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Transcriptional alteration of cytoskeletal genes induced by microcystins in three organs of rats

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ABSTRACT

This study explored the mechanisms of toxicity of microcystins by measuring the transcription levels of nine cytoskeletal genes (actin, tubulin, vimentin, ezrin, radixin, moesin, MAP1b, tau, stathmin) in the liver, kidney and spleen of male Wistar rats treated with microcystins at a dose of 80 μg MC-LReq kg^{-1} bw. Microcystins disrupted the transcriptional homeostasis of cytoskeletal genes in these organs. Changes in the transcription of four genes (β-actin, ezrin, radixin and tau) in liver, one gene (stathmin) in kidney, and one gene (radixin) in spleen were significantly correlated with the tissue concentration of microcystins. However, the influences on the transcription of most genes we studied were greater in the liver than in the kidney or spleen. The effects of microcystins on the transcription of cytoskeletal genes may explain some of the morphological and pathological changes observed in these organs and provide new information on the hepatotoxicity of these compounds. Additionally, transcriptional changes in tumor-associated cytoskeletal genes (ezrin, moesin and stathmin) that were observed in the present study provide a possible clue to the tumor-promoting potential of microcystins and their influences on the transcription of MAP1b and tau imply possible neurological toxicity of microcystins in vertebrates.

1. Introduction

Cyanobacterial blooms are mostly dominated by *Microcystis* species, which are well-studied producers of a group of hepatotoxins known as microcystins. Microcystins can cause lethal poisoning of fish, wildlife, livestock and pets (Carmichael and Falconer, 1993; Dawson, 1998). Human illnesses attributed to cyanobacterial toxins to date may be categorized as: gastroenteritis and related diseases, allergic and irritation reactions, and liver diseases (Bell and Codd, 1994; Chorus et al., 2000; Hitzfeld et al., 2000). In a recent study, microcystins were found to be transferred from contaminated lake water and aquatic animals to a chronically exposed human population (fishermen at Lake Chaohu, China) (Chen et al., 2009).

Microcystins (MCs) comprise a family of more than 80 structurally related toxins. The most common are MC-LR, MC-RR and MC-YR (Svrcek and Smith, 2004). These cyclic heptapeptides preferentially accumulate in vertebrate liver cells due to the presence of organic anion transporting proteins (OATPs) that are highly expressed in this organ (Fischer et al., 2005). However, accumulation of microcystins has also been reported in the intestine, kidney, spleen, gonad, heart, and muscles of fish and mammals (Cazenave et al., 2005; Kagalou et al., 2008; Kankaanpää et al., 2005; Qiu et al., 2009). Recently, several researchers have observed the accumulation of microcystins in the brains of fish and mammals (Cazenave et al., 2005; Falconer et al., 1986; Lei et al., 2008a,b; Wang et al., 2008) and abnormal behavior associated with brain damage, suspected

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to be caused by microcystins, has been reported (Baganz et al., 2004; Cazenave et al., 2008; Ernst et al., 2006).

The toxicity of microcystins has been attributed to the highly specific inhibition of the phosphatases PP1 and PP2A (Honkanen et al., 1990; MacKintosh et al., 1990), which in turn leads to an increase in protein phosphorylation. Accumulating evidence also implies that MC-dependent damage in mammals is accompanied by oxidative stress in the liver, kidney and intestinal mucosa (Botha et al., 2004; Ding et al., 1998, 2001; Moreno et al., 2003, 2005). It has been suggested that either of the above mechanisms could induce cytoskeletal damage leading to morphological or pathological changes (Ding et al., 2001; Hooser et al., 1991; Toivola et al., 1997).

The cytoskeleton consists of three major structural elements: microfilaments (MFs), intermediate filaments (IFs) and microtubules (MTs). MFs and MTs form highly dynamic cytoskeletal networks composed, respectively, of actin and tubulin polymers, and a large number of associated proteins (Andersen, 2000; Louvet-Vallée, 2000). The ERM family (ezrin, radixin and moesin) and stathmin, which are proteins associated with MFs and MTs, respectively, are involved in the promotion of malignant tumors and metastases, including astrocytomas, uveal melanomas, ovarian carcinoma, leukemia, lymphoma and neuroblastoma (Geiger et al., 2000; Mäkitie et al., 2001; Moilanen et al., 2003; Rana et al., 2008). MAP1b and tau, which play important roles in the neurological disorder Alzheimer's disease (AD), are classified as MT-associated proteins (Alonso et al., 1994; Hasegawa et al., 1990).

In recent decades, morphological changes induced by microcystins, and by cyanobacterium extracts have been described in MFs, MTs and IFs. Hyperphosphorylation of keratins 8 and 18 was stimulated by MC-LR in vivo and in vitro, eventually leading to the disruption of the IFs (Toivola et al., 1997, 1998). Several researchers have observed that MC-LR-induced changes in fibroblasts, kidney cells, and some hepatocytes, first occurred in IFs, followed by MTs, and later MFs (Khan et al., 1996; Wickstrom et al., 1995). Ding et al. (2001) observed that MC-LR induced changes in MFs and MTs in primary rat hepatocytes and they suggested that the binding of MC-LR to the cysteine residues of tubulin subunits resulted in an increase in the quantity of free tubulin, thereby reducing the stability of the tubulin mRNA and decreasing the quantity of cellular tubulin. Clark et al. (2007) studied transcriptional changes using a microarray from the liver of mice exposed to a prolonged sub-lethal dose of microcystin. Changes in the expression of many cytoskeletal genes were detected. Recently, protein expression changes also have been detected by 2D gel electrophoresis. For example, changes in the expression of several cytoskeletal proteins were reported in medaka fish (*Oryzias latipes*) exposed to MC-LR at relatively high doses (Mezhoud et al., 2008a,b) and changes have also been observed in human amniotic epithelial FL cells (Fu et al., 2009). However, there has been little focus on quantitative changes of cytoskeletal gene expression and almost nothing is known about MC-induced changes of MT-associated proteins and MF-associated proteins.

To reveal the mechanisms underlying cytoskeletal alterations stimulated by microcystins, we intravenously injected rats with microcystin extracts and investigated the transcription of three cytoskeletal proteins, three MF-associated proteins and three MT-associated proteins in liver, kidney and spleen.

2. Materials and methods

2.1. Toxin

The cyanobacteria (mainly composed of *Microcystis* spp. *Microcystis aeruginosa*) used in the experiment was collected from Lake Dianchi, Yunnan Province of China. Crude microcystins in the cyanobacteria were extracted three times with 75% (V/V) methanol and suspended in distilled water for the toxic experiment. Prior to use, the material was analyzed for toxin content via reverse-phase High Performance Liquid Chromatography (HPLC, LC-20A, Shimadzu Corporation, Nakagyo-ku, Kyoto, Japan) equipped with an ODS column (Cosmosil 5C18-AR, 4.6 × 150 mm, Nacalai, Japan) and an SPA-10A UV–vis spectrophotometer set at 238 nm. MC concentrations were determined by comparing the peak areas of the test samples with standards (MC-LR and MC-RR, Wako Pure Chemical Industries, Japan). As is described by Gupta et al. (2003), the intraperitoneal (i.p.) medium lethal dose (LD50) in mice for MC-RR and MC-YR is about 5-fold and 2.5-fold that of MC-LR, respectively, and the coefficients used to convert MC-RR and MC-YR into the MC-LR equivalent (MC-LReq) were 0.2 and 0.4, respectively. In the present experiment, the MC content was 80 μg MC-LReq/mL, where MC-RR and MC-LR constituted 167.7 μg/mL and 47.0 μg/mL, respectively. Crude microcystin extracts were finally suspended in physiological saline solution (0.9% NaCl).

2.2. Experimental protocol

Healthy male Wistar rats weighing 200 ± 20 g were obtained from the Center for Disease Control of Hubei Province, China. The rats were kept in stainless steel cages at 20 °C under a 12 h light–dark cycle, and given free access to standard rodent pellet diet and water. All procedures carried out on animals were approved by the Institutional Animal Care and Use Committee, and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory.

1 mL suspension of extracted solution of microcystins in physiological saline solution was intravenously injected in the tail of 60 rats at a dose of 80 μg MC-LReq kg^{-1} bw. In addition, 60 rats were intravenously injected with 1 mL physiological saline solution as control.

Five rats were collected from each group at 1, 2, 4, 12 and 24 h after injection for total RNA isolation samples. Each rat was killed and the liver, kidney and spleen were quickly removed, washed, minced, and stored frozen in liquid nitrogen until RNA isolation.

2.3. Total RNA extraction and reverse transcription

Total RNA was isolated from 30 to 50 mg sections of rat organs using Trizol reagent (Invitrogen, America). RNA was

extracted according to the manufacturer's protocol, re-suspended in 50 μL RNase-free water, and stored at −80 °C. Quantification was done using Eppendorf Biophotometer (Hamburg, Germany). Reverse transcription was performed with oligo(dT) primer using First Strand cDNA Synthesis Kit (Fermentas, Lithuania).

2.4. Quantitative real-time PCR (Q-PCR)

Some mRNA sequences of rat (*Rattus norvegicus*) were searched in GenBank, according to which, the primers used in real-time PCR were designed. The specification of each pair of primers was confirmed by randomly sequencing six clones, and further confirmed by the melting curve analysis using real-time PCR. The amplification efficiency of each pair of primers was tested by constructing the corresponding plasmid and only primers with similar amplification efficiencies were used in this experiment. The housekeeping gene GAPDH was analyzed in samples, and the level of it was stable in the present experiments. Therefore GAPDH was used as the endogenous assay control.

SYBR Green Q-PCR kit (Toyobo, Japan) was used as the fluorescent dye for real-time quantitative PCR on a Chromo4 96-well reactor with optical caps (MJ Research, Cambridge, MA). The reactions were performed in a 20 μL volume mix containing 10 μL SYBR Green I mixture (Toyobo, Japan), 1 μL primers, 1 μL cDNA and 7 μL DEPC-treated water. The thermal cycling program was: 3 min denaturation at 95 °C; for amplification and quantification 45 cycles of 30 s at 95 °C, 30 s at 61–69 °C (the annealing temperature varied depending on the gene specific primers), 30 s at 72 °C, 1 s at 76–81 °C (plate read for targeted gene, temperature varied depending on the gene specific primers), 1 s at 78 °C (plate read for GAPDH), a 10 min extension at 72 °C, and then a melt curve from 70 to 90 °C with a heating rate of 0.3 °C s^{-1}.

Real-time PCRs were performed in triplicate for each cDNA sample, and data were analyzed with the Option Monitor Software 2.03 Version (MJ Research, Cambridge, MA). Transcription of the nine genes in the control remained stable throughout the 24 h and showed negligible inter-individual variations during the experiment. All the primers used in the real-time PCR are listed in Table 1.

2.5. Statistical analysis

All values are expressed as mean ± standard deviation for each treatment group. Statistical analysis of differences between treatment groups and control groups was done by one-sample *T*-test. Correlation between transcriptional level of each gene and the microcystin concentrations in the different organs was done using Pearson's correlation analysis. Software used for statistical analysis in the present study is the SPSS (Chicago, IL, USA) for Windows (Ver. 13.0). Differences were considered significant if $p \leq 0.05$.

3. Results

3.1. Changes in the transcription of actin, tubulin and vimentin

3.1.1. Liver

The transcription of β-actin in the liver was significantly positive when correlated with the concentrations of microcystins (Figs. 1 and 2, and Table 2). In the first 2 h after exposure, the α-tubulin transcriptional level was similar to the control, it was significantly higher at 4 and 6 h, and was significantly lower at 12 and 24 h. Microcystins induced a time-dependent increase of vimentin transcription, which was at a minimum (0.58-fold) after 1 h and at a maximum level at 24 h (7.56-fold).

3.1.2. Kidney

No significant alteration was detected in the transcription levels of the three genes in the kidney (Fig. 1) during the first hour following microcystin treatment of rats. Significant changes in the transcription of β-actin and α-tubulin were observable at 2 h and the transcription of vimentin had increased significantly at 12 and 24 h.

3.1.3. Spleen

The transcription of β-actin in spleen tissue (Fig. 1) was significantly augmented by microcystins in the first 2 h, with a peak at 1 h, but it was depressed significantly from 4 h onward. Transcription of α-tubulin in the spleen was significantly lower than controls throughout the entire experiment. Vimentin transcription was depressed significantly at 1, 2, 6 and 24 h.

3.2. Changes in the transcription of ERM family

3.2.1. Liver

The transcription of ezrin was significantly higher than in controls from 2 to 24 h except for the 12 h measurement (Fig. 3). The transcription of radixin was significantly depressed throughout the entire experiment except for a small elevation at 2 h (1.16-fold). The transcriptional

Table 1
Primer sequences (5′–3′) used in this study.

	Forward	Reverse
Tau	GGACAGGAAATGACGAGAAGAAAGCC	GGGAGCGACTGCCAGGGGTT
Tubulin	ACCTGACCGTAGCCGCCATT	CGGTGCTGTTGCCAATGAA
Moesin	CTGCGGGCTGATGCTATGG	GCAGGGTCTTGTATTTGTCTCGTC
Radixin	AGTGAGGGCGTGACGAACC	GAATCTGCCGCAGAGTCTTGTAC
Ezrin	CTGACTTCGTGTTCTACGCTCCC	CGGTTTCCAACTGTTGTCGC
Vimentin	CAGGCAAAGCAGGAGTCAAACG	GCCATCTTTACATTGAGCAGGTCC
Actin	GACCTCTATGCCAACACAGTGCTGT	CTAGAAGCATTTGCGGTGCACGATG
Stathmin	GGAGGTGAAGATGAGCCCGAGGAC	CAGGGTAGGTTTCACTCCATCCAGAC
MAP1b	CGTTACGCAGCCCTCCCCTTAT	GCCTTGCTTTCCATTCTTCCCTTC

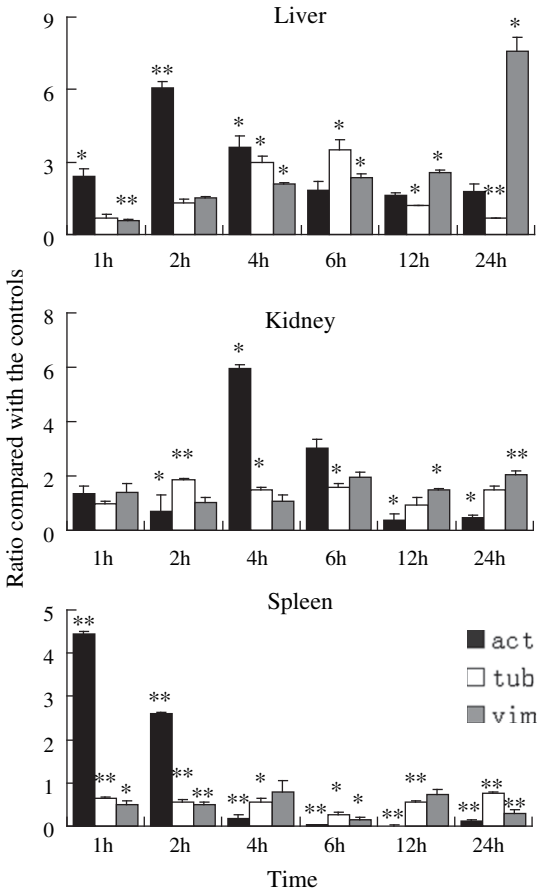

Fig. 1. Relative mRNA expressions of β-actin, α-tubulin and vimentin in liver, kidney and spleen after an i.v. injection with 80 μg MC-LReq kg^{-1} bw. The significance levels observed are $p < 0.05$ (*) and $p < 0.01$ (**). act: β-actin; tub: α-tubulin; vim: vimentin.

changes of these two genes were significantly correlated with the concentrations of toxins (Figs. 2 and 3 and Table 2). Significant changes of moesin transcriptional level were detected at 6 h (3.5-fold), and thereafter.

3.2.2. Kidney

Changes in the transcription of ezrin and moesin were detected from 6 h (Fig. 3). Ezrin transcription was significantly elevated from 6 to 12 h and recovered to the level of the controls at 24 h. Moesin transcription was significantly inhibited from 6 to 24 h. The transcription of radixin was significantly elevated at 1 h, it was significantly depressed from 2 to 6 h, and it recovered thereafter.

Table 2
Results of Pearson's correlated analysis.

		RR	LR	Total
Liver	Actin	0.874*	0.927**	0.930**
	Tau	0.924**	0.828*	0.905*
	Ezrin	0.844*	0.827*	0.852*
	Radixin	0.828*	0.833*	0.848*
Kidney	Stathmin	0.831*	0.554	0.786
Spleen	Radixin	0.928**	0.676	0.917*

* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

3.2.3. Spleen

No significant induction of the transcription of the three ERM family genes was detected. In fact, minima for ezrin, radixin and moesin occurred at 6 h, by factors of 0.13, 0.01 and 0.04 relative to controls, respectively. The change in radixin transcription was significantly correlated with the tissue concentrations of microcystins (Figs. 2 and 3, and Table 2).

3.3. Changes in the transcription of MAPs

3.3.1. Liver

Transcription of MAP1b was depressed significantly in the first 2 h, with one third less of template abundance than controls (Fig. 4). A significantly increased level of transcription of MAP1b was observed from 4 h onward with an approximately 4-fold increase from 4 to 12 h and a greater than 16-fold increase at 24 h. The transcription of tau was significantly depressed at all times except for 12 h and was significantly correlated with the tissue concentrations of microcystins. The transcription level of stathmin increased significantly and time-dependently from 4 to 12 h with a 102-fold peak at 12 h. At the end of the experiment (24 h), the relative abundance of stathmin was still more than 60 times that of controls.

3.3.2. Kidney

The influence of microcystins on the transcription of MAP1b was much lower in kidney tissue than in liver. A significant alteration of MAP1b in kidney was only detected at 24 h (Fig. 5). The transcription of tau was depressed throughout the experiment and this depression was significant at 1, 2 and 24 h. In contrast to tau, the transcription of stathmin was significantly higher in the treated

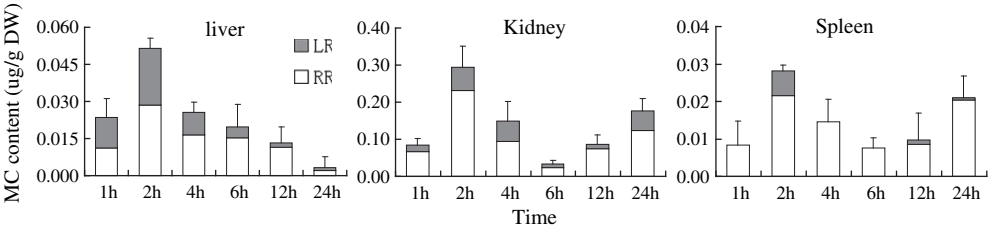

Fig. 2. Microcystin contents in Wistar rat tissues after an i.v. injection with 80 μg MC-LReq kg^{-1} bw (Wang et al., 2008).

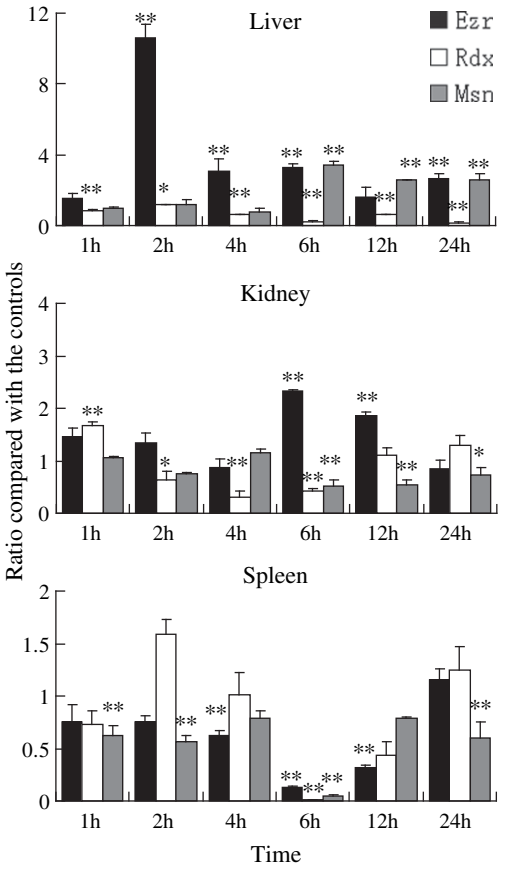

Fig. 3. Relative mRNA expressions of ezrin, radixin and moesin transcripts in liver, kidney and spleen after an i.v. injection with 80 μg MC-LReq kg^{-1} bw. The significance levels observed are $p < 0.05$ (*) and $p < 0.01$ (**). Ezr: ezrin; Rdx: radixin; Msn: moesin.

rats than in the controls at 2, 6 and 24 h. Additionally, the transcriptional change in stathmin was significantly correlated with the tissue concentration of MC-LR (Figs. 2 and 5 and Table 2).

3.3.3. Spleen

Significantly increased transcription of MAP1b was detected at 1 and 6 h only (Fig. 5). As in the kidney, transcription of tau significantly decreased in the treated rats relative to the controls at 2, 4 and 24 h. Transcription of stathmin showed a time-dependent increase from 1 to 6 h, it was significantly lower in the treated rats than in the controls at 12 h, and then recovered at 24 h.

4. Discussion

Microcystins in recreational and drinking waters represent a potential hazard to the health of human populations. The cytoskeleton, a basic structural element of all cell types, plays key roles in the maintenance of cell architecture, adhesion, migration, differentiation, division, and organelle transport. Data from this study clearly show that microcystins could disrupt the transcriptional balance of some cytoskeletal proteins in the liver, kidney, and spleen of rats. Moreover, transcriptional modulation of four genes (β-actin, ezrin, radixin and tau) in the liver, one gene (stathmin) in the kidney and one gene (radixin) in the spleen was significantly correlated with the concentration of microcystins in these organs. Microcystins induce morphological and pathological injuries (Li et al., 2005, 2008; Liu et al., 2010; Milutinović et al., 2006; Qiu et al., 2007, 2009) through redistribution of microfilament (MF) (Eriksson et al., 1989; Falconer and Yeung, 1992; Ghosh et al., 1995; Hooser et al., 1991), intermediate filaments (IF) (Falconer and Yeung, 1992) and microtubules (MT) (Khan et al., 1996; Wickstrom et al., 1995), and our results suggest potential molecular mechanisms underlying these phenomena.

Actins are central elements in the MF system. They are highly conserved eukaryotic proteins, which occur both in monomeric (globular or G-actin) and polymerized (filamentous or F-actin) forms (Stossel, 1984). The relative proportions of F- and G-actins are determined by the concentration of monomeric actin protein (Papakonstanti and Stournaras, 2008). Previous studies have observed MF destruction induced by microcystins, as indicated by loss of arrays of stress fibers, shortening of MFs, and detachment from the plasma membrane (Batista et al., 2003; Ding et al., 2000, 2001; Ghosh et al., 1995; Wickstrom et al., 1995). Each of these alterations could result from a change in monomeric actin concentration, which could in turn disrupt the transcriptional homeostasis of actin. In our experiment, changed transcription of β-actin in three organs was detected. Levels of transcription in the liver were positively correlated with the tissue concentrations of microcystins, suggesting that transcriptional changes played a critical role in microcystin-induced MF destruction. Besides β-actin, transcriptional change of three other

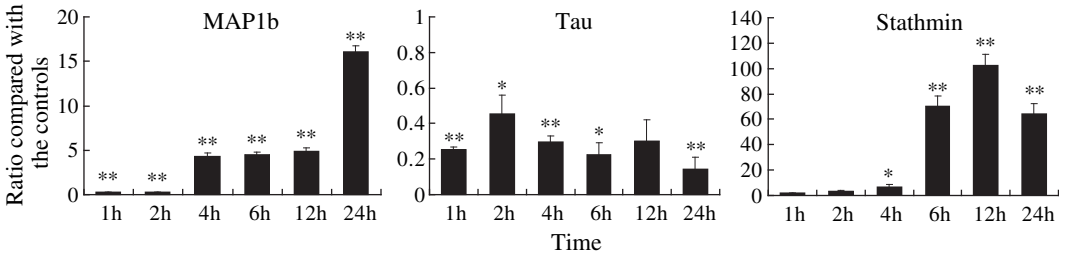

Fig. 4. Relative mRNA expressions of MAP1b, tau and stathmin in liver of rats after an i.v. injection with 80 μg MC-LReq kg^{-1} bw. The significance levels observed are $p < 0.05$ (*) and $p < 0.01$ (**).

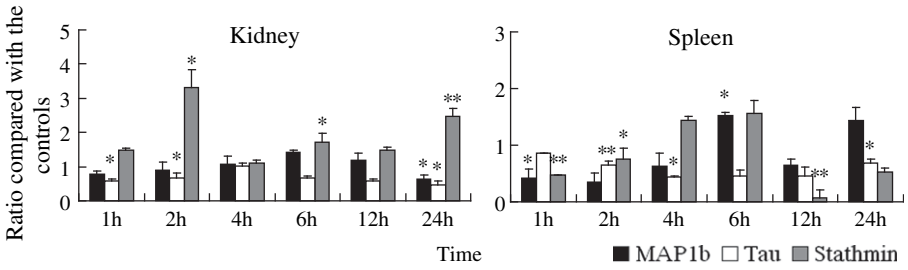

Fig. 5. Relative mRNA expressions of MAP1b, tau and stathmin in kidney and spleen of rats after an i.v. injection with 80 μg MC-LReq kg^{-1} bw. The significance levels observed are $p < 0.05$ (*) and $p < 0.01$ (**).

genes (ezrin, radixin and tau) was also positively correlated with the concentration of microcystins in liver but only one gene was correlated with microcystin concentrations in kidney and spleen. Moreover, the influences of microcystins on the transcription of most of the genes observed in the present research were more pronounced in liver than in kidney or spleen, which provides new evidence for the hepatotoxicity of microcystins.

Ezrin, radixin and moesin (ERM family) involve in cell adhesion (Sato et al., 1992). Takeuchi et al. (1994) observed that the suppression of ERM expression leads to the destruction of both cell–cell and cell–substrate adhesion in thymoma cells and mouse epithelial cells. The similar effect was observed in two colo-rectal cancer cell lines in which the expression of ezrin was inhibited at both protein and mRNA levels (Hiscox and Jiang, 1999). Besides this function, the sub-cellular redistribution of these proteins and the expression levels of these genes are associated with tumorigenesis as well as motility and invasive behavior of malignant cells (Carmeci et al., 1998; Moilanen et al., 2003; Tokunou et al., 2000). Ezrin is the most studied of these three genes and its crucial role in tumor progression seems reasonably certain (Zeng et al., 2006). Its abnormal expression has been detected in many malignant tumors at both transcription and protein levels (Elliott et al., 2005; Geiger et al., 2000; Guan et al., 2002; Ilmonen et al., 2005; Moilanen et al., 2003; Tokunou et al., 2000; Weng et al., 2005; Zeng et al., 2006). Likewise, the higher expression of moesin in the cell line with the more malignant phenotype at both transcription and protein levels was detected by Carmeci et al. (1998). In the present study, transcription of two ERM family genes (ezrin and radixin) was positively correlated with the concentration of microcystins in the liver of treated rats and the transcription of radixin was correlated with the concentration of microcystins in both liver and kidney. In contrast, all significant changes in the transcription of these three genes in the spleen were inhibitory. Many studies have shown that inhibition of PP (Chen et al., 1995; Louvet-Vallée, 2000; Nakamura et al., 1995) and activation of calpain or in turn increased concentrations of Ca^{2+} (Shcherbina et al., 1999; Shuster and Herman, 1995; Yao et al., 1993) can all influence the activity, expression, and even transcription of these three genes. The effects of PP inhibition and calpain activation could both be results of microcystin stimulation (Ding et al., 2002; Honkanen et al., 1990; MacKintosh et al., 1990). Therefore, we suggest that the explanation of microcystin-induced cell adherence loss (Ding et al., 2000; Khan et al., 1996; Li et al., 2004, 2005; Qiu et al., 2009; Wickstrom et al., 1995) is the influence on the expression of ERM family in mRNA or protein level. Meanwhile, our observations also provide new insights into the potential carcinogenesis of microcystins.

Stathmin, one type of microtubule-associated protein, also known as oncoprotein 18 (op18), regulates microtubule dynamics by promoting depolymerization of MTs and/or preventing polymerization of tubulin heterodimers (Cassimeris, 2002; McNally, 1999). Its over-expression was detected across a broad range of human malignancies (leukemia, lymphoma, neuroblastoma; ovarian, prostatic, breast and lung cancers and mesothelioma) and it is up-regulated in normally proliferating cell lines (Rana et al., 2008). Moreover, in lung adenocarcinoma, protein over-expression is correlated with Affymetrix mRNA expression (Chen et al., 2003). In the present study, the transcription of stathmin was significantly elevated in the liver and kidney of microcystin-treated rats and transcriptional changes in the kidney were significantly correlated with the concentration of MC-LR. Therefore, changed transcription of stathmin induced by microcystins is another possible mechanism of MC-mediated tumor-promotion.

Unlike stathmin, the major known biological functions of MAP1b and tau are to promote assembly of microtubules and to stabilize microtubules by binding to them. In our research, the transcription of tau was depressed throughout the experiment and, in the liver, changes in the transcription level were significantly correlated with the concentration of microcystins. The transcription of MAP1b was significantly elevated in liver 2 h after MC treatment and after 24 h it was more than 15-fold higher than the controls. These transcriptional changes could be caused by negative feedback regulation from changed expression of the protein. It is reported that after being treated with another PP2A inhibitor, okadaic acid, protein expression of tau in rat brain was significantly increased, and expression of MAP1b was reduced (Gong et al., 2000a,b). The major MAPs present in brain are phosphoproteins MAP1, MAP2 and tau (Kirkpatrick and Brady, 1999). Phosphorylation alters their interaction with microtubules (Alexa et al., 1996; Jameson and Caplow, 1981; Pedrotti et al., 1996). Several studies have observed abnormal phosphorylation of MAP1b and tau in AD brain (Alonso et al., 1994; Hasegawa et al., 1990; Iqbal et al., 1994; Ulloa et al., 1994; Wang et al., 1995, 1996). Correspondingly, a decreased activity of PP2A was

found in AD brains (Gong et al., 1993, 1995; Speciale et al., 1995). As a potent inhibitor of PP2A, microcystins have been shown to be able to cross through the blood–brain barrier and accumulate in brains of both mammals and fish (Cazenave et al., 2005; Falconer et al., 1986; Lei et al., 2008a,b; Wang et al., 2008). Furthermore, in the Brazilian hemodialysis event, patients developed predominantly neurological symptoms shortly after dialysis, in some cases within hours. Thus, change of MAPs (phosphorylation modification, protein expression or transcription) in brain might provide an explanation for the abnormal behavior of fish stimulated by microcystins (Baganz et al., 2004; Cazenave et al., 2008; Ernst et al., 2006). Changes in the transcription of MAP1b and tau that we demonstrated in our research suggest a possible mechanism for neurological toxicity of microcystins in vertebrates.

In conclusion, we showed that microcystins affected the homeostasis of the transcription of nine cytoskeletal genes (β-actin, α-tubulin, vimentin, ezrin, radixin, moesin, tau, MAP1b and stathmin) in the liver, kidney and spleen of rats injected intravenously with microcystins at a dose of 80 μg MC-LReq kg^{-1} bw. Our observations suggest that the effects of microcystins on the transcriptional level of these genes could be a factor leading to morphological changes, abnormal behavior, and increased tumor-promoting activity in mammals exposed to microcystins. Transcriptional changes of MAP1b and tau shown in our research provide evidence for the neurological toxicity of microcystins in vertebrates. Due to the wide occurrence of cyanobacterial blooms containing microcystins, and the identification of microcystins in the serum of a chronically exposed human population (Chen et al., 2009), further research should be directed to the potential tumorigenesis and neurological toxicity of microcystins.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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