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Effects of microcystin-containing cyanobacterial extract on hematological and biochemical parameters of common carp (*Cyprinus carpio* L.)

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Abstract The aim of the study was to assess the effects of a cyanobacterial extract containing microcystins (MCs) on selected hematological and biochemical parameters in common carp (*Cyprinus carpio* L.), as well as to determine the accumulation of toxins in fish tissues. The fish were immersed for 5 days in water containing toxins at a final concentration of 12 μg/L of microcystin LR equivalent. Microcystin LR residues were detected in fish liver, reaching 207, 238 and 260 ng/g f.w. of the tissues taken 24 h, 72 h and 5 days after the end of intoxication, respectively. The most substantial changes were found in fish plasma, including increases in creatine kinase, lactate dehydrogenase, ammonia, glucose, aspartate aminotransferase and alanine aminotransferase levels. A decline of about 50% in lysozyme activity was observed by the end of the experimental period. Moreover, a marked increase in ceruloplasmin activity was detected 24 h after the end of intoxication with a subsequent decrease in its activity after 72 h and 5 days. This study concludes that not only consumption of food containing toxins but also MCs dissolved in water may pose a threat to fish health. Additionally, detected changes in lysozyme and ceruloplasmin activity may have distinct effects in fish resistance against pathogens or oxidative stress, which should be taken into account in the future studies.

Keywords Cyanobacterial water blooms · Microcystin LR · *Cyprinus carpio* · Hematological profile · Biochemical profile · Microcystin tissue accumulation

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Introduction

The increasing eutrophication of surface waters is one of the main causes of massive cyanobacterial water blooms observed worldwide in the last few decades. Some species of cyanobacteria produce toxins, which may have different chemical structures and toxic activity and induce various biological effects. It is estimated that, of the 2,000 described cyanobacterial species, nearly 50 may be toxic, and the variability in this regard relates even to the various strains of the

same species (Ernst 2008). Approximately 75% of water blooms are found to be toxic (Sivonen and Jones 1999). Cyanotoxins, according to the nature of their toxic activity, are divided into hepatotoxins, neurotoxins, cytotoxins, dermatotoxins, irritants and gastrointestinal toxins (Briand et al. 2003). Hepatotoxic microcystins (MCs), with the most toxic isoform, microcystin LR (MC-LR), are among the most frequently detected cyanotoxins of high stability in the environment (Blaha et al. 2009; Briand et al. 2003). MCs are widely known to accumulate in the food chain (Xie et al. 2005; Chen et al. 2009a), negatively affecting both wild life and human beings (Matsunaga et al. 1999; Carmichael et al. 2001; Qiu et al. 2007; Chen et al. 2009b).

Due to their natural environment, fish can be exposed to cyanotoxins via multiple routes, at different concentrations and during different periods of time. There are many reports on the negative effects of MCs on fish, including disturbances in developmental processes, behavioral changes, hepatotoxic, nephrotoxic or immunotoxic effects (Oberemm et al. 1999; Malbrouck and Kestemont 2006; Baganz et al. 2004; Ernst 2008). Changes in biochemical and hematological parameters were described in certain studies, in which fish were exposed to MCs either naturally (Kopp et al. 2005, 2009, 2010; Qiu et al. 2009) or experimentally, via oral or intraperitoneal (i.p.) route (Li et al. 2004; Zhang et al. 2007). The first route of exposure is more natural, but the observed changes may result not only from MC toxicity but also from such factors occurring during cyanobacterial blooms, as hypoxia, a high pH level, high ammonia concentrations or other, untested agents present in water. On the other hand, i.p. injection does not reflect actual kinetics and trends of changes arising during MC intoxication of fish in the environment. Oral intoxication, although considered the main way of exposure on cyanotoxins, at least for herbivorous and omnivorous fish, does not include other possibilities of toxin uptake, such as entering via the gills. Moreover, when whole cyanobacteria cells are administered orally, the expected level of exposure to cyanotoxins may be overestimated, as it was described that viable cells were detected after their passage through the alimentary tract in cyprinids (Jancula et al. 2008).

The aim of the study was to assess the influence of the immersion of *Cyprinus carpio* L. in water containing MCs at environmentally relevant concentrations (Blaha et al. 2010) on fish blood indices, as well as to establish the toxin accumulation in selected tissues after 5 days of exposure. Moreover, the study intended to investigate the possibility of the toxins to affect the activity of lysozyme, one of the basic components of fish innate immunity, and ceruloplasmin, the acute phase protein.

Materials and methods

MC-containing extract

MC-containing extract was obtained from the cyanobacterial scum consisting of *Microcystis* spp. (*M. aeruginosa, M. flos-aquae, M. natans*) and, to a lesser extent, *Aphanizomenon flos-aquae*, collected from a dam reservoir located in SE Poland. The total MC content in the cyanobacterial scum was determined as the equivalent of MC-LR by gas chromatography/mass spectrometry (GC/MS, Varian) according to the method described by Pawlik-Skowrońska et al. (2008). The calibration curve for quantitative analysis was prepared with standard MC-LR (Alexis Biochemicals).

Fish and experimental setup

The studies were conducted on 48 healthy common carp (*C. carpio* L.) of either sex, with a mean body weight of 30.1 ± 8.5 g, which were originally bred at the Research Institute of Fish Culture and Hydrobiology, Vodnany, University of South Bohemia, the Czech Republic. In this study, the principles of laboratory animal care and the national laws 246/1992 "Animal Welfare" on the protection of animals were followed (Ref. no. 22761/2009-17210). Fish were divided into three groups—two experimental and one control group, 16 carp in each—and kept in 40-L tanks at 17.5 ± 1°C, pH 7.7 ± 0.1 and dissolved oxygen 81.3 ± 2% with 10 h: 14 h light/dark cycle. Fish were not fed during the whole experimental period. MC-containing cyanobacterial extract was added to the experimental tanks in order to gain the final concentration of 12 μg/L of MC-LR equivalent. Water was changed daily. MC concentration in the tank water was confirmed daily with the use of ELISA kit (EnviroGard, USA), in accordance with the producer protocol. After 5 days of exposure, fish were

transferred to the MC-free water. Blood and tissue sampling was conducted after 24 h, 72 h and 5 days once exposure had ceased.

Sampling procedure

Blood samples for further analyses were taken from *vena caudalis* with heparin at 50 IU/mL. Whole livers, spleens, kidneys, as well as muscle and gill samples of the same size from each fish were taken, pooled inside each experimental group and frozen at −80°C for the determination of MC concentrations.

MC determination in fish tissues

Toxin identification and quantification in fish tissues were carried out using a high-performance liquid chromatography (HPLC) system (Prominence, Shimadzu) equipped with a Diode Array Detector (DAD SPD M20A) operating at 238 nm. MC-LR, MC-LA, MC-YR, MC-LY, MC-RR, MC-LW and MC-LF standards (Alexis, Biochemicals) were used for calibration curve preparation.

Pooled samples of each tissue were weighed and extracted through homogenization in 75% methanol (Merck, pure p.a.) containing 0.002 M HCl and ultrasonication (2–3 times for 10 min). In order to remove an excess of lipid compounds, the methanol extracts were treated two times with n-hexane (1:1, v/v). The hexane layers were discarded, and the obtained extracts were evaporated to dryness in glass vials and stored at −20°C. HPLC analysis of MCs in the purified extracts was performed on LiChroCART 125-3 Purospher RP-18 column (5 µm, Merck) with mobile phases: A—water acidified with 0.05% trifluoroacetic acid (TFA, Merck) and B—aqueous acetonitrile with 0.05% TFA, according to Lawton et al. (1994). The gradient of phase B was 30–100% with a flow rate 0.7 mL/min. The MC detection limit was 60 ng/g f.w.

Blood assays

The following parameters in blood were tested: the erythrocyte count (RBC), hemoglobin concentration (Hb), hematocrit value (PCV), mean erythrocyte volume (MCV), mean erythrocyte hemoglobin (MCH), mean color concentration (MCHC), leukocyte count (WBC) and differential leukocyte count. All determinations were carried out according to the methods described in Svobodova et al. (1991). Blood plasma, obtained by blood centrifugation at 400*g* for 15 min at 4°C, was stored at −80°C for further biochemical analyses.

Biochemical determinations of the glucose (GLU), the total amount of proteins (TP), albumins (ALB), the total amount of globulin (GLOB), ammonia (NH_3), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatine kinase (CK), lactate dehydrogenase (LDH), calcium (Ca^{2+}), magnesium (Mg^{2+}), inorganic phosphate (PHOS), alkaline phosphatase (ALP), triglycerides (TRIG) and gamma-glutamyl transferase (GGT) were conducted with a VET-TEST 8008 analyzer (IDEXX Laboratories Inc., USA).

Lysozyme activity in fish plasma was determined by a turbidimetric assay according to Day et al. (2007), with slight modifications. For standard curve preparation, lyophilized lysozyme from the hen egg white (Sigma) was used. The standard concentrations of the enzyme, studied samples and PBS (as a negative control) were added in triplicate to the 96-well microtiter plate. Then, a fresh suspension of *Micrococcus lysodecticus* (0.2 mg/mL in 0.1 M phosphate buffer, pH 6.2) was added quickly to each well. The initial OD (T0) was taken immediately at 450 nm on the BioRad 550 microplate reader, and the plate was left for 20 min at room temperature until the next OD reading (T20). OD values at T20 were subtracted from OD at T0 and converted to µg/mL using the linear equation from the standard curve.

Ceruloplasmin (Cp) activity in plasma was measured as p-phenylenediamine (PPD) oxidase activity, according to Pelgrom et al. (1995). In short, fish plasma was mixed with 0.1% PPD (Sigma) solution in 0.2 M acetate buffer (pH 5.5) and incubated for 30 min at 37°C in the dark. The reaction was stopped by the addition of 0.1% sodium azide (Sigma), and the Cp activity was measured colorimetrically at 540 nm using a BioRad SmartSpec 3000 spectrophotometer.

Statistical analysis

Statistical analysis was performed by a nonparametric Mann–Whitney *U* test with Statistica 8.0 software (StatSoft, USA). P values < 0.05 were considered to be statistically significant.

Results

During the experiment, both control and MC-exposed carp showed normal behavior. There were no signs of respiratory distress such as rapid ventilation, increased rate of gill opercular movements or fish floating at the surface of the water. There were no mortalities during the experiment.

MC determination in fish tissues

Among all examined isoforms of MCs, only MC-LR was detected and exclusively in fish liver, in which it reached 207 ± 128, 238 ± 7 and 260 ± 43 ng/g f.w. after 24 h, 72 h and 5 days, respectively. In the other studied organs, that is, spleens, kidneys, gills, as well as in the muscle samples, the toxin was below the limit of detection.

Blood assays

The visible changes in blood cell parameters after the end of intoxication included a significant decrease ($P < 0.05$) in the monocyte count seen after 24 h, gradually increasing during the ensuing days. A statistically significant increase in MCHC after 24 h was detected, while MCV and MCH remained unchanged, compared to the control group (Table 1). The values for RBC, Hb, PCV and WBC were similar among all groups.

More pronounced changes were detected in biochemical indices (Table 2). A significant ($P < 0.05$) increase in the GLU level was seen, but only 24 h after the intoxication had ceased. Levels of CK and LDH in intoxicated fish plasma were significantly ($P < 0.05$) higher during the entire period of analysis, with an observed upward trend. The activity of CK detected in the plasma of experimental fish constituted 134, 151 and 170% of the enzyme activity in the control group after 24 h, 72 h and 5 days, respectively. Similar increase was seen in the LDH level. Moreover, after 24 h, also a tendency to increase the activity of AST ($P = 0.058$), ALT ($P = 0.066$) and NH_3 ($P = 0.052$) was observed. After 72 h and 5 days, respectively, the changes were already statistically significant, reaching 216 and 250% of the level of AST activity in control fish and 300 and 364% of ALT activity. In the case of NH_3, the observed increase was rather stable and reached about 120% of the level in control fish. The remaining parameters (TP, ALB, GLOB, Ca^{+2}, Mg^{2+},

Table 1 Blood cell parameters of the fish intoxicated with the cyanobacterial extract containing 12 μg of MC-LR equivalent per 1 L for 5 days in the comparison with the control fish

Parameter	Units	24 h		72 h		5 days	
		Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
RBC	T/L	1.50 ± 0.26	1.43 ± 0.18	1.58 ± 0.31	1.67 ± 0.30	1.57 ± 0.34	1.51 ± 0.23
Hb	g/L	64.39 ± 11.48	67.76 ± 10.71	61.17 ± 11.59	62.33 ± 11.26	67.42 ± 12.93	72.06 ± 10.93
PCV	l/L	0.29 ± 0.04	0.28 ± 0.05	0.29 ± 0.06	0.29 ± 0.05	0.27 ± 0.06	0.29 ± 0.06
MCV	fl	198.62 ± 17.14	198.24 ± 56.24	188.31 ± 59.99	180.83 ± 42.29	178.38 ± 49.12	195.62 ± 36.68
MCH	pg	43.07 ± 3.83	48.35 ± 11.42	40.55 ± 14.12	37.97 ± 7.92	43.76 ± 7.85	48.22 ± 6.64
MCHC	g/L	217.88 ± 23.05	246.94 ± 26.42*	216.67 ± 27.94	212.68 ± 26.03	251.50 ± 32.73	249.07 ± 20.70
WBC	G/L	9.26 ± 2.95	10.51 ± 3.92	17.86 ± 10.21	13.66 ± 3.86	11.75 ± 4.27	11.86 ± 5.71
Lymphocytes	G/L	7.89 ± 3.22	9.78 ± 3.81	16.72 ± 10.07	12.82 ± 3.86	10.81 ± 3.93	12.95 ± 4.46
Monocytes	G/L	0.56 ± 0.41	0.13 ± 0.17*	0.30 ± 0.19	0.18 ± 0.16	0.33 ± 0.31	0.24 ± 0.17
Band neutrophils	G/L	0.71 ± 0.71	0.51 ± 0.48	0.53 ± 0.36	0.52 ± 0.17	0.32 ± 0.29	0.18 ± 0.18
Segmented neutrophils	G/L	0.10 ± 0.08	0.10 ± 0.10	0.13 ± 0.11	0.13 ± 0.13	0.17 ± 0.22	0.14 ± 0.16
Basophiles	G/L	0.00	0.00	0.21 ± 0.26	0.04 ± 0.12	0.12 ± 0.33	0.00

$n = 5$, mean ± SD. Time after the end of intoxication

* Statistically significant at $P < 0.05$

Table 2 Biochemical parameters of the fish intoxicated with the cyanobacterial extract containing 12 μg of MC-LR equivalent per 1 L for 5 days in the comparison with the control fish

Parameter	Units	24 h		72 h		5 days	
		Control group	Experimental group	Control group	Experimental group	Control group	Experimental group
GLU	mmol/L	3.96 ± 0.90	5.05 ± 0.87*	3.95 ± 0.32	3.88 ± 0.72	3.86 ± 0.26	3.82 ± 0.38
TP	g/L	36.50 ± 7.13	37.25 ± 6.73	36.12 ± 6.29	36.25 ± 3.28	36.87 ± 3.80	37.00 ± 2.88
ALB	g/L	6.87 ± 3.31	6.12 ± 3.44	7.00 ± 4.00	6.24 ± 2.19	6.12 ± 2.75	6.37 ± 2.45
GLOB	g/L	29.37 ± 3.38	31.12 ± 3.56	30.25 ± 3.41	30.00 ± 1.51	31.87 ± 3.6	30.62 ± 1.06
NH_3	μmol/L	549.75 ± 73.29	648.12 ± 109.83	552.25 ± 34.76	657.12 ± 81.16*	561.37 ± 40.94	664.62 ± 44.00*
AST	μkat/L	3.82 ± 2.23	5.64 ± 1.67	3.52 ± 0.66	7.62 ± 0.69*	3.64 ± 0.42	9.09 ± 0.56*
ALT	μkat/L	0.10 ± 0.04	0.20 ± 0.13	0.10 ± 0.04	0.30 ± 0.13*	0.11 ± 0.04	0.40 ± 0.12*
CK	μkat/L	11.98 ± 1.91	16.06 ± 1.95*	12.05 ± 1.26	18.20 ± 1.27*	12.17 ± 1.28	20.70 ± 0.77*
LDH	μkat/L	17.80 ± 0.80	19.14 ± 0.67*	17.72 ± 0.47	22.00 ± 1.43*	17.66 ± 0.72	24.25 ± 0.81*
Ca^{2+}	mmol/L	2.51 ± 0.17	2.56 ± 0.24	2.54 ± 0.16	2.58 ± 0.37	2.65 ± 0.32	2.67 ± 0.28
Mg^{2+}	mmol/L	1.02 ± 0.22	1.06 ± 0.21	1.01 ± 0.17	0.97 ± 0.17	0.95 ± 0.23	0.96 ± 0.14
PHOS	mmol/L	1.34 ± 0.11	1.40 ± 0.11	1.37 ± 0.09	1.43 ± 0.10	1.50 ± 0.23	1.52 ± 0.21
ALP	μkat/L	0.75 ± 0.12	0.77 ± 0.12	0.73 ± 0.11	0.80 ± 0.15	0.82 ± 0.19	0.87 ± 0.21
TRIG	mmol/L	0.93 ± 0.10	0.95 ± 0.08	0.93 ± 0.11	0.96 ± 0.20	0.98 ± 0.14	0.96 ± 0.20
GGT	nmol/L	12.56 ± 7.75	10.47 ± 8.67	12.75 ± 7.87	18.37 ± 13.30	6.37 ± 8.80	16.25 ± 14.82
Lysozyme	μg/mL	5.52 ± 1.25	2.21 ± 0.69*	6.05 ± 0.87	2.48 ± 0.77*	4.82 ± 0.96	2.64 ± 0.99*
Cp	OD_{540nm}	0.48 ± 0.06	0.69 ± 0.10*	0.51 ± 0.07	0.31 ± 0.08*	0.46 ± 0.04	0.32 ± 0.09*

$n = 5$, mean ± SD. Time after the end of intoxication

* Statistically significant at $P < 0.05$

PHOS, ALP, TRIG and GGT) were similar to those found in the control fish plasma.

MC-intoxicated fish demonstrated a strong decrease in lysozyme levels after 24 h and 72 h, reaching about 40% of its activity in the control group, with a slow rise detected after 5 days, when nearly 55% of the control activity was noted.

Marked increase in Cp activity, reaching 144% of the OD value of the enzyme activity in control fish, was detected in plasma sampled 24 h after the end of intoxication. However, after 72 h, Cp activity was lower than that in the control group, with the OD value reaching 61% of the control level, and the reduction was still observed after 5 days.

Discussion

It is believed that fish can be exposed to MCs during feeding with food containing cyanotoxins and to a lesser extent, through passive means, absorbing the toxins directly from the water (Butler et al. 2009; Cazenave et al. 2005). The second means of exposure is seldom used in experimental settings for MC toxicity assessment. In our study, for the first time, toxin-induced changes in blood parameters, including Cp and lysozyme activity, were assessed, after common carp immersion in water containing MCs under experimental conditions. The obtained results indicate that not only consumption of food containing toxins but also MCs dissolved in water may pose a threat to fish health.

After entering the body, MCs are transported via the bloodstream. The cellular uptake of the toxins is mediated by multispecific organic anion-transporting peptides (rodent Oatps; human OATPs) found to be expressed, for example, in liver or kidney (Campos and Vasconcelos 2010), which makes these organs susceptible to MC intoxication. MCs tend to accumulate in fish liver, reaching higher concentrations than in the other tissues (Li et al. 2007; Papadimitriou et al. 2010; Romo et al. 2011). The exception was the fish fed on cyanobacteria containing toxins, in which higher MC concentrations were detected in the intestinal walls than in the livers (Zhang et al. 2009; Chen et al. 2009a). In our study, the only organ in

which the toxin was found was the liver. This confirmed the absorption of MCs into bodies of fish under applied experimental conditions. MCs were not detected in the other studied organs, which is inconsistent with previous studies indicating that the toxins may accumulate in relatively high concentrations in kidneys, while in gills and muscles, it may also be found, though at lower levels (Papadimitriou et al. 2010; Chen et al. 2009a; Cazenave et al. 2005). Cazenave et al. (2005) observed high MC concentrations in gills after *Jenynsia multidentata* and *Corydoras paleatus* exposure for 24 h to 50 μg/L of MC-RR dissolved in water, which were 0.56 and 1.40 μg/g f.w., respectively. On the other hand, the toxin amounts in livers were also a few-fold higher (1.62 and 19.63 g/g f.w.) than those detected in our study. There could be several reasons for these differences. The first one is the toxin dose, which in our experiment was more than four times lower. The second one is the toxin variant, as different variants can have varying accumulation abilities. It cannot be ruled out, however, that the toxin may have been present in other studied organs, though in much lower concentrations, being below the limit of detection of the used method.

Most of the observed changes in our study were connected with liver dysfunctions, which confirms toxic potency of MCs administered by immersion. As the only MC isoform detected in fish tissues was MC-LR, it can be concluded that this toxin was responsible for the alterations observed in fish blood. The earliest symptom of hepatotoxicity was increased LDH activity, showing the tendency to rise in time, indicating the progressive damage of the organ. Besides high LDH levels, which are considered to be a useful marker of hepatocyte collapse (Hudder et al. 2007), also CK activity showed a similar profile. Circulating CK is removed by degradation in the liver, so the increase in the enzyme activity in plasma may be treated as a consequence of liver failure; however, it is rather a muscle or brain damage marker. The most distinct symptoms of hepatotoxic effects in exposed fish were highly elevated levels of AST and ALT, coupled with increased NH_3 level, which also indicated the escalation of the liver damage process, lasting until the end of the experiment.

To some extent, similar biochemical changes were also found in the studies on crucian carp (*Carassius auratus*) (Zhang et al. 2007) or common carp (Kopp et al. 2009, 2010). In the study by Zhang et al. (2007), fish were injected intraperitoneally with MCs, which resulted in faster and more pronounced changes in the liver profile than those observed in our experiment. Besides the increase in LDH, AST and ALT levels, seen already in the first hours after toxin injection, also ALP, TP and TRIG exhibited significant changes. Moreover, depending on the used toxin dose, LDH, AST, ALT and ALP tended to decrease toward the end of the experiment, indicating a recovery trend. No such effects were seen in our study; on the contrary, rather the escalation of the liver damage was observed, which shows differences in MC toxicodynamics dependent on the exposure route.

The lack of more pronounced changes in blood cell parameters of intoxicated fish under the applied experimental protocol seems to confirm the conclusion reached by Kopp et al. (2010) that changes in blood morphology after fish intoxication with MCs observed in other studies (Palíková et al. 1998, Kopp et al. 2010) are probably the effects of extensive hemorrhaging caused by high doses of toxins or may be the result of oxygen decline during cyanobacterial blooms.

Lysozyme is one of the main components associated with the first line of immune defense in fish. The main sources of the enzyme are monocytes, macrophages and polymorphonuclear granulocytes, from which it is released into the blood (Murray and Fletcher 1976). Lysozyme activity in fish plasma is one of the sensitive biomarkers of environmental contamination (Bols et al. 2001). Decreased enzyme activity was reported after fish intoxication with, for example, heavy metals, but that drop was concomitant with a decrease in the leukocyte count. In our experiment, no changes in the total number of WBC were detected; however, a significant decrease in the monocyte count after 24 h was seen, with a subsequent slow increase later in the experimental period, which might reflect the lysozyme kinetics in fish blood plasma. Moreover, as previously found, MC-LR is able to modulate some vital functions of phagocytic cells in fish (Palíková et al. 1998; Sieroslawska et al. 2007), which may also result in changed enzyme production and release. Although it is difficult to speculate on the mechanisms of lysozyme activity depression, as they are probably complex, it should be noted that observed effects may result in weaker nonspecific defense reactions in fish living in the environment containing MC-LR and thus their increased susceptibility to bacterial diseases.

Cp belongs to acute phase proteins, whose level changes in response to such stimuli as tissue damage or inflammation (Bols et al. 2001). In fish, the main function of Cp, like in mammals, is copper transport in the blood. It also acts as an antioxidant of ferroxidase activity, which can oxidize ferrous iron (Fe^{2+}) into the nontoxic form of ferric (Fe^{3+}) (Fox et al. 2000). Ferrous iron, in the presence of hydrogen peroxide or molecular oxygen, possesses the ability to generate highly toxic hydroxyl and superoxide free radicals. The increase in Cp activity observed 24 h after the end of intoxication, in connection with other observed changes, confirms liver and possibly also other organ toxin-induced damages. Moreover, as MC-LR is known to generate oxidative stress in exposed cells (Gehringer 2004; Ding et al. 2000), we can speculate that the induction of Cp activity might be the part of protective mechanisms in fish, preventing iron-catalyzed hydroxyl radical formation. Such prevention against reactive oxygen species (ROS) formation by inhibition of Fenton reaction in hepatocytes intoxicated with MC-LR was reported after cell pretreatment with deferoxamine (DFO) (Ding et al. 2000). DFO belongs to specific iron chelators of effects similar to those induced by Cp. However, later in the experiment, a severe decrease in the enzyme activity was observed, lasting to the end of the experimental period.

Initially, Cp is synthesized in hepatocytes as an apo-ceruloplasmin. Copper incorporation occurs in the liver later, before enzyme secretion into the blood. The failure of copper incorporation into Cp results in a loss of enzymatic activity and leads to rapid degradation by plasma proteases (Squitti et al. 2008 and references herein), so the observed effects may be a consequence of the impairment of liver functions manifested by disturbances in enzyme formation.

The oxidative stress is a consequence of both ROS overproduction and a decrease in antioxidant levels. The decrease in Cp activity observed in our study may contribute to the potentiation of the oxidative effects of MC-LR, as also another antioxidant, GSH, is known to be depleted by MCs (Ding and Ong 2003).

In conclusion, MC-LR appeared to be able to induce biochemical changes in carp exposed to the toxin by immersion, which confirms that fish may undergo intoxication by other means than consuming food containing cyanotoxins. Liver damage accounts for most of the observed changes, which is a common result of MC-LR poisoning. Additionally, detected changes in lysozyme and Cp activity may have distinct effects on resistance of fish to pathogens or oxidative stress.

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