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Preliminary Study of the Distribution and Accumulation of GSH/Cys Metabolites of Hepatotoxic Microcystins-RR in Common Carp from a Lake with Protracted Cyanobacterial Bloom (Lake Taihu, China)

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Abstract Tissue distributions and seasonal dynamics of glutathione and cysteine S-conjugates of microcystin-RR in feral fish from Lake Taihu were studied. High MC-RR-Cys was found in tissues, Mean MC-RR-Cys in kidney (0.253 μg g^{-1} DW) was 4 times of that in liver (0.063 μg g^{-1} DW). Ratios of MC-RR-Cys/MC-RR in liver/kidney reached as high as 5.3/39.8, respectively, meanwhile, kidney showed low accumulation of MC-RR and higher formation efficiency of MC-RR-Cys than liver (7.51× to liver), this suggested that MC-RR-Cys were significantly accumulated with the depletion of MC-RR, and it was selectively biotransformed to MC-RR-Cys in kidney for further excretion.

Keywords MC-RR-GSH · MC-RR-Cys · Common carp · LC–MS · Risk assessment

Microcystins (MCs) are a family of potential hepatotoxins produced by cyanobacteria. There were over 80 analogues of MCs (Dietrich and Hoeger 2005), among which MC-LR (leucine arginine), -RR (arginine arginine) and -YR (tyrosine arginine) are the most common variants. As strong inhibitors of PP-1 and 2A, MCs can act as skin and liver tumor promoters in laboratory animals. Extensive studies have documented the distribution and bioaccumulation of MCs in various aquatic organisms (snail, mussel, and various fish) (Chen et al. 2007), and could potentially threaten the health of aquatic animals and humans (Chen et al. 2009). Studies have shown that the reduced glutathione (GSH) plays important roles in the desintoxication of MCs by formation of a hydrophilic conjugation via GST (Li et al. 2011). Glutathione conjugates of MC-LR can be synthesized by enzyme extracts containing GST of aquatic macrophyte, invertebrates, fish eggs and fish (Pflugmacher et al. 1998). They have reported that the hydrophilic cysteine S-conjugates are effectively formed, transported and eliminated after treated with MC-LR in vivo. MC-LR-GSH was detected both in vivo and vitro (Chen et al. 2007; Zhang et al. 2009), and suggested that efficiency of MC-LR-Cys formation differs among species. However, studies on MC-S-conjugates have focused on MC-LR for the absence of analytical technologies on MC-RR-GSH, MC-RR-Cys. Actually, MC-RR was especially taken up into tissues of aquatic biota for its hydrophily. Carps cultured in Lake Taihu for consumption also showed high bioaccumulation of MC-RR, They found liver and kidney of feral fish were severely affected by cyanobacterial blooms containing high MC-RR. Hence, it is worthwhile to investigate the metabolism of MC-RR of fish tissues, especially in liver and kidney.

In this study, metabolites of MC-RR (MC-RR-GSH and MC-RR-Cys) were quantified for the first time in liver and kidney of common carp. The contents of MC-RR and its metabolites in muscle were also determined for the potential risk in fish consumption. Therefore, the main purposes of this study were to 1) to examine distributions and seasonal changes of microcystins-RR and its metabolites in

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metabolic organs (liver and kidney) and eatable part [(muscle), 2] to discuss the relationship between bioaccumulation of the above compounds in natural conditions (frequently exposed to the cyanobacterial blooms), so as to identify the roles of glutathione and cysteine conjugates in the MCs detoxification process.

Materials and Methods

Gonghu Bay, an important bay of Lake Taihu, situated on the ancient Yangtze River is generally utilized for water supply, flood control, fisheries, tourism and recreation, and shipping. Recent studies show that water quality is deteriorating increasingly due to rapid economic development and intensive water use, thus heavy cyanobacterial blooms often occur over wider areas in warm seasons.

MC-RR were isolated and purified from *Microcystis aeruginosa* collected from Lake Dianchi MC-RR-GSH and MC-RR-Cys were prepared based the method described by Kondo et al. (1992).

Three *Cyprinus carpio* (body weight 0.52 ± 0.13 kg; body length 26.1 ± 2.7 cm) samples were prepared from Gonghu Bay monthly from January to December 2008. The collected fish were measured, weighed and sacrificed immediately, and then, the liver, kidney and muscle were dissected in the field, each sample was mixed with three fish and stored frozen at −20°C immediately.

Samples were lyophilized by a Christ@ Alpha 2–4 freeze dryer (Martin Christ, Osterode, Germany). The lyophilized samples Analyses (0.2 g dry weight for each sample) were performed by a Finnigan LC–MS/MS were performed according to the methods described by our previous work (Wu et al. 2010).

Results and Discussion

At the end of May 2007, an outbreak of cyanobacterial blooms in Gonghu Bay led to the rapid deterioration of water quality in Gonghu waterworks, seriously affecting its water supply for 2 million inhabitants of Wuxi city (Yang 2008). We determined the seasonal dynamics of *Microcystis* spp. biomass and intra/extracellular MCs in Gonghu Bay from January to December 2008 in our recent work, and found *M. aeruginosa* biomass ranged from 0.001 to 32.1 mg L^{-1} with a mean value of 13.38 mg L^{-1}, comprised a large proportion of phytoplankton community (>99 %) in this area. Average concentration of MC-RR in surface cyanobacterial blooms were 1.62 μg L^{-1} (ranged from 0.001 to 6.94 μg L^{-1}). Dissolved MCs in the water column was relatively low with a mean content of 0.017 μg L^{-1}. Both intra- and extra-cellular MCs reached peaks (6.94–0.925 μg L^{-1}, respectively) in October. Common carp samples were collected monthly within this water during the year of 2008.

Quantification analysis was performed at the selected reaction monitoring (SRM) in positive mode (Fig. 1). For quantification purposes, mass spectra of the product ions were monitored at *m/z* 440.0, 453.0 and 887.2 from the parent ion at *m/z* 520.0 $[(M + 2H)^{2+}]$ for MC-RR, and daughter ions at m/z 609.6 and 537.2 were from the parent ion of MC-RR-GSH at *m/z* 673.5 $[(M + 2H)^{2+}]$ by loss of a neutral fragmentation 129–273 Da. And *m/z* 513.4 $[(M\text{-}C_9H_{11}O + 2H)^{2+}]$, 519.8 $[(M\text{-Cys} + H)^{2+}]$, 1008.4 $[(M\text{-}C_9H_{11}O\text{-}NH_3 + H)^{+}]$ were the cleavages from MC-RR-Cys. TIC chromatography was showed in (Fig. 1b), retention times for MC-RR-GSH, MC-RR-Cys and MC-RR were 10.25, 10.69, 11.41 min, respectively.

Several studies demonstrated the accumulation of MC-RR in various tissues of aquatic animals via blood steam. In this study, MC-RR was detected in most of liver and kidney samples, and ultimately depurated when it comes to muscle. Thus, relative distribution of MC-RR shown the highest amounts in liver (Fig. 2a), followed by kidney (Fig. 2b) and muscle (Fig. 2c). In addition, the measured MC-RR in liver and kidney correlated significantly ($r = 0.846$, $p < 0.01$; $n = 12$). Dietrich and Hoeger (2005) suggested MC-RR tended to be transported to kidney by selective transport systems and excreted. Liver/kidney ratio of the determined MC-RR was 2, it is likely that kidney was active in depuration of MC-RR, and this results in the less accumulation of MC-RR in kidney. Low concentration of MC-RR was reported to be related to its higher hydrophilicity (Xie et al. 2004), which facilitates its transportation and clearance.

It is widely reported that intracellular tripeptide GSH, a phase II detoxification enzyme system, plays a key role in cellular defense against oxidative damage (Dekant 2001) and participates in the detoxification of microcystins via glutathione S-transferases (GST) and glutathione peroxidase(GPx) (Ding et al.2000). Zhang et al. (2009) have detected the content of MC-LR-Cys in various aquatic animals, and suggested efficiency of MC-LR-Cys formation differs among species. Similar to MC-LR, Cazenave et al. (2005) observed that glutathione-S-transferase (GST) activity was inhibited by MC-RR in fish. Therefore, we detect the seasonal variation of MC-RR-Cys. MC-RR and MC-RR-Cys were recorded in liver and kidney in most month of the year, the proportions (MC-RR-Cys/MC-RR) varied from month to month, contents of MC-RR-Cys was much higher in liver (0.063 μg g^{-1} DW) and kidney(0.253 μg g^{-1} DW), as 5.3 × 39.8 as that of MC-RR contents, respectively. The above results suggest that MC-RR-Cys take up to 80 % of the three methanol-extractable types. As described in previous sections, MC-RR-Cys was

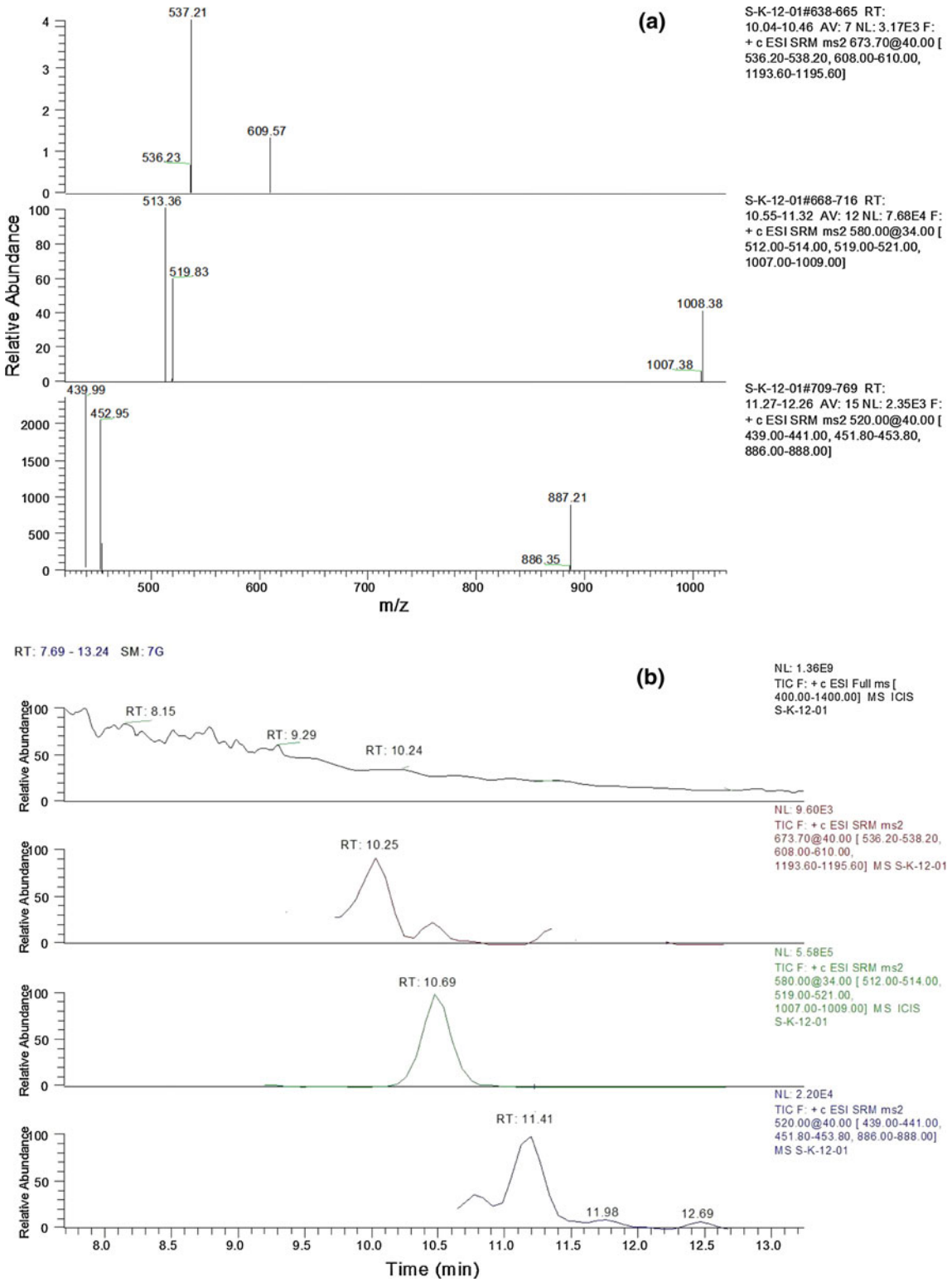

Fig. 1 ESI-LC/MS2 in selected reaction monitoring (SRM) chromatograms and product ion mass spectrum for MC-RR and its metabolites (MC-RR-GSH and MC-RR-Cys) in the liver of common carp from December 2008, Lake Taihu, China. Shown are: **a** product ion mass spectrum for MC-RR-GSH, MC-RR-Cys and MC-RR; **b** total ion and SRM chromatograms for MC-RR and its two metabolites (MC-RR-GSH and MC-RR-Cys)

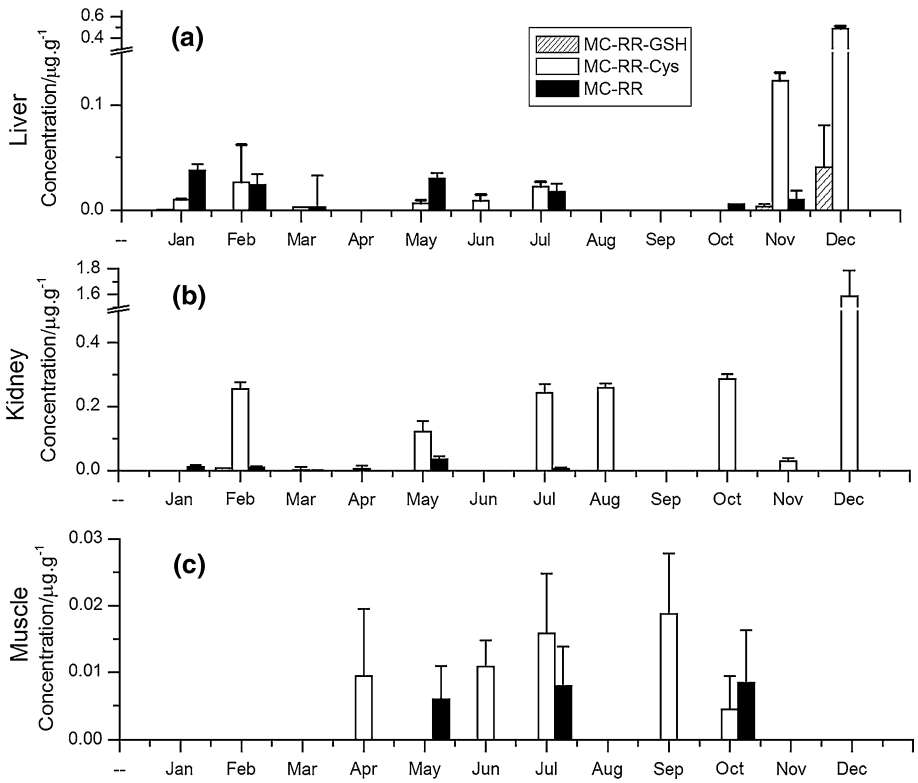

Fig. 2 Seasonal changes for MC-RR, MC-RR-GSH, and MC-RR-Cys contents in **a** liver, **b** kidney, **c** muscle of common carp (*C. carpio*) in Gonghu Bay, Lake Taihu, China, from January to December 2008. LOD (limit of detection) = 0.004 μg g^{-1} DW

detected in both kidney and liver, but mainly accumulated in kidney with a 4 times ratio relative to in liver. Prieto et al. (2006) have found kidney being the most sensitive tissue to MC-RR for LPO value change. And ratio of MC-RR-Cys/MC-RR in liver was 5.3, it significantly increased to 39.8 in kidney. Results in Fig. 2 revealed that MC-RR-Cys was accumulated with the exhaustion of MC-RR, especially in kidney, the most likely explanation for such change of distribution was that MC-RR was tend to biotransform to MC-RR-Cys in kidney before its metabolism/depuration. MC-RR also tended to be transported to kidney by selective transport systems and excreted (Dietrich and Hoeger 2005). The formation efficiency of MC-RR-Cys (caculated by MC-RR-Cys/MC-RR ratio, Zhang et al. 2009) in kidney was 7.5× faster than in liver. High ratio of MC-RR-Cys/MC-RR was a good evidence that common carp formed MC-RR-Cys efficiently in a long term bloom exposure, suggesting that (1) cysteine S-conjugates were formed as important metabolites with the depuration of MC-RR. (2) Kidney showed an active mechanism in depuration of MC-RR by formation of MC-RR-Cys, leading to the accumulation of MC-RR-Cys.

Tolerable daily intake (TDI) of 0.04 μg kg^{-1} body weight/day for free MC-LR was proposed by the World Health Organization (WHO) (Chorus and Bartram 1999). However, the Cys-S-conjugates still remained toxic (Kondo et al. 1992; Dekant 2001). The risk associated with consuming aquatic animals contaminated with MCs may have been underestimated. Our results showed that MC-RR and MC-RR-Cys (about twofold of the MC-RR) were present in most of the muscle samples. Therefore, in our future studies, a reasonable assessment on human consumption of aquatic animals is needed to evaluate the potential hazard of the measured cysteine conjugates of microcystins.

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