-1} dose group, it reached a maximum value of 1.592 μg g^{-1} DW (0.14% of injected MCs) at 48 h post-injection. Williams et al. (1995) detected rather high MC-LR content (1.67% of the injected dose at 46 h) in the kidney of Atlantic salmon after a sublethal i.p. injection of 0.1 mg/100 g of ^{3}H-MC-LR. Mohamed et al. (2003) estimated MCs accumulation in *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom and found that the highest MCs level was in the guts (0.821 μg g^{-1} fresh weight), followed by the livers (0.532 μg g^{-1}) and kidneys (0. 400 μg g^{-1}). Chen et al. (2006) reported that annual mean MCs content in kidney of bighead carp was even higher than that in liver when the fish was feeding naturally on toxic *Microcystis* blooms in Taihu Lake. S.X. Li et al. (2007) reported that the highest contents of MCs (0.094–4.641 μg g^{-1} DW) were found at 3 h post-injection in the kidney of bighead carp injected i.p. with extracted MCs at two doses of 200 and 500 μg MC-LReq. kg^{-1} bw, indicating that the kidney may have capacity to transport MCs. An immunostaining study found that the injected cysteine conjugate of MC-LR (MCLR-Cys) was the most actively excreted from the kidney (Ito et al., 2002).

Although it comprised 14.3% of the total toxins i.p.-injected to crucian carp, no MC-LR in the kidney was detectable in both dose groups. Based on the results of distribution of MCs in various tissues of crucian carp in the previous study (see Lei et al., 2008 in detail), we demonstrated that MCs (MC-RR + MC-LR) entered into the blood circulatory system rapidly after injection and MC-RR were transported to various tissues, while MC-LR was found only in the liver, heart, gallbladder, gonad and gill. A subchronic toxicity experiment where silver carp were fed with toxic fresh *Microcystis viridis* cells (MC-LR and -RR contents were 110–292 and 268–580 μg kg^{-1} DW, respectively) found that the transportation of MC-LR across the intestines was probably inhibited selectively, whereas MC-RR in the gut might have been massively transported across the intestine and embedded in the fish body (Xie et al., 2004). Prieto et al. (2006) suggested that susceptibility of kidney to MC-RR exposure compared to liver and gills in tilapia under acute (i.p.) administration could be related to its higher hydrophilicity than MC-LR, which facilitated its distribution to kidney. Such difference between MC-RR and MC-LR in kidney of crucian carp in the present study might be due to organ specificity of different MCs. That is, MC-RR rather than MC-LR is easier accumulated in kidney of crucian carp.

In the present study, ultrastructural lesions in the kidney of crucian carp in both dose groups progressed in severity and extent with increasing accumulated MC-RR and exposure times within 48 h post-injection: swollen mitochondria in proximal tubules were firstly noticed at 1 h in both dose groups and partial fusion of foot processes was also were found in the 200 μg kg^{-1} dose group; at 3 h, swelling of mitochondria in proximal tubules became more serious, extensive fusion of foot processes and marked widening of glomerular basement membrane were observed in the 200 μg kg^{-1} dose group; At 12 h, similar changes (hydropic

mitochondria in proximal tubules and partial fusion of podocyte foot processes in glomeruli) occurred in the 50 μg kg^{-1} dose group, while degenerative loose of glomerular basement membrane and hydropic mitochondria in proximal tubules were prominent with nuclear deformation and lymphocytic infiltration in proximal tubules in the 200 μg kg^{-1} dose group; between 24 h and 48 h, ultrastructural changes in both dose groups were characterized with hydropic degeneration of mitochondria and proliferation of lysosomes in proximal tubules, and extensive fusion of podocyte foot processes, degenerative loose and disruption of the glomerular basement membrane and proliferation of mesangial cells in glomeruli. Some of these alterations are consistent with those of Kotak et al. (1996) and Molina et al. (2005), who tested the effects of MCs on rainbow trout injected with a lethal dose of 1000 μg MC-LR kg^{-1} bw and tilapia fish exposed to cyanobacterial cells (60.0 mg MC-LR/fish per day), respectively. These results are also similar to our previous observation in bighead carp, in which dilated Bowman's space, partial fusion of foot processes and proliferation of mesangial cells in glomeruli, and hydropic mitochondria and nuclear deformation in renal tubules were found when bighead carp were exposed to a naturally cyanobacteria blooms in Taihu Lake (Li et al., 2008). Apparently, our results together with previous studies suggest that the membranous structure is the main action site of MCs in the kidney, which is consistent with the toxic mechanism of MCs. The integrity of the structure and ingredient of the cytoskeleton protein networks plays an important role in morphology and function of cell. Membranaceous structures of cell and organelle contain lots of lipid polyunsaturated fatty acids (PUFA), which are vulnerable to ROS attack. The toxic mechanisms of MCs are to potent inhibit protein phosphatases 1 and 2A and/or increase reactive oxygen species (ROS), which consequently cause disruption of cell cytoskeleton and may induce apoptosis or even necrosis finally (Ito et al., 2002; Li et al., 2003). Thus, it is not surprising to find pathological changes in renal membranous structure in our study. In addition, in the present study, the recovery of renal ultrastructural lesions was evident in the 50 μg kg^{-1} dose groups at 168 h with a remarkable decrease of MCs in the kidney. There were few reports about the ultrastructural recovery of kidney in fish exposed to MCs. L. Li et al. (2007) reported that considerable recovery was observed in liver and kidney of silver carp collected from fish pen in Taihu lake on December (the post-bloom period), as indicated by normal proximal tubules with abundant intact mitochondria, and normal morphology of foot processes in epithelial cells of glomeruli. This recovery suggests that both kidney and liver have a mechanism to degrade or bind MCs actively to resist the external threat.

In the present study, the early ultrastructural changes of renal tubules induced by MCs, swollen mitochondria was first observed in both dose groups at 1 h post-injection, when MC-RR content in the kidney was only 0.039 μg g^{-1} DW in the 50 μg kg^{-1} dose group, and in glomeruli, changes were not seen in the 50 μg kg^{-1} dose group until after 12 h of MCs exposure. Ultrastructural alterations in the 200 μg kg^{-1} dose group indicated tubular widespread swelling and vacuolization, resulting from hydropic degeneration of

mitochondria, with this severe injury possibly causing renal necrosis. It appears that mitochondria damage in the tubules is an early, and presumably a critically important effect of MCs in our study. Mitochondria are known to be vulnerable targets of various toxins because of their important role in maintaining cellular structures and functions (Robertson et al., 2004). It is reported that MC-LR could induce morphological changes of mitochondria, uncoupling of mitochondrial electron transport and production of ROS, leading to cell apoptosis in in vitro cultured hepatocytes (Li et al., 2003; Ding and Ong, 2003; Boaru et al., 2006) and in vivo liver (Li et al., 2004; Qiu et al., 2007). Furthermore, in our study, the severity of the injury in renal tubules was much higher than that in glomeruli, suggests that renal tubules are the more sensitive, vulnerable parts of the kidney tissues in fish exposed to MCs. By histopathological observation of kidney damage in carp gavaged with a single sublethal bolus dose of *Microcystis aeruginosa* at a dose of 400 μg MC-LReq. kg^{-1} bw, Fischer and Dietrich (2000) indicated that pathological changes in glomeruli, especially in the mesangia, were secondary to the pathological changes in the proxima. This difference in response to MCs exposure by renal different parts is most likely due to differences in the ability to internalize the toxin.

Blood biochemical indices are useful and sensitive for the diagnosis of diseases and monitoring of the physiological status of fish exposed to toxicants (Adhikari et al., 2004). In fish, 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). CR is a waste product of the phosphorylation of adenosine diphosphate (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). Concurrently with MC-RR accumulation and pathological alterations in the kidney, in the present study, the elevation of levels of BUN and CR suggested the occurrence of renal dysfunction in crucian carp. Such increases were described for two planktivorous filter-feeding fishes, silver carp and bighead carp exposed to naturally *Microcystis* blooms in Taihu Lake for 9 months (Qiu et al., 2009). In conclusion, our study indicates that MC-RR rather than MC-LR is easier accumulated in kidney, which mainly promotes ultrastructural pathological alterations in renal membranous structure and causes renal dysfunction.

Ethical statement

The authors guaranteed that all procedures in this experiment have been performed according to the guidelines of the Ethical Committee for Animal Experiments at Huazhong Agricultural University, which is in accordance with the China Animal Welfare Legislation.

Acknowledgments

Conflict of interest

The authors declare that there are no conflicts of interest in the present experiment.

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