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DISTRIBUTION OF TOXINS IN VARIOUS TISSUES OF CRUCIAN CARP INTRAPERITONEALLY INJECTED WITH HEPATOTOXIC MICROCYSTINS

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Abstract—An acute toxicity experiment was conducted to examine the distribution and depuration of microcystins (MCs) in crucian carp (*Carassius aurutus*) tissues. Fish were injected intraperitoneally with extracted MCs at a dose of 200 μg MC-LR (where L = leucine and R = arginine) equivalent/kg body weight. Microcystin concentrations in various tissues and aquaria water were analyzed at 1, 3, 12, 24, and 48 h postinjection using liquid chromatography coupled with mass spectrometry. Microcystins were detected mainly in blood (3.99% of injected dose at 1 h), liver (1.60% at 1 h), gonad (1.49% at 3 h), and kidney (0.14% at 48 h). Other tissues, such as the heart, gill, gallbladder, intestine, spleen, brain, and muscle, contained less than 0.1% of the injected MCs. The highest concentration of MCs was found in blood (526–3,753 ng/g dry wt), followed by liver (103–1,656 ng/g dry wt) and kidney (279–1,592 ng/g dry wt). No MC-LR was detectable in intestine, spleen, kidney, brain, and muscle, whereas MC-RR was found in all examined fish tissues, which might result from organ specificity of different MCs. Clearance of MC-RR in brain tissue was slow. In kidney, the MC-RR content was negatively correlated with that in blood, suggesting that blood was important in the transportation of MC-RR to kidney for excretion.

Keywords—Microcystins Intraperitoneal injection Crucian carp Tissue distribution Depuration

INTRODUCTION

Microcystins (MCs) are a family of hepatotoxins produced by cyanobacteria and pose a worldwide health threat to humans and animals [1–3]. Structurally, MCs are monocyclic heptatides containing two laevo amino acids and five dextro amino acid. Currently, more than 80 structural variants of MCs are known, and these variants generally differ in the nature of the two L-amino acids at positions 2 and 4 and in the degree of methyl substitution [4]. The most common, and also the most extensively studied MCs are MC-LR (position 2, leucine; position 4, arginine), MC-RR (position 2, arginine; position 4, arginine), and MC-YR (position 2, tyrosine; position 4, arginine), with an order of toxicity of MC-LR > MC-YR > MC-RR based on laboratory toxicity tests in which the median lethal dose (LD50) was obtained in mice using intraperitoneal injection [5]. Microcystins severely inhibit protein phosphatase types 1 and 2A, causing functional and structural disturbances of the liver [6–8].

Fish can be exposed to MCs either during feeding or passively, when the toxins pass through the gills during breathing, and fish mortality is reported in ponds and lakes where toxic cyanobacterial blooms have collapsed [3,9]. Some field studies demonstrate that MCs can accumulate in fish tissues (especially in the liver) and may be transferred farther up the food chain [10–13]. The effects of MCs on fish have been investigated through intraperitoneal injection, oral gavage, or immersion in water containing purified MCs or lysates or whole cells of cyanobacteria. Microcystins affect growth rate and osmoregulation, increase heart rate and liver enzyme activities in the serum, modify behavior, and cause histopathological changes in the liver, intestine, kidney, heart, spleen, and gill [14]. Moreover, the probable neurotoxicity of MCs has been considered [15,16].

Until now, few studies have documented the distribution and depuration of MCs in tissues of fish to assess acute toxicity. Williams et al. [17] determined the distribution and clearance of [^{3}H]MC-LR in the liver, kidney, flesh, gill, and intestine of Atlantic salmon via intraperitoneal injection (0.1 mg MC/100 g). Williams et al. [18] reexamined the distribution and clearance of radiolabeled MC-LR in liver and flesh of Atlantic salmon using [^{14}C]MC-LR. Tencalla and Dietrich [19] documented the dynamics of MCs in liver and blood of rainbow trout that were gavaged with toxic doses of MC-producing *Microcystis aeruginosa* PCC 7806. Bury et al. [20] studied the distribution of [^{3}H]MC-LR in liver, muscle, spleen, and kidney of rainbow trout at 24 h after oral gavage. (Both Atlantic salmon and rainbow trout are cold-water fish.) Cazenave et al. [15] investigated the accumulation of MC-RR in the liver, muscle, gallbladder, brain, blood, and gill of *Jenynsia multidentata* and *Corydoras paleatus* that were exposed to 50 μg/L of MC-RR dissolved in water for 24 h.

The present study used an omnivorous fish, crucian carp (*Carassius auratus*), which is a dominant freshwater species in China and is widely used as a food source by the Chinese people. The purposes of the present study were to examine the distribution and depuration of two common MCs (MC-RR and MC-LR) in various tissues (blood, heart, gill, liver, gallbladder, intestine, spleen, gonad, muscle, brain, and kidney) of crucian carp via intraperitoneal injection with extracted MCs and to discuss the possible mechanisms underlying these patterns. To date, very limited information is available regarding MC accumulation in heart, brain, and gonad in fish.

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MATERIALS AND METHODS

Toxin

The cyanobacterial material used in this experiment was collected from surface blooms of Lake Dianchi (Yunnan, China). Freeze-dried, crude algal matter was 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 toxicity experiments. Before use, the toxin-containing solution was analyzed for MC concentration via high-performance liquid chromatography (LC-20A; Shimadzu, Kyoto, Japan). Microcystin concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR and MC-RR; Wako Pure Chemical Industries, Osaka, Japan). The MC-containing solution was finally diluted with distilled water to 136.5 μg/ml of MC-RR and 22.7 μg/ml of MC-LR.

Fish

Healthy crucian carp with a mean weight of 265 ± 22.6 g (mean ± standard deviation throughout) (n = 90) were purchased from a local fish hatchery in Wuhan City (Hubei, China). Fish were acclimated for two weeks in six aquaria (150 L, n = 15 fish/aquarium) containing dechlorinated tap water and fed with commercial crucian carp food at a rate of 2% of body weight per day. Feeding was terminated 2 d before initiation of the experiment, and no food was supplied to fish during the experimental period. Water temperature was controlled at 25 ± 1°C, and dissolved oxygen was 6.8 ± 0.7 mg/L.

Sample preparation

A dose of an approximately 1-ml suspension of extracted solution of MCs in plain distilled water was injected intraperitoneally along the ventral midline into the peritoneum of fish, amounting to 600 μg/kg body weight of MC-RR plus MC-LR. Because the intraperitoneal LD50 in mice for MC-RR is approximately fivefold higher than that for MC-LR [5], the dose of 600 μg/kg injected with extracted compound toxins of MC-RR and MC-LR in the present study is equivalent to 200 μg/kg of purified MC-LR.

Fifteen test fish were collected for MC analysis at 1, 3, 12, 24, and 48 h postinjection. Fish without administration of MCs are referred to as 0 h. The fish were killed by a blow to the head. Blood was collected from a cut across the tail of the fish. After sampling the blood, the fish were dissected and separated, with the liver, kidney, spleen, intestine, brain, heart, gallbladder, gill, gonad, and muscle (taken from the back) being collected. For the analysis of fish tissues, resources did not allow analysis of individual replicate fish for some organs (e.g., brain, heart, and gallbladder). Instead, materials were pooled such that all the liver, kidney, spleen, intestine, brain, heart, gallbladder, gill, gonad, muscle, and blood of five dissected fish at each sampling time were analyzed. Each reported value represents an average quantity of MCs in the tissues of five individuals. All samples were immediately frozen and lyophilized using a Christ® Alpha 2-4 freeze dryer (Martin Christ, Osterode, Germany). Two liters of surrounding water were taken at each sampling time per aquarium.

Extraction and determination of MCs

For MC extraction from fish tissues, the method described by Xie et al. [21] was used with some modification. Briefly, lyophilized sample was homogenized in a mortar and extracted three times with butanol:methanol:water (1:4:15, v:v:v), then sonicated for 3 min (50% amplitude, 65 W, 20 kHz; Sonics VC130PB; Sonics and Materials, Newtown, CT, USA). The extract was centrifuged (36,290 *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 MCs was washed with 10 ml of 100% methanol and then eluted with 20 ml of 70% methanol. The MC-containing fraction also was evaporated to dryness. This fraction was dissolved with 100 μl of distilled water and used for the final detection and identification of MCs by liquid chromatography–mass spectrometry (LC-MS).

For analysis of the aquarium water, 1 L of water was filtered on a glass filter (mesh size, 1.2 μm; GF/C Whatman International, Maidstone, UK) to separate MCs dissolved in water (dissolved MCs) and MCs bound to particles (MCs in seston). The filter papers were extracted three times with 75% methanol, and the suspensions were centrifuged (36,290 *g,* 4°C, 1 h). The supernatant liquors were diluted 1:5 with distilled water, and the filtrates were directly concentrated on C18 cartridges (0.5 g), which were previously activated with 10 ml of 100% methanol and 10 ml of distilled water. Then, cartridges were washed with 10 ml of distilled water. Cartridges were eluted with 10 ml of 100% methanol, and eluates were evaporated to dryness. The residue was reconstituted with 100 μl of distilled water and used for the final detection and identification of MCs by LC-MS.

Qualitative and quantitative analysis of MCs was performed using a Finnigan LC-MS system (Thermo Electron, Waltham, MA, USA) comprising a Thermo Surveyor Autosampler, a Surveyor mass spectrum pump, a Surveyor photodiode-array system, and a Finnigan LCQ-Advantage MAX ion-trap mass spectrometer equipped with an electrospray ionization source (Thermo Electron). The instrument control, data processing, and analysis were conducted by using Xcalibur™ software (Ver 3; Thermo Electron). Separation was carried out in reverse phase using an Agilent Zorbax SB-C18 column (length, 100 mm; inner diameter, 2.1 mm; film thickness, 3.5 μm; Agilent Technologies, Santa Clara, CA, 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 was as follows: 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 typically were 10 μl. The mass spectrometer was set to electrospray ionization positive-ion mode, and mass spectrometer tuning and optimization were achieved by infusing MC-RR with ion of

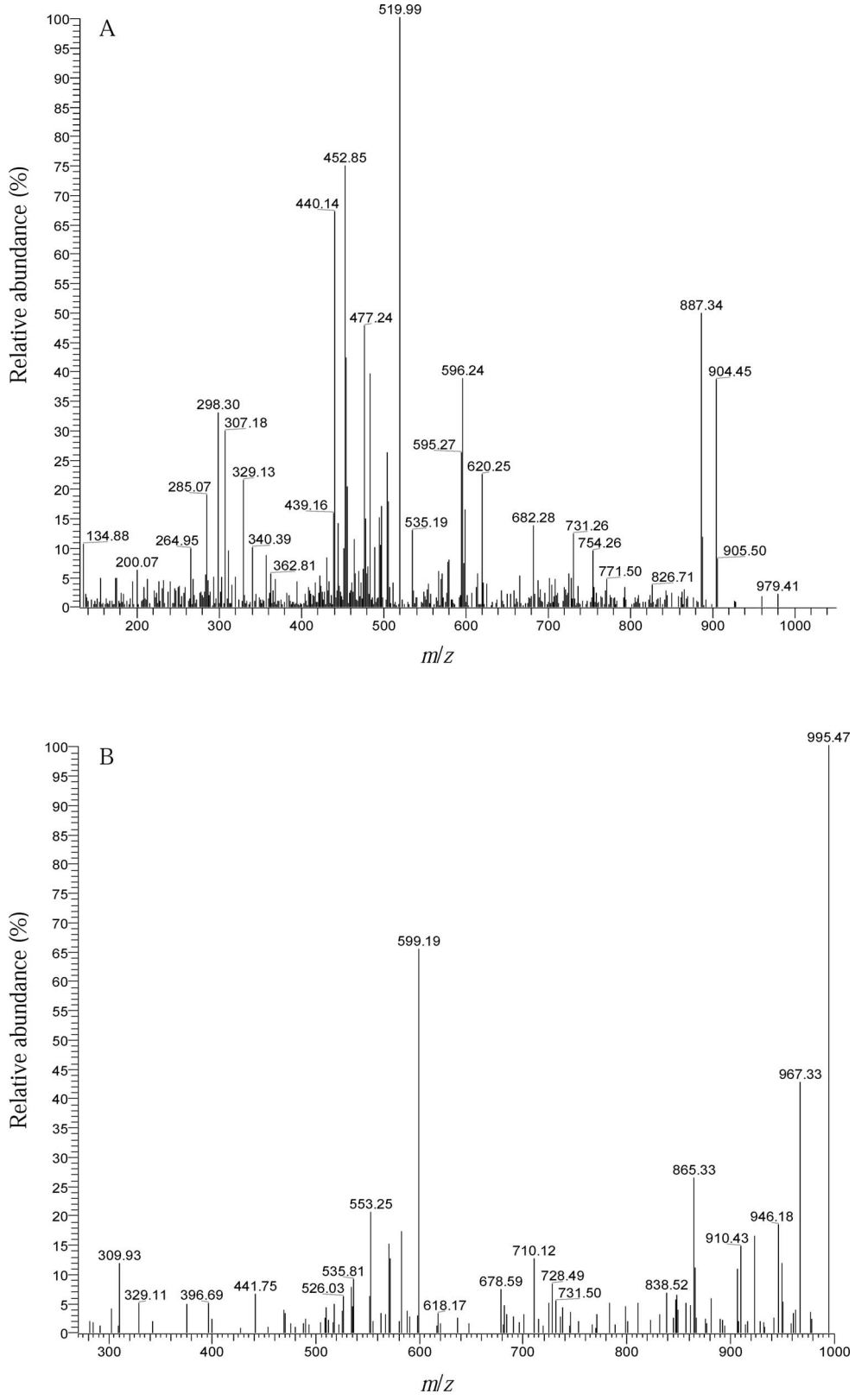

Fig. 1. Liquid chromatography–tandem mass spectrometry analysis of microcystins (MCs) in the liver of crucian carp (*Carassius auratus*) at 1 h postinjection. (**A**) Mass spectrum at 8.21 min (MC-RR, where R = arginine). (**B**) Mass spectrum at 10.98 min (MC-LR, where L = leucine).

$[M+2H]^{2+}$ at *m*/*z* 520. Quantification of MCs was achieved through the total signal of tandem MS (MS/MS). The precursor ion was $[M+2H]^{2+}$ at *m*/*z* 520 for MC-RR, whereas the precursor ion was $[M+H]^{+}$ at *m*/*z* 995.5 for MC-LR. Collision energy was 37% for both MC-RR and MC-LR. All the values presented in the text were measured by LC-MS/MS.

The extraction procedures and the method used to analyze the samples are not suitable to detect MCs bound to glutathione

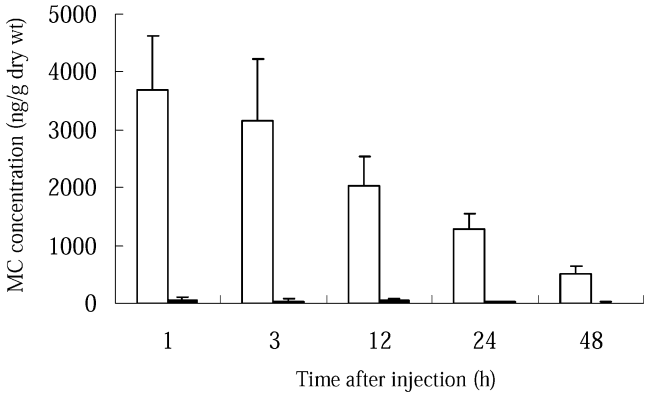

Fig. 2. Microcystin (MC) concentration in blood of crucian carp (*Carassius auratus*) after intraperitoneal injection with 200 μg MC-LR (where L = leucine and R = arginine) equivalent/kg body weight (n = 3 per time point). □ = MC-RR, ■ = MC-LR.

or protein phosphatase. Therefore, all results in the present study refer to free MCs in the fish tissues.

Statistical analysis

The relationships among MC concentrations in fish tissues and aquaria water were examined by correlation analysis using SPSS® software (Chicago, IL, USA). The differences between MC concentrations in fish tissues and aquaria water in relation to time were analyzed by one-way analysis of variance (ANOVA; SPSS software). The differences were considered to be significant at $p < 0.05$.

RESULTS

Figure 1 shows a LC-MS/MS measurement of MCs in liver of crucian carp at 1 h postinjection. Based on the total ion chromatogram, mass chromatograms monitored at m/z 520, and the presence of $[M+H]^+$ ion at m/z 452 and 887, it was confirmed that the peak at 8.21 min was derived from MC-RR. Similarly, the peak at 10.98 min was derived from MC-LR, both because the peak was detected by monitoring with m/z 995.5 and because the mass chromatogram showed $[M+H]^+$ ion at m/z 995.5 and 599 for MC-LR.

At 0 h, no MCs were detected in blood and tissues of crucian carp and aquarium water. After injection, MCs appeared rapidly in blood (Fig. 2), tissues (Fig. 3), and aquaria water (Fig. 4). The highest concentration of MCs (MC-RR + MC-LR) was found in blood (526–3,753 ng/g dry wt), followed by liver (103–1,656 ng/g dry wt), kidney (279–1,592 ng/g dry wt), and heart (20–1,045 ng/g dry wt). Microcystin levels in other tissues, especially muscle, were relatively low. No MC-LR was detectable in the intestine, spleen, kidney, brain, and muscle. Depending on the coefficient in Table 1, we transferred the MC content in fish tissues to values as a percentage of the injected dose. The MC content in the blood, liver, gonad, kidney, and aquaria water are shown in Table 2 as a percentage of injected dose. The heart, gill, gallbladder, intestine, spleen, brain, and muscle contained less than 0.1% of injected MCs.

A decreasing tendency of MC concentration was observed with time in the blood, heart, gill, liver, gallbladder, intestine, and spleen (ANOVA, $p < 0.05$). The MC-RR content in blood was correlated significantly with that in gill ($r = 0.951$, $p < 0.05$), liver ($r = 0.908$, $p < 0.05$), and kidney ($r = -0.968$, $p < 0.01$) (Table 3). Significant positive correlation was present among MC-RR contents in the heart, liver, gallbladder, intestine, and spleen (Table 3). The maximum MC contents were found at 3 h in the gonad and at 12 h in the brain. No significant correlations existed between MC-RR concentration in the gonad (or brain) and other tissues (Table 3). The MC concentrations in the kidney and aquaria water increased during the experimental period (ANOVA, $p < 0.05$). The MC-RR concentration in aquaria water was correlated positively with that in kidney ($r = 0.943$, $p < 0.05$) but negatively with that in blood ($r = -0.973$, $p < 0.01$) and gill ($r = -0.977$, $p < 0.01$) (Table 3).

Table 1. Tissue weight per body weight (TW/BW) and dry weight per wet weight (DW/WW) in crucian carp (n = 15)[a]

Tissue	TW/BW(%)	DW/WW
Heart	0.10 ± 0.03	0.18 ± 0.02
Gill	2.28 ± 0.44	0.20 ± 0.01
Liver	2.34 ± 0.71	0.23 ± 0.04
Gallbladder	0.16 ± 0.06	0.14 ± 0.01
Gonad	7.95 ± 3.93	0.28 ± 0.02
Intestine	1.33 ± 0.33	0.17 ± 0.02
Spleen	0.25 ± 0.09	0.21 ± 0.02
Brain	0.18 ± 0.04	0.19 ± 0.01
Kidney	0.43 ± 0.10	0.20 ± 0.04
Muscle	66[b]	0.21 ± 0.01
Blood	5[b]	0.22 ± 0.05

[a] Mean ± standard deviation.
[b] Approximation.

DISCUSSION

In the present study, when crucian carp were injected intraperitoneally with 200 μg MC-LR equivalent/kg body weight, MCs were detected mainly in blood (3.99% of injected dose at 1 h), liver (1.60% at 1 h), gonad (1.49% at 3 h), and kidney (0.14% at 48 h). Other tissues, such as the heart, gill, gallbladder, intestine, spleen, brain, and muscle, contained less than 0.1% of injected MCs. This is comparable to the results of previous studies in Atlantic salmon [17,18] and rainbow trout [19,20]. When Atlantic salmon were injected with 0.1 mg/100 g of [^{3}H]MC-LR, 4.9% of the injected dose was found in liver at 5 h, 1.67% in kidney at 46 h, and low levels in flesh, gill, pyloric caeca, and intestine [17]. With 0.1 mg/100 g of [^{14}C]MC-LR, 16.6% of the injected radiolabel was detected in liver at 5 h, whereas the relative level in liver compared to flesh tissue was 30:1 [18]. When rainbow trout were exposed to MC via gavage at a dose of 5,700 μg MC-LR/kg body weight, 4.4 and 1.5% of the applied dose reached the blood and liver, respectively, in 3 d [19]; at a dose of 5,873 μg [^{3}H]MC-LR/kg body weight, 1.24 and 0.05% of the applied dose were in liver and muscle, respectively, at 24 h [20].

In the present study, MCs appeared rapidly in blood, with a maximum value of 3,757 ng/g dry weight at 1 h postinjection, and then decreased over time. This indicates that MCs entered into the circulatory system rapidly after injection and were transported to various organs, which subsequently resulted in different levels of MCs in the fish tissues (liver > kidney > heart > gonad > spleen > brain > intestine > gill > gallbladder > muscle). When rainbow trout were gavaged with 5,700 μg MC-LR/kg body weight, absorption of MC-LR from the gastrointestinal tract into the blood occurred rapidly, and MC-LR was quickly taken up into the liver. The MC-LR was detected in both blood and liver at 1 h, with a maximum of 517 ng/ml in blood and 524 ng/g wet weight in liver at 3 h [19].

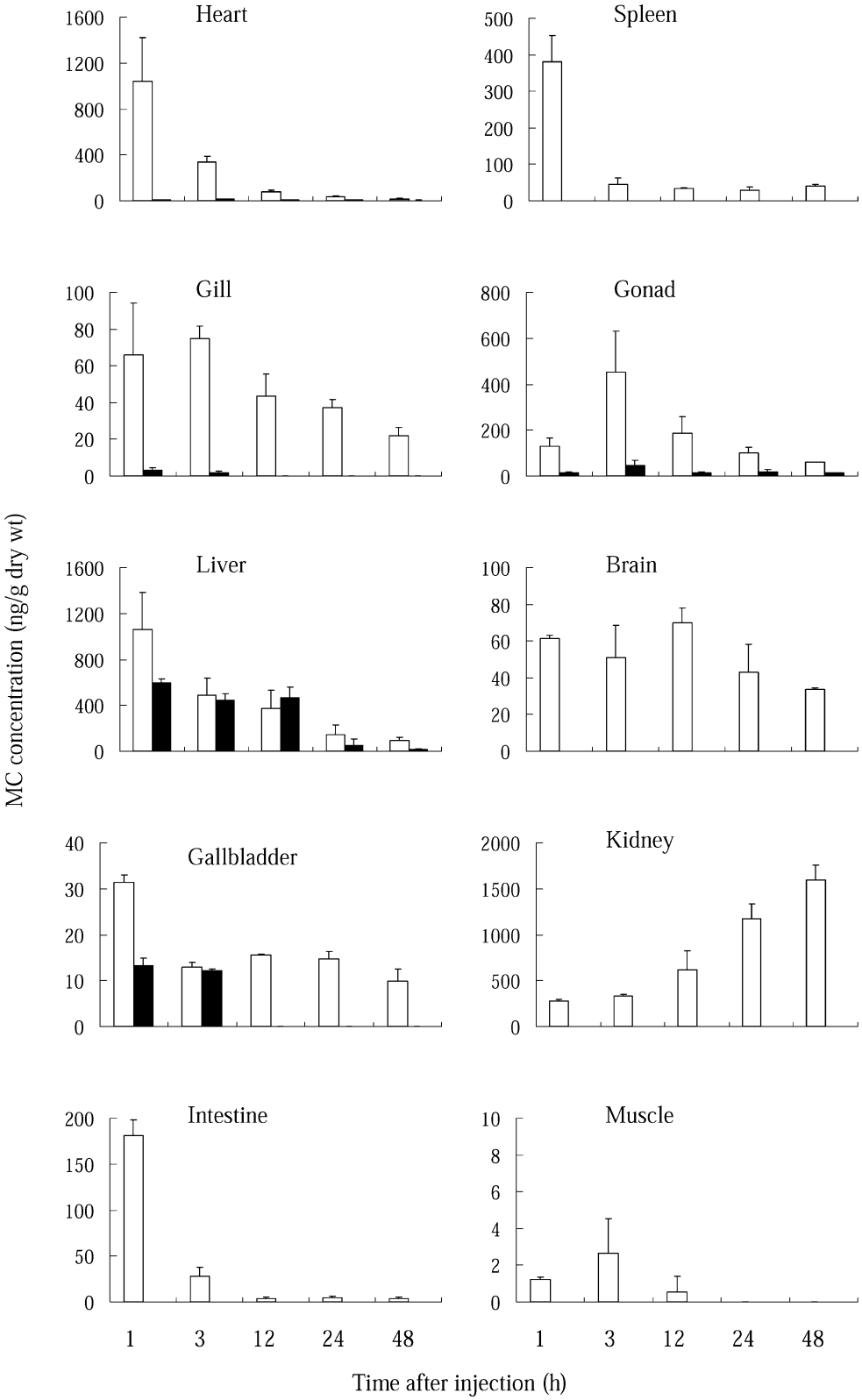

Fig. 3. Microcystin (MC) levels detected in crucian carp (*Carassius auratus*) tissues after intraperitoneal injection with 200 μg MC-LR (where L = leucine and R = arginine) equivalent/kg body weight (n = 3 per time point). □ MC-RR, ■ MC-LR.

The present study revealed a decrease in MC content (from 1,657 ± 360 ng/g at 1 h to 103 ± 43 ng/g at 48 h) in the liver of crucian carp during the experimental period. Several other studies also have shown a decrease in MC content in the liver of fish with time [18,19]. In Atlantic salmon, Williams et al. [18] noted a progressive decrease in the amount of [^{14}C]MC-LR (from 6.98 ± 0.64% to 0.13 ± 0.008% of radiolabel) extracted from liver using methanol. In rainbow trout, an apparent decrease of extractable MC (from 524 ± 197 to 40 ± 8 ng/g) occurred 3 h postadministration in the liver [19]. The

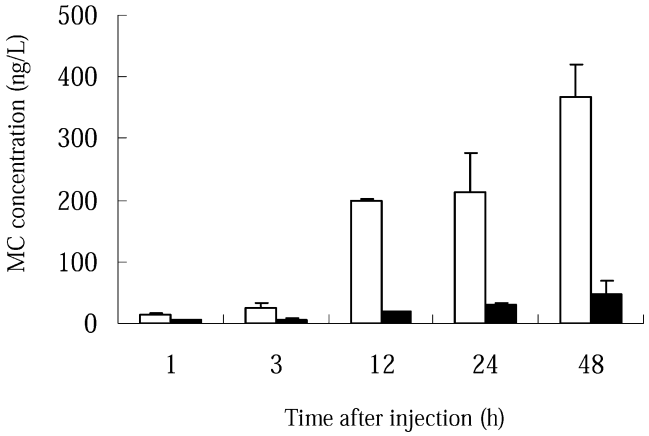

Fig. 4. Microcystin (MC) concentration in the aquaria water (n = 2 per time point). □ = MC-RR (where R = arginine), ■ = MC-LR (where L = leucine).

apparent decrease of extractable MC might be caused by two factors: Biliary excretion of MC or its metabolites, and the slow covalent addition of MC to the catalytic subunit of protein phosphatases and other thiol-containing cellular proteins (e.g., glutathione) [22]. Whereas biliary excretion would progressively reduce the amount of free hepatic MC, covalent binding of MC to the protein phosphatases would prevent extractability of MC and, therefore, contribute to the underestimation of actual amounts of MC in the liver [22].

It is suggested that bile plays an important role in the elimination and recirculation of excessive MCs from the liver of fish [19]. In the present study, the MC-RR content in the gallbladder was significantly correlated with that in liver, although the values were very low (only 10–45 ng/g dry wt). When rainbow trout were orally dosed with 1,220 mg/kg body weight of *M. aeruginosa* (corresponding to 5.60 mg/kg body wt of MC-LR), the MC content in gallbladder (3.5 μg/ml) was severalfold higher than that in liver [23]. When *J. multidentata* and *C. paleatus* were exposed to 50 μg/L of water-dissolved MC-RR, however, no MC was detected in gallbladder tissues [15]. During periods of cyanobacterial blooms in Lake Chaohu (Anhui, China), MCs in the bile of various fish at different trophic levels varied between 0 and 22.6 μg/g dry weight [11]. The inconsistent results described above might result from differences in fish species, exposure routes, and analytical methods.

In the present study, no MC-LR was detectable in the intestine, spleen, kidney, brain, and muscle, whereas MC-RR was found in all the examined fish tissues, with the highest concentration in blood, followed by kidney and liver. Such differences between MC-RR and MC-LR in their distribution in fish tissues may be explained by organ specificity of MCs. Prieto et al. [24] found that under acute intraperitoneal administration, both MC-LR and MC-RR increase lipid peroxidation values in tilapia fish, with the liver being the tissue most sensitive to MC-LR and the kidney the most sensitive to MC-RR. It was suggested that susceptibility of the kidney to MC-RR exposure compared to liver and gill could be related to its higher hydrophilicity compared with that of MC-LR, which facilitates its distribution to the kidney [24]. In a subchronic experiment in which silver carp were fed with toxic, fresh *Microcystis viridis* cells (MC-LR and MC-RR contents were 110–292 and 268–580 μg/kg dry wt, respectively), Xie et al. [21] suggested that MC-RR in the gut fluids might have been transported en masse across the intestines and embedded in the fish body, whereas the transportation of MC-LR across the intestines probably was inhibited selectively. In addition, minor structural changes, characteristic of the different MC congeners, may have major effects on the uptake, organ distribution, and excretion of these toxins [25].

It is known that MCs can accumulate in the gonads of invertebrates, such as mussels [26], snails [27–29], and shrimp [30]. Chen et al. [30] suggested that the reproductive systems of freshwater invertebrates are the second most important target organ of MCs. In the present acute experiment, substantial amounts of MC-RR and MC-LR were detected in the gonad of crucian carp after injection. Microcystin exposure reduced the spawning activity and success in zebrafish (*Danio rerio*) [31] and had harmful effects on the embryonic development of fish [32–34]. Recently, the effects of MCs on the reproductive system in mammals were reported by Ding et al. [35]. When male mice were intraperitoneally treated with *M. aeruginosa* cell extracts containing MCs, the testes were damaged, the quality of mature sperm in the seminiferous tubules decreased, and the motility and viability of the sperm from MC-treated mice decreased compared with control mice [35].

In the present study, small amounts of MC-RR were detected in brain tissue, and the clearance of MC-RR in the brain was slow. Evidence for the presence of MCs in fish brain is limited. Fischer and Dietrich [36] first detected MC-LR in the brain tissue of *Cyprinus carpio* at 48 h after bolus dosing of freeze-dried algae. Cazenave et al. [15] confirmed the presence of MC-RR in the brain tissue of *J. multidentata* exposed to water-dissolved MC-RR. The brain was considered to be the most affected organ to MC-RR because of increased lipid peroxidation levels in the brain of *Corydoras paleatus* exposed to water-dissolved MC-RR [16]. Rainbow trout injected with broken *M. aeruginosa* 7820 cells showed severe meningeal edema and occasional foci of neuronal necrosis within the cerebellar and optic areas of the brain [37].

During our experiment, the MC-RR content in kidney in-

Table 2. Microcystin (MC) content in blood, liver, gonad, and kidney of crucian carp (*Carassius auratus*) and aquaria water as a percentage of injected MCs after intraperitoneal injection with 200 μg MC-LR (where L = leucine and R = arginine) equivalent/kg body weight[a]

Time after injection (h)	% Injected MC				
	Blood	Liver	Gonad	Kidney	Aquaria water
1	3.99 ± 1.01	1.60 ± 0.19	0.45 ± 0.06	0.02 ± 0.00	0.22 ± 0.04
3	3.33 ± 1.34	1.07 ± 0.13	1.49 ± 0.64	0.03 ± 0.00	0.33 ± 0.09
12	2.29 ± 0.62	1.07 ± 0.12	0.58 ± 0.12	0.05 ± 0.02	1.82 ± 0.00
24	1.38 ± 0.28	0.16 ± 0.09	0.42 ± 0.14	0.10 ± 0.01	2.05 ± 0.46
48	0.57 ± 0.21	0.06 ± 0.02	0.28 ± 0.00	0.14 ± 0.01	3.25 ± 0.77

[a] Values are presented as the mean ± standard deviation (n = 3).

Table 3. Correlation (r) among microcystin-RR (where R = arginine) concentrations in tissues of crucian carp (*Carassius auratus*) and aquarium water[a]

	Aquarium water	Blood	Heart	Liver	Gallbladder	Intestine	Spleen	Gill	Brain	Gonad
Blood	−0.973**									
Heart	−0.762	0.838								
Liver	−0.820	0.908*	0.974**							
Gallbladder	−0.643	0.724	0.919*	0.917*						
Intestine	−0.655	0.743	0.985**	0.942*	0.948*					
Spleen	−0.578	0.676	0.963**	0.913*	0.951*	0.995				
Gill	−0.977**	0.951*	0.685	0.750	0.500	0.555	0.467			
Brain	−0.551	0.647	0.411	0.601	0.551	0.369	0.350	0.497		
Gonad	−0.637	0.567	0.096	0.196	−0.156	−0.076	−0.176	0.770	0.259	
Kidney	0.943*	−0.968**	−0.696	−0.818	−0.622	−0.589	−0.519	−0.932*	−0.776	−0.644

[a] * $p < 0.05$, ** $p < 0.01$.

creased to a relatively high level, and it was negatively correlated with that in blood, suggesting that blood was important in the transportation of MC-RR to kidney for excretion. Previous studies have shown that MC exposure through natural or experimental routes resulted in accumulation of MC [12,13] and damage in the kidney tissue of fish [38,39]. Recently, Zhang et al. [40] found that extracted MCs induced kidney impairment, representing hypofunction of hemopoiesis and, thus, causing anemia in the crucian carp.

In the present study, the highest accumulation of MC in muscle was 2.7 ng/g dry weight. A coefficient of five was used to convert dry weight to wet weight (Table 2), and because the intraperitoneal LD50 in mice for MC-RR is approximately fivefold higher than that for MC-LR [4], a coefficient of 0.2 was used to convert MC-RR into the MC-LR equivalent. If an adult person weighing 60 kg eats 300 g of fish muscle a day, then the daily intake of MCs would be only 0.00054 μg/kg, which is much lower than the accepted tolerable daily intake for MC-LR suggested by the World Health Organization (i.e., 0.04 μg/kg body wt) [2]. Nevertheless, MC content in the muscle of wild fish can be rather high, posing a potential risk for human consumption [10–13]. For example, in Lake Chaohu, high MC content (0.497 μg/g dry wt) was found in wild crucian carp during periods of cyanobacterial blooms—approximately 25-fold the recommended tolerable daily intake for MC-LR [11].

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REFERENCES

1. Carmichael WW. 1997. The cyanotoxins. In Callow JA, ed, *Advances in Botanical Research*, Vol 27. Academic, London, UK, pp 211–256.
2. Chorus I, Bartram J. 1999. *Toxic Cyanobacteria in Water: A Guide to Public Health Consequences, Monitoring, and Management.* E&FN Spon, London, UK.
3. Zimba PV, Khoo L, Gaunt P, Carmichael WW, Brittain S. 2001. Confirmation of catfish mortality from *Microcystis* toxins. *J Fish Dis* 24:41–47.
4. Svrcek C, Smith DW. 2004. Cyanobacteria toxins and the current state of knowledge on water treatment options: A review. *J Environ Eng Sci* 3:155–185.
5. Gupta N, Pant SC, Vijayaraghavan R, Rao PVL. 2003. Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin MC variants (LR, RR, YR) in mice. *Toxicology* 188:285–296.
6. Eriksson JE, Toivola D, Meriluoto JAO, Karaki H, Han Y, Hartshorne D. 1990. Hepatocyte deformation induced by cyanobacterial toxin reflects inhibition of protein phosphatases. *Biochem Biophys Res Commun* 173:1347–1353.
7. Falconer IR, Yeung DSK. 1992. Cytoskeletal changes in hepatocytes induced by *Microcystis* toxins and their relation to hyperphosphorylation of cell proteins. *Chem Biol Interact* 81:181–196.
8. Runnegar MT, Kong S, Berndt N. 1993. Protein phosphatase inhibition and in vivo hepatotoxicity of microcystins. *Am J Physiol* 265:224–230.
9. Rodger HD, Turnbill T, Edwards C, Codd GA. 1994. Cyanobacterial (blue-green algal) bloom associated pathology in brown trout, *Salmo trutta* L., in Loch Leven, Scotland. *J Fish Dis* 17: 177–181.
10. Mohamed ZA, Carmichael WW, Hussein AA. 2003. Estimation of microcystins in the freshwater fish *Oreochromis niloticus* in an Egyptian fish farm containing a *Microcystis* bloom. *Environ Toxicol* 18:134–141.
11. Xie LQ, Xie P, Guo LG, Li L, Miyabara Y, Park HD. 2005. Organ distribution and bioaccumulation of microcystins in freshwater fish at different trophic levels from the eutrophic Lake Chaohu, China. *Environ Toxicol* 20:293–300.
12. Chen J, Xie P, Zhang DW, Ke ZX, Yang H. 2006. In situ studies on the bioaccumulation of microcystins in the phytoplanktivorous silver carp (*Hypophthalmichthys molitrix*) stocked in Lake Taihu with dense toxic *Microcystis* blooms. *Aquaculture* 261:1026–1038.
13. Chen J, Xie P, Zhang DW, Lei HH. 2007. In situ studies on the distribution patterns and dynamics of microcystins in a biomanipulation fish—Bighead carp (*Aristichthys nobilis*). *Environ Pollut* 147:150–157.
14. Malbrouck C, Kestemont P. 2006. Effects of microcystins on fish. *Environ Toxicol Chem* 25:72–86.
15. Cazenave J, Wunderlin DA, Bistoni MA, Ame MV, Krause E, Pflugmacher S, Wiegand C. 2005. Uptake, tissue distribution, and accumulation of microcystin-RR in *Corydoras paleatus*, *Jenynsia multidentata*, and *Odontesthes bonariensis*: A field and laboratory study. *Aquat Toxicol* 75:178–190.
16. Cazenave J, Bistoni MA, Pesce SF, Wunderlin DA. 2006. Differential detoxification and antioxidant response in diverse organs of *Corydoras paleatus* experimentally exposed to microcystin-RR. *Aquat Toxicol* 76:1–12.
17. Williams DE, Kent ML, Andersen RJ, Klix H, Holmes CFB. 1995. Tissue distribution and clearance of tritium-labeled dihydromicrocystin-LR epimers administered to Atlantic salmon via intraperitoneal injection. *Toxicon* 33:125–131.
18. Williams DE, Craig M, Dawe SC, Kent ML, Andersen RJ, Holmes CFB. 1997. ^{14}C-labeled microcystin-LR administered to Atlantic salmon via intraperitoneal injection provides in vivo evidence for covalent binding of microcystin-LR in salmon livers. *Toxicon* 35: 985–989.
19. Tencalla F, Dietrich D. 1997. Biochemical characterization of microcystin toxicity in rainbow trout (*Oncorhynchus mykiss*). *Toxicon* 35:583–595.

20. Bury NR, Newlands AD, Eddy FB, Codd GA. 1998. In vivo and in vitro intestinal transport of ^{3}H-microcystin-LR, a cyanobacterial toxin, in rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol* 42:139–148.
21. Xie LQ, Xie P, Ozawa K, Honma T, Yokoyama A, Park HD. 2004. Dynamics of microcystins-LR and -RR in the phytoplanktivorous silver carp in a sub-chronic toxicity experiment. *Environ Pollut* 127:431–439.
22. Fischer WJ, Hitzfeld BC, Tencalla F, Eriksson A, Mikhailov A, Dietrich DR. 2000. Microcystin-LR toxicodynamics, induced pathology, and immunohistochemical localization in livers of bluegreen algae exposed rainbow trout (*Oncorhynchus mykiss*). *Toxicol Sci* 54:365–373.
23. Sahin A, Tencalla FG, Dietrich DR, Naegeli H. 1996. Biliary excretion of biochemically active cyanobacteria (blue-green algae) hepatotoxins in fish. *Toxicology* 106:123–130.
24. Prieto AI, Jos A, Pichardo S, Moreno I, Camean AM. 2006. Differential oxidative stress responses to microcystins LR and RR in intraperitoneally exposed tilapia fish (*Oreochromis* sp.). *Aquat Toxicol* 77:314–321.
25. Dietrich D, Hoeger S. 2005. Guidance values for microcystins in water and cyanobacterial supplement products (blue-green algal supplements): A reasonable or misguided approach? *Toxicol Appl Pharmacol* 203:273–289.
26. Watanabe MF, Park HD, Kondo F, Harada K, Hayashi H, Okino T. 1997. Identification and estimation of microcystins in freshwater mussels. *Nat Toxins* 5:31–35.
27. Chen J, Xie P. 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in two freshwater shrimps, *Palaemon modestus* and *Macrobrachium nipponensis*, from a large, shallow, eutrophic lake of the subtropical China. *Toxicon* 45:615–625.
28. Xie LQ, Yokoyamab A, Nakamuraa K, Park HD. 2007. Accumulation of microcystins in various organs of the freshwater snail *Sinotaia histrica* and three fish in a temperate lake, the eutrophic Lake Suwa, Japan. *Toxicon* 49:646–652.
29. Zhang DW, Xie P, Liu YQ, Chen J, Liang GD. 2007. Bioaccumulation of the hepatotoxic microcystins in various organs of a freshwater snail from a subtropical Chinese lake, Taihu Lake, with dense toxic *Microcystis* blooms. *Environ Toxicol Chem* 26: 171–176.
30. Chen J, Xie P, Guo LG, Zheng L, Ni LY. 2005. Tissue distributions and seasonal dynamics of the hepatotoxic microcystins-LR and -RR in a freshwater snail (*Bellamya aeruginosa*) from a large, shallow, eutrophic lake of the subtropical China. *Environ Pollut* 134:423–430.
31. Baganz D, Staaks G, Steinberg C. 1998. Impact of the cyanobacteria toxin, microcystin-LR on behavior of zebrafish, *Danio rerio*. *Water Res* 32:948–952.
32. Oberemm A, Fastner J, Steinberg CEW. 1997. Effects of microcystin-LR and cyanobacterial crude extracts on embryolarval development of zebrafish (*Danio rerio*). *Water Res* 31:2918–2921.
33. Jacquet C, Thermes V, Luze A, Puiseux-Dao S, Bernard C, Joly JS, Bourrat F, Edery M. 2004. Effects of microcystin-LR on development of medaka fish embryos (*Oryzias latipes*). *Toxicon* 43: 141–147.
34. Wang PJ, Chien MS, Wu J, Chou HN, Lee SJ. 2005. Inhibition of embryonic development by microcystin-LR in zebrafish, *Danio rerio*. *Toxicon* 45:303–308.
35. Ding XS, Li XY, Duan HY, Chung IK, Lee JA. 2006. Toxic effects of *Microcystis* cell extracts on the reproductive system of male mice. *Toxicon* 48:973–979.
36. Fischer WJ, Dietrich DR. 2000. Pathological and biochemical characterization of microcystin-induced hepatopancreas and kidney damage in carp (*Cyprinus carpio*). *Toxicol Appl Pharmacol* 164:73–81.
37. Phillips MJ, Roberts RJ, Stewart JA, Codd GA. 1985. The toxicity of the cyanobacterium *Microcystis aeruginosa* to rainbow trout, *Salmo gairdneri* Richardson. *J Fish Dis* 8:339–344.
38. Kotak BG, Semalulu S, Fritz DL, Prepas EE, Hrudey SE, Coppock RW. 1996. Hepatic and renal pathology of intraperitoneally administered microcystin-LR in rainbow trout (*Oncorhynchus mykiss*). *Toxicon* 34:517–525.
39. Li L, Xie P, Chen J. 2007. Biochemical and ultrastructural changes of the liver and kidney of the phytoplanktivorous silver carp feeding naturally on toxic *Microcystis* blooms in Taihu Lake, China. *Toxicon* 49:1042–1053.
40. Zhang XZ, Xie P, Li DP, Shi ZC. 2007. Hematological and plasma biochemical responses of crucian carp (*Carassius auratus*) to intraperitoneal injection of extracted microcystins with the possible mechanisms of anemia. *Toxicon* 49:1150–1157.