Bull Environ Contam Toxicol (2007) 79:297–300
DOI 10.1007/s00128-007-9207-6

Tissue Distribution of Microcystins in Bighead Carp via Intraperitoneal Injection

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Received: 17 November 2006 / Accepted: 30 April 2007 / Published online: 1 August 2007

Hepatotoxic microcystins (MCs) are natural toxins produced by freshwater cyanobacteria such as *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* (Carmichael 2001). It can cause illness and death in wild and domestic animals and humans (Jochimsen et al. 1998; Zimba et al. 2001). Fishes, as higher trophic consumers, are known to accumulate microcystins. Many toxic experimental studies in fish were focused on documenting the toxicity of microcystin exposure through gastrointestinal and blood circular systems. There were only a few studies considering the tissue distribution and depuration of microcystin in fish (Williams et al. 1995, 1997; Tencalia and Dietrich 1997; Bury et al. 1998; Xie et al. 2004). Acute or subchronic toxic experiments have been conducted to study tissue distribution of microcystin on cold-water carnivorous fishes such as Atlantic salmon (Williams et al. 1995, 1997) and rainbow trout (Tencalia and Dietrich 1997; Bury et al. 1998), and warm-water phytoplanktivorous fishes such as *Tilapia rendalli* (Soares et al. 2004) and silver carp (Xie et al. 2004).

Bighead carp, *Aristichthys nobilis*, is one of the most important freshwater planktivorous fish and has been introduced worldwide for agriculture, comprising 6% of the total freshwater fish production of the world (Xie 2003).

Microcystin-producing algae are usually found in its gut content (Xie, 2003). However, there is still no information available on the content and distribution of microcystins in the tissues of bighead carp. The purpose of this study was to report the tissue distribution and deputation of extractable MCs in various organs of bighead carp after the intraperitoneal injection with extracted compound toxins of MCs. The possible mechanisms underlying these patterns were also discussed.

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Materials and Methods

Bighead carp (n = 36) with mean weight 51.27 ± 1.76 g were purchased from a local fish hatchery (Wuhan, China). Before experimentation, fish were acclimated for 3 days in 100 l aquaria containing dechlorinated tap water. Water temperature was 20 ± 1°C and no food was fed to the fish during the experiment.

Algae cells, of which total microcystin content was 1.41 mg g^{-1} DW and MC-RR, -LR and -YR contents were 0.84, 0.50 and 0.07 mg g^{-1} DW, respectively, were freeze-dried, extracted with methanol, and then suspended in distilled water. 0.5 ml suspension, amounting to equivalent of 400 and 1,000 μg MC-LR + MC-RR kg^{-1} BW, were directly injected (i.p.) along the ventral midline into the peritoneum using syringes. According to Li et al. (2005), these two dose were equivalent to 200 and 500 μg kg^{-1} purified MC-LR, respectively. Three test fish in each dose groups were killed at 1, 3, 12, 24 and 48 h post-injection, respectively. Three control fish were killed only at 0 and 48 h. All muscle, kidney, intestine, gallbladder and spleen samples were immediately frozen and freeze-dried for microcystin analysis.

Extraction and analysis of the microcystins in the fish tissue followed the method of Xie et al. (2004). Freeze-dried samples were extracted 3 times with 10 ml of BuOH:MeOH:H_2O (1:4:15) for 24 h and the suspensions were centrifuged at 18,000 rpm (1 h at 4°C). Each supernatant liquor diluted 1:1 with distilled water was directly

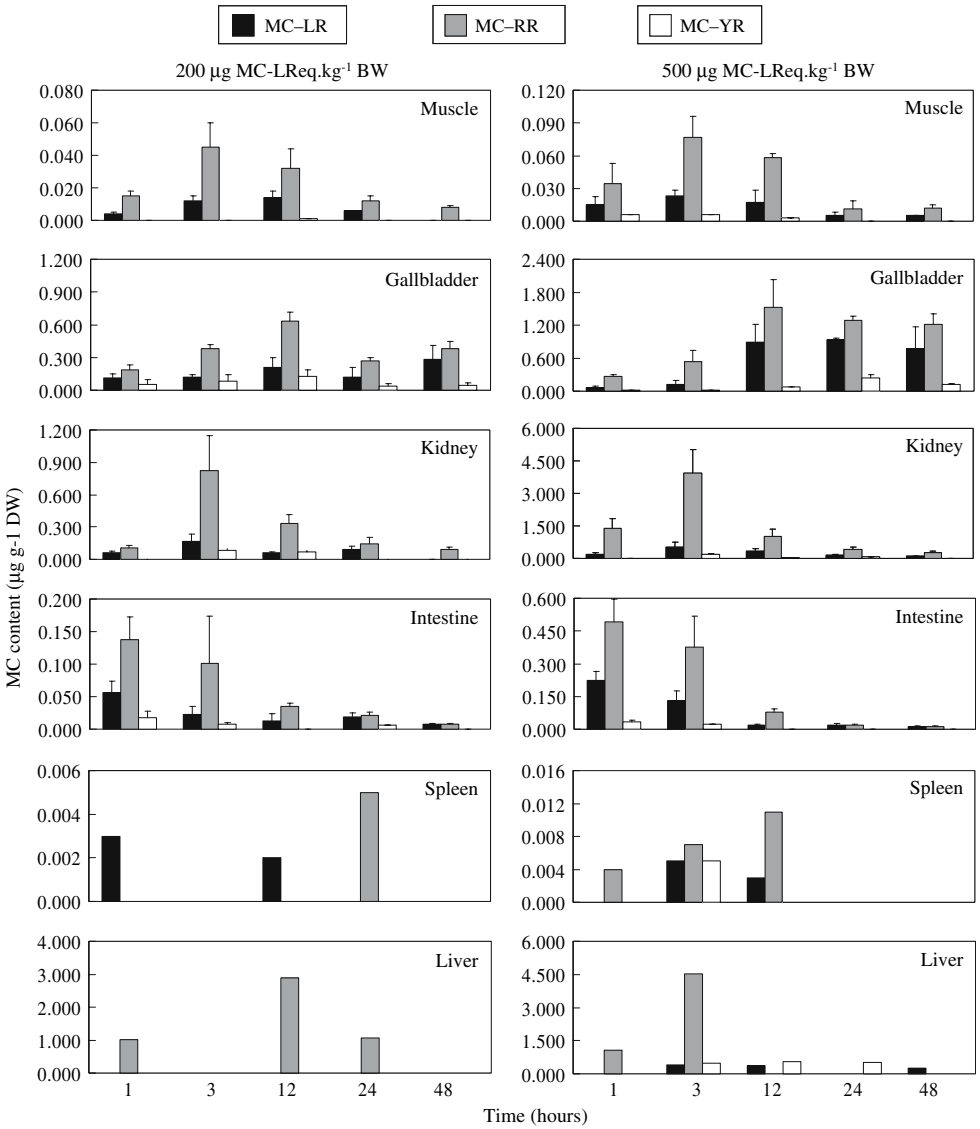

Fig. 1 MC contents in muscle, gall bladder, kidney, intestine, spleen and liver of bighead carp after i.p. injection with microcystins equivalent to 200 and 500 µg MC-LReq kg^{-1} BW, respectively. MC contents of liver were cited from Li et al. (2005)

concentrated on a SPE cartridge (C18, 5 g), which was previously activated with 50 ml of 100% methanol and distilled water. The cartridge was washed with 50 ml of distilled water and 100 ml of 20% methanol. Elution from the cartridge with 100 ml 90% methanol was evaporated to dryness. The residue was dissolved with 5 ml of methanol and applied to a silica cartridge, which was preconditioned with 10 ml of methanol. After the cartridge was washed with 10 ml methanol, elution from the cartridge with 20 ml of 70% methanol was evaporated to dryness. The residue was dissolved with 100 µl distilled water and used for the final detection and identification of MC by LC-MS.

MC concentration was measured using Finnigan LC-MS system consisting of a thermo surveyor auto sampler, a survey or MS pump, a survey or PDA system, and a Finnigan LCQ-Advantage MAX ion trap mass spectrometer equipped with an atmospheric pressure ionization fitted with an electrospray ionization source (ESI). The sample was separated on Hypersil GOLD 5 µm column (2.1 mm i.d. × 150 mm) with a linear gradient run of acetonitrile (15–95%) and acidified water. Both water and acetonitrile were acidified with 0.05% trifluoroacetic acid. Data acquisition was in the positive ionization centroid mode with full mass mode at a mass range between 500 and 1,100. MS tuning and optimization were achieved by infusing microcystin-RR and monitoring the $[M + H]^+$ ion at m/z 520 (Chen and Xie, 2005).

Results and Discussion

Figure 1 showed the distribution of MCs in the organs of bighead carp. In both dose groups, the highest contents of MCs were found in the kidney (0.094–4.641 µg g^{-1} DW), followed by the gallbladder (0.328–2.474 µg g^{-1} DW) and intestine (0.014–0.751 µg g^{-1} DW) (Turkey *t*-test,

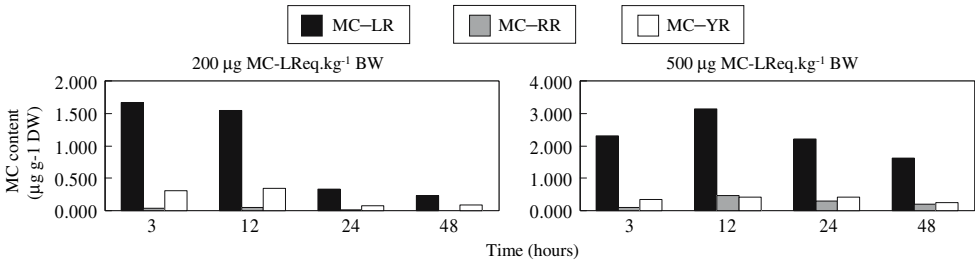

Fig. 2 MC content in aquaria water after i.p. injection with microcystins equivalent to **a** 200 and **b** 500 µg MC-LReq kg^{-1} BW, respectively

$p < 0.05$). Small amounts of MCs were detected in the muscle (0.008–0.057 µg g^{-1} DW in the 200 µg MC-LReq kg^{-1} group and 0.016–0.106 µg g^{-1} DW in the 500 µg MC-LReq kg^{-1} group, respectively). MC contents in the spleen were occasionally detected (0–0.017 µg g^{-1} DW). The maximum MC contents were found at 3 h in the muscle and kidney, at 12 h in the gallbladder, and at 1 h in the intestine (Turkey *t*-test, $p < 0.05$). MC contents in the aquaria water at 3, 12, 24 and 48 h were shown in Fig. 2. The maximum MC concentration were occurred at 3 h in the 200 µg MC-LReq kg^{-1} group and at 12 h in the 500 µg MC-LReq kg^{-1} group, respectively. A small quantity of MC-RR and -YR was detected in both dose groups (0–0.047 and 0–0.243 µg l^{-1}, respectively).

There was only limited information on tissue distribution of microcystin in acute toxic experiments on fishes. Williams et al. (1995, 1997) determined tissue distribution and clearance of ^{3}H- and ^{14}C-labeled microcystin-LR in Atlantic salmon via intraperitoneal injection (0.1 mg of toxin/100 g). Tencalla and Dietrich (1997) conducted the kinetics and biochemical effects of microcystins in rainbow trout (*Oncorhynchus mykiss*) by introduced directly into stomachs in vivo. Bury et al. (1998) studied intestinal transport of microcystin-LR in rainbow trout (*O. mykiss*), which was administered a single dose of 1 mM ^{3}H-microcystin-LR by oral gavaging. The present study first reported MC contents (by HPLC-MS) in various organs of warm-water planktivorous bighead carp through the acute toxic experiment. This was comparable to previous studies in the cold-water carnivorous salmonid Atlantic salmon (Williams et al. 1995, 1997) and rainbow trout (Tencalla and Dietrich 1997; Bury et al. 1998).

MCs content in the intestine decreased dramatically after 1 h post-injection, which suggested that MCs entered the blood system and were transported rapidly to various organs of bighead carp, and subsequently resulted in different levels of MCs in the fish tissues (kidney > gallbladder > intestine > muscle > spleen). Rapid accumulation and slow degradation of MCs were observed in this experiment. This finding supported the results of Williams et al. (1995) and Bury et al. (1998). In both dose groups, the maximum MC contents were found at 3 h in the muscle and kidney, at 12 h in the gallbladder and at 1 h in the intestine.

High level of MC contents in the kidney was found in bighead carp, while low MC contents were detected in the spleen. Williams et al. (1995) detected rather

high MC-LR content in the kidney of Atlantic salmon after a sublethal i.p. injection (0.1 mg/100 g) of ^{3}H-MC-LR. However, no radioactivity was detected in the kidney or spleen of rainbow trout administered a dose of 1 mM ^{3}H-MC-LR via oral gavage (Bury et al. 1998). Inconsistent data may be due to different MC doses, injection modes, analytical methods and organ specificity of MC between fish species. Relatively high MC content in the kidney in our study suggested that the kidney may have capacity to transport microcystin.

The decrease of MC content in the gallbladder occurred more slowly than uptake, and extremely slow decrease occurred in the 500 µg MC-LReq kg^{-1} group. Sahin et al. (1996) detected in the gallbladder of rainbow trout gavaged with hepatotoxic *Microcystis aeruginosa* by methanol extraction and protein phosphatase assays, and proved that biochemically active microcystin molecules were excreted into biliary tract of poisoned fish. Our results also suggested that the clearance of MCs in gallbladder seems more difficult than other organs.

A small quantity of MC-LR can be absorbed and transported across the intestine of fishes in previous studies. In rainbow trout, less than 5% and 1.5% of the applied dose of MC-LR reached the blood and liver in 3 days (Tencalla and Dietrich 1997), and only ca. 0.28–1.29% of the applied dose in the liver and muscle in 24 h (Bury et al. 1998). The maximum MC-LR concentration in the liver of Atlantic salmon was just 4.9 ± 0.5% of the injected dose, and the aquarium water contained 30.6% of tritium labeled MC-LR (Williams et al. 1995). In a subchronic toxicity experiment where the phytoplanktivorous 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), Xie et al. (2003) reported that planktivorous silver carp probably had a mechanism to degrade MC-LR actively and to inhibit MC-LR transportation across the intestines. In the present study, MC concentrations in the aquaria water showed that most MC-LR but a small quantity of MC-RR were excreted. Although MC detected in the experiment was extractable MC, rather low MC-LR content in the intestine and other organs indicated the

intestine of bighead carp can severely inhibit the transportation of MC-LR. The elimination of MC-LR seemed occurred at the intestine while MC-RR degraded in the liver after entering blood system. Further studies are needed to clarify the degradation mechanisms of MC-LR and RR in these planktivorous fishes.

Acknowledgments This research was supported by the Key Project of Chinese Academy of Sciences (KSCX2-SW-129) and a fund from the National Natural Science Foundation of China (30530170).

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