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IMPACTS OF MICROCYSTIN, A CYANOBACTERIAL TOXIN, ON LABORATORY RODENTS *IN VIVO*

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Abstract

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Cyanobacterial water blooms became a global problem/issue because beside a dramatic deterioration of water quality parameters they also produce cyanobacterial toxins being harmful for animals and humans. Cyanotoxins especially the most prominent one, microcystin-LR (MC-LR), are of major concern and they have been reported to cause even death of mammals following ingestion or ingurgitation due to hepatotoxic modes of action. The aim of the recent study is to summarize briefly the impacts of microcystin on laboratory rodents, mice and rats, being used as models for other mammals including human beings. Most experimental approaches used intraperitoneal rather than oral and intratracheal application of microcystins, especially MC-LR, being the most efficient way to induce adverse impacts on different target organs. However, no matter how the exposure of rodents was performed, microcystins induced severe harmful impacts on the different target organs, preferentially the liver, for instances hemorrhages and apoptosis in liver, liver tumours, adverse effects on gut, kidney, testis and epididymis including spermatogenesis, on lung, on serum parameters and on progeny. In addition to these histological findings, microcystin was found to affect specifically biochemical parameters of target organs such as enzymes e.g. GST, CAT, GR, GPX, SOD, AST, ALT, γ-GT, protein phosphatases, SDH, SoDH and LDH or stress proteins such as HSP-70 and further parameters such as hepatic sulfhydryl content, GSH depletion, total bilirubin, urea nitrogen, and creatinine. Gene array analyses revealed that microcystin affects genes related to actin organization, cell cycle, apoptosis, cellular redox potential, cell signalling, albumin metabolism, glucose homeostasis pathway and organic anion transport polypeptide system. In combination with a further proteomics approach the proteomic analyses indicate that liver apoptosis induced by microcystin can be induced by two pathways: the BID-BAX-BCL2 and the reactive oxygen species pathway. The reviewed data clearly show that microcystin, especially MC-LR is able to cause severe adverse impacts on laboratory rodents and therefore there is an emerging need for further research to cover the major concern about cyanobacterial water blooms affecting mammals including human beings.

microcystin; mice; rats; intraperitoneal, oral, intratracheal application

ALT = alanine amino transferase, AST = aspartate aminotransferase, CAT = catalase,

GPX = glutathione peroxidase, GR = glutathione reductase, GSH = glutathione synthetase,

GST = glutathione-S-transferase, HSP-70 = heat shock protein 70, LDH = lactate dehydrogenase, SDH = succinate dehydrogenase, SOD = superoxide dismutase,

SoDH = sorbitol dehydrogenase, γ-GT = γ-glutamyl transpeptidase

INTRODUCTION

Since several years the occurrence of water blooms caused by mass development of cyanobacteria in fresh and in sea water has became an important issue concerning environmental research (Carmichael, 1989; Demir, 2007; Fonseca and Bicudo, 2008). The biomass production of cyanobacteria is related to massive input of nutrients especially of nitrogen and phosphorus into water bodies by human activities such as agricultural fertilization and sewage effluents and, in addition, the higher mean temperature caused by cli-

mate change. Besides negative effects such as deterioration of physicochemical parameters of water environment (oxygen depletion and bad odour from decaying cyanobacterial biomass), cyanobacteria are able to produce a wide range of bioactive compounds (Carmichael, 2001). These substances are called cyanotoxins and can be classified into three groups concerning their structure: cyclic peptides (e.g. microcystins, nodularins), alkaloids (e.g. anatoxins, saxitoxins) and lipopolysaccharides that are produced by all cyanobacterial species (Sivonen and Jones, 1999). Cyanotoxins have severe effects on vertebrates due to their chemical structures. Cyclic peptides are mainly associated with hepatotoxicity whereas alkaloids are known to be neurotoxic and lipopolysaccharides have the potential to be irritants. Microcystins, the most common cyanotoxins in freshwaters, are a family of toxins produced by species primarily *Microcystis aeruginosa* but also by other *Microcystis* species and other genera, namely *Anabaena*, *Oscillatoria* and *Nostoc* (Dawson, 1998). By far the main occurring microcystin is microcystin–LR, a hepatotoxin being a threat to health of other animals and even humans. There have been reports of several cases of acute animal poisoning episodes (Done and Bain, 1993; Fitzgerald and Popenga, 1993; Carbis et al., 1994; Van Halderen et al., 1995; Frazier, 1998; Boaru et al., 2006). The microcystins have been also associated with human toxicity. Exposure to these cyanotoxins resulted in acute sickness and death in dialysis patients in Brazil (Jochimsen et al., 1998) and it has been suggested that microcystins may play a role in the high incidence of hepatocellular carcinoma present in specific regions of China (Yu, 1995). Many investigations of microcystins revealed that they cause severe hepatotoxic effects in diverse mammalian species (Hermansky et al., 1993; Chernoff et al., 2002; Weng et al., 2007; Ito et al., 1997; Nishawaki-Matsushima et al., 1992). Various ways of exposure are applied in order to test the toxicity of microcystin-LR in mammals including intraperitoneal, oral and intratracheal application. The aim of this article is to summarize and review the impacts of microcystins, mainly of microcystin-LR (MC-LR) on rodents, used as models for mammals including human beings, considering toxicity related to different exposure routes (cf. Tab. I-III).

DISCUSSION AND CONCLUSIONS

MC-LR is the most common cyanotoxin known to be hepatotoxic in mammals; however, summarized data presented in Tab. I-III demonstrate besides its hepatotoxicity several further adverse biological impacts on various target organs. Laboratory rodents are generally accepted as good models for mammalian toxicology including humans. Because of several severe impacts of cyanotoxins on mammals (Done and Bain, 1993; Frazier, 1998; Fitzgerald and Popenga, 1993; Carbis et al., 1994; Van Halderen et al., 1995) including humans (Jochimsen et al., 1998; Yu, 1995) many investigations were undertaken in order to elucidate the potential impacts of microcystins, especially MC-LR or cyanobacterial extracts and scums using different experimental approaches dealing with various exposure routes. The most common exposure pathways for microcystin application are: intraperitoneal injection (cf. Tab. I), oral administration (cf. Tab. II) and intratracheal application (cf. Tab. III). Results from different studies presented in Tab. I–III. clearly demonstrated that intraperitoneal injection caused most efficiently adverse impact of MC-LR on various target organs, preferentially the liver, for instances hemorrhages and apoptosis in liver and liver tumours. Adverse histological effects were also detected in gut, kidney, testis and epididymis including spermatogenesis, as well as in lung, in serum parameters and in same target organs of progeny. Besides these histological findings biochemical evaluation of various parameters in target organs were determined. Adversely affected were enzymes such as GST, CAT, GR, GPX, SOD, AST, ALT, γ-GT, protein phosphatases, SDH, SoDH and LDH, and the stress protein HSP-70. In addition, further parameters such as hepatic sulfhydryl content, GSH depletion, total bilirubin, urea nitrogen, and creatinine became negatively influenced. More recently, gene array analyses revealed that MC-LR affects genes related to actin organization, cell cycle, apoptosis, cellular redox potential, cell signalling, albumin metabolism, glucose homeostasis pathway and organic anion transport polypeptide system (Chen et al., 2005). In combination with an advanced proteomics approach (Weng et al., 2007) the proteomic analyses indicate that liver apoptosis induced by MC-LR can be induced by two pathways: the BID-BAX-BCL2 and the reactive oxygen species pathway.

Comparison of different experimental approaches concerning various MC-LR applications revealed that intraperitoneal injection is the most efficient way to induce adverse impacts. However, natural exposure routes of MC-LR in mammals are rather due only to oral or tracheal ingestion. Recent results concerning oral (cf. Tab.II) and tracheal ingestion (cf. Tab. III) confirmed that much higher concentrations of microcystins are needed to evoke similar adverse effects compared to intraperitoneal injection. However, different exposure routes are causing different impacts on target organs as seen by oral administration of microcystins affecting primarily gastrointestinal tract. Inhalation or tracheal ingestion demonstrated severe impacts on inhalation tract (cf. Tab. III). Therefore because of different exposure routes causing different impacts on target organisms more emphasis should be taken to investigate the effects of natural exposure routes such as oral, tracheal, and even transdermal ones instead of testing intraperitoneal application, that is not happening under natural conditions.

Results of the various exposures are presented in detail by Tab. I–III.

Tab. I consists of data obtained by intraperitoneal injections. Tab. II shows the results from oral administration of MC-LR and Tab. III represents findings from intratracheal application, inhalation and tail vein injection of MC-LR.

I: *Intraperitoneal injection*

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
Balb/c mice	♀, 7 weeks	75% LD_{50} pure MC-LR	8, 16, 24, 32 h pe	liver-detoxification of MC-LR by increase in GST activity, synthesis of GSH, GPX.	**Gehringer et al. (2004)**
Swiss mice	♂, 4 and 12 weeks	48.2 μg.kg^{-1}	2, 8 h, 1, 2, 3, 4 d pe sublethal dose of MCs	lungs-increase of alveolar collaps and polymorphonuclear cells due to lesion time, maximum value reached earlier in young animals.	**Picanço et al. (2004)**
Balb/c mice	♀, 6 and 9 weeks	40, 48, 57.6, 60 μg.kg^{-1} pure MC-LR	24 h experiment 4, 7, 11, 17, 21 h pe	liver-hemorrhage within 7 h, paracentral necrosis, apoptosis in the centrilobular and midlobular regions.	**Yoshida et al. (2001)**
Kunming mice	pregnant ♀, 23-28 g	3, 6, 12 μg.kg^{-1} cell extract of *M. aeruginosa*	18 d experiment from gestational days 6-15 daily	decrease in body weight gain of pregnant mice (12 μg.kg^{-1}), fetuses-decreased body weight, body length, tail length (12 μg.kg^{-1}), fetuses liver- petechial hemorrhage, hydropic degeneration (6, 12 μg.kg^{-1}).	**Bu et al. (2006)**
KM mice	♂, 25-30 g, 8 weeks	3.33, 6.67 μg.kg^{-1} cell extract of *M. aeruginosa*	14 d, daily 8 h pe	decrease of mean body weight, mean absolute weight of the testes and epididymis, increased relative mean weight of the testes, damaged testes-more pronounced space between the seminiferous tubules, decreased sperm quality, reduced sperm motility and viability.	**Ding et al. (2006)**
Wistar rats	♂, about 200 g	100 μg.kg^{-1} pure MC-LR		liver-increased weight, higher specific activity of sucrase, lactase, maltase and ALP not modified; ACP, SDH, lysosomal and mitochondrial membrane markers were increased, increased peroxidative status in serum and intestinal mucosa.	**Moreno et al. (2003)**
mice		101 μg.kg-1 [^{3}H]-MC-LR	death, 6 h pe	liver contained 56 ± 1%, intestine 7 ± 1%, kidney 0.9 ± 0.2%, carcass 10 ± 1% of injected dose; heart, spleen, lung and skeletal muscle contained < 1% of [^{3}H]-MC-LR.	**Robinson et al. (1989)**
Balb/c mice	♂, adult, 20-22 g	100 μg.kg^{-1} lethal 12, 23, 45 μg.kg^{-1} sublethal dose of MC-LR		hepatic nuclear exctract-inhibition of nuclear protein phosphatase activity within lethal dosing.	**Guzman et al. (2003)**
Balb/c mice	♀, 7 weeks	75% LD_{50} MC-LR	8, 16, 24, 32 h pe	GIT – duodenum-the most significant increase in apoptotic index, followed by jejunum and ileum; increase of apoptoses index in time.	**Botha et al. (2004)**

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
Swiss albino mice	♀, 24-26 g	38.31 $\mu g.kg^{-1}$ MC-LR 76.62 $\mu g.kg^{-1}$ MC-LR	15, 30, 60 min, and 1, 2, 3 d pe	no effect of other antioxidant enzymes except CAT and GR at 38.31 $\mu g.kg^{-1}$. Time-dependent increase in HSP-70 expression; Liver body weight index, hepatic lipid peroxidation and GSH depletion increase. Decrease in the activity of GPX, SOD, CAT, GR, GST.	**Jayaraj et al. (2006)**
Swiss mice	♂, 25-30 g, 6-8 weeks	40 $\mu g.kg^{-1}$ MC-LR	2, 8 h, 1, 2, 4 d pe	no MC or inhibition of PPases detected in mice lungs. Rapid increase in lung impadence and an inflammatory response with interstitial edema and inflammatory cell recruitment.	**Soares et al. (2007)**
Swiss albino mice	♀, 24-26 g	43 $\mu g.kg^{-1}$ MC-LR	30 min pe and MDT	significant increase in liver body weight index, increase in serum levels of hepatic enzymes (AST, ALT and γ-GT), enhanced LDH leakage, DNA fragmentation, depletion of hepatic glutathione. liver histology-time dependent pathological lesions like congestion, hemorrhage, portal mononuclear cell infiltration and obliteration of chromatin material. Lung lesions in bronchi and parenchyma.	**Gupta et al. (2003)**
Rats	fed	LD_{50} (122 $\mu g.kg^{-1}$) 100, 150, 200 $\mu g.kg^{-1}$	MDT: 11.2, 18.2, 31.9 h	increase in liver weight and serum levels of SoDH, plasma membranes isolated from liver exhibited toxin-induced cytoskeletal elements changes.	**Miura et al. (1991)**
Rats	fasted	LD_{50} (72 $\mu g.kg^{-1}$) 100, 150, 200 $\mu g.kg^{-1}$	MDT: 1.8, 1.7, 1.5 h	increase in liver weight and serum levels of SoDH; plasma membranes isolated from liver exhibited toxin-induced cytoskeletal elements changes. Complete inhibition of state 3 respiration in liver mitochondria.	**Miura et al. (1991)**
Wild type mice B6.129-*Trp*53$^{+,+}$ N5 mice		40 $\mu g.kg^{-1}$ MC-LR	daily for 28 d 4, 24 h, 4, 14, 28 d after	increase in plasma hepatic enzyme activities, decrease in total protein, first dose albumin and glucose concentration. Gene array analysis revealed in the actin organization, cell cycle, apoptotic, cellular redox, cell signalling, albumin metabolism, glucose homeostasis pathway, and the organic anion transport polypeptide system.	**Clark et al. (2007)**

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
Swiss albino mice	6 weeks (24-26 g) 12 weeks (31-34 g) 18 weeks (34-38 g) 24 weeks (37-42 g) 30 weeks (42-45 g) 36 weeks (43-46 g)	LD_{50} (43 μg.kg^{-1})	24 h	time to death in toxin treated animals decreased with age of mice; significant increase in liver body mass index and increases in serum enzymes (LDH, AST, ALT, γ-GT, SoDH)	**Rao et al. (2005)**
NIH non-Swiss mice	♀, 20-24 g	12.5, 25, 50, 100 μg.kg^{-1} MC-LR	15, 30, 45, 60 min, 2 h pe	liver weight increased, 50 and 100 μg.kg^{-1} Kupffer cell hyperplasia, loss of hepatic architecture and necrosis. Serum enzymes increased (LDH, ALT, AST). Hepatic sulfhydryl content decreased. 100 μg.kg^{-1} lethal dose.	**Hermansky et al. (1990)**
Balb/c mice	♀, adults	100 μg.kg^{-1} MC-LR	15, 30, 60, 90 min pe	liver lesions, higher liver weight, necrosis, diassociation and rounding of centrilobular hepatocytes. Kidney weight increased and vacuolization of proximal tubular epithelium with tubular dilatation, increase of ALT, ALP, total bilirubin, urea nitrogen, creatinine, 100 μg.kg^{-1} lethal dose	**Hooser et al. (1989)**
Sprague-Dawley rats	♀; ♂; 175-200 g	20-1,200 μg.kg^{-1} MC-LR	5-60 min, 3-24 h	liver lesions, higher liver weight, necrosis, diassociation and rounding of centrilobular hepatocytes. Kidney weight increased and vacuolization of proximal tubular epithelium with tubular dilatation, increase of ALT, ALP, total bilirubin, urea nitrogen, creatinine, 160 μg.kg^{-1} lethal dose.	**Hooser et al. (1989)**
CD-1 mice	♀ pregnant	2-160 μg.kg^{-1}	during gestation days 7-8, 9-10, or 11-12 sampling at d 17	lethality at 128 and 160 μg.kg^{-1}, liver necrosis with hemorrhage, fetuses were not affected by teratogenicity	**Chernoff et al. (2002)**
Balb/c mice	♂, 20-35 g	20-40 μg.kg^{-1}	24 h	lethality of 25, 32.5, and 40 μg.kg^{-1} was 0, 50, and 100% increase in weight of liver and kidney hepatic hemorrhage	**Lovell et al. (1989)**
Swiss Webster mice	♂, 6 weeks, 22-28 g	1st 55 μg.kg^{-1} MC-LR 2nd 75 μg.kg^{-1}	1st sublethal dose 2nd LD 2 or 3 d later	after priming by sublethal dose LD caused increased survivorship causing weakness, recumbency, anorexia, icterus, and gross liver lesions	**Lovell et al. (1989)**

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
Balb/c mice	♀, 16-20 g	50, 60, 70 μg.kg^{-1} MC-LR	24 h	liver- transcriptomics: affecting 61 genes related to apoptosis; proteomics: 35 proteins were up and 30 down regulated; computer simulation of data revealed that 50 μg.kg^{-1} caused apoptosis mainly by BID-BAX-BCL2-pathway; 70 μg.kg^{-1} impacted apoptosis mainly by ROS pathway	**Chen et al. (2005)**
NIH non-Swiss mice	♀, 22-24 g	100 μg.kg^{-1} MC-LR	15-60 min	liver-hepatocyte distortion, mitochondrial aggregation, accumulation of endoplasmic reticulum, no damage of sinusoidal endothelial cells	**Hermansky et al. (1993)**
ICR mice	♂, 20-27 g	35 μg.kg^{-1} MC-LR	1-45 min, 1-24 h	MC-LR appeared in serum and liver after 15 min and was present up to 24 h, hepatic protein phosphatase 2A decreased after 1-2 h	**Lin & Chu (1994)**
Fisher 344 rats	♂, 7 weeks	priming by diethylnitrosamine 1, 10 μg.kg^{-1} MC-LR	twice per week over weeks 2-8	liver-tumor promoting, GST foci increased, inhibition of protein phosphatase type 1 and 2A	**Nishiwaki-Matsushima et al. (1992)**
ICR mice	♀, 14 weeks	[^{3}H]-dihydro-MC-LR	5, 15, 30, 60 min	distribution of [^{3}H]-dihydro-MC-LR preferentially in liver (71.5% after 1 h) all other organs less than 1.4%	**Nishiwaki et al. (1994)**
Balb/c mice	20 g	45 μg.kg^{-1} MC-LR	2-24 h	liver-hepatocellular hypertrophy, loss of vacuolation, glycogen depletion, apoptosis	**Guzman & Solter (2002)**
Crl:CD-1 (ICR)BR mice	♀, ♂	50, 158, 500 μg.kg^{-1} MC-LR	up to 14 d	LD 50 between 50 and 158 μg.kg^{-1}, liver hemorrhage, pallor of kidney, spleen, and adrenal	**Fawell et al. (1999)**
ICR mice	♂, 8 weeks	60 μg.kg^{-1} MC-LR	12 h pe	large amount of ROS generation in mice liver, upregulated the expression of BAX and BID, caused the mitochondrial membrane protein loss and hepatocyte apoptosis as well as liver injury	**Weng et al. (2007)**

II: *Oral administration of MC-LR*

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
Cr1:CD-1 (ICR)BR mice	♀, ♂	500; 1,580; 5,000 $\mu g.kg^{-1}$ MC-LR	single dose up to 14 d daily	LD 50 at 5,000 $\mu g.kg^{-1}$, liver hemorrhage, necrosis, vacuolation	**Fawell et al. (1999)**
Cr1:CD-1 (SD)BR rats	♀, ♂	500; 1,580; 5,000 $\mu g.kg^{-1}$ MC-LR	single dose up to 14 d daily	not lethal, liver hemorrhage, necrosis, vacuolation	**Fawell et al. (1999)**
Cr1:CD-1 (ICR)BR mice	♀, pregnant	200; 600; 2,000 $\mu g.kg^{-1}$ MC-LR	during gestation d 6-15 daily	not lethal, liver hemorrhage, necrosis, vacuolation, 2,000 $\mu g.kg^{-1}$ caused developmental toxicity on progeny	**Fawell et al. (1999)**
Balb/c mice	♀, 6 weeks	20 $\mu g.l^{-1}$ MC-LR in drinking water	3-18 months	calculated intake ca. 35 µg/mouse, no toxic effect by chronic exposure to MC-LR	**Ueno et al. (1999)**
Mice	25 g	*M. aeruginosa* in drinking water a.l. or in scum 15,000 cells ml^{-1}		scum containing *M. aeruginosa* was preferentially chosen for drinking and caused lethality of 50% and 100% after 48 h and 6 d, respectively	**Rodas & Costas (1999)**
ICR mice	♀, 8 weeks	[^{3}H]-dihydro-MC-LR	3, 6, 19 h, 3, 6 d	distribution of [^{3}H]-dihydro-MC-LR preferentially in GIT (37.6% after 19 h) but only 0.68% in liver	**Nishiwaki et al. (1994)**
Balb/c mice ICR mice		500 $\mu g.kg^{-1}$ MC-LR	0, 1, 6, 7, 12, 13 weeks	Balb/c mice had lethality > 50%, ICR mice nearly no lethality	**Ito et al. (2000)**
Swiss albino mice	6 weeks (24-26 g), 36 weeks (43-46 g)	3.5 g $MCE.kg^{-1}$ (LD50)	24 h	similar to i.p. administration, time to death in toxin treated animals decreased with age of mice, glutathione depletion and increases of hepatic lipid peroxidation, DNA fragmentation, liver mass index, and serum enzymes (LDH, AST, ALT, γ-GT, SoDH)	**Rao et al. (2005)**
Balb/c mice	♀, 6 weeks	8-20 $mg.kg^{-1}$ MC-LR	single dose	medial lethal dose was 10.9 $mg.kg^{-1}$. Hepatocellular injuries with hemorrhage and necrosis. Apoptotic cell death of hepatocytes. Lethality of MC-LR much lower in oral dosage than by i.p. administration but toxic effects are similar.	**Yoshida et al. (1997)**
ICR mice	♂, 32 weeks, 48.2±3.8 g 5 weeks, 27.4±1.2 g	500 $\mu g.kg^{-1}$ MC-LR	2 ,5 19 h pe gastric intubation	aged mice (32 weeks) changes in the liver, rough surface of the stomach, small intestinal mucosa. Liver changes were not found in young mice (5 weeks). Hepatotoxicity by oral administration of MC-LR is deeply related to aging, and particularly to conditions in the small intestine such as permeability of capillaries in the villi	**Ito et al. (1997)**

III: *Intratracheal and tail vein administration, inhalation exposure*

Experiment. Animals	Sex, weight, age	Exposure concentr.	Exposure time	Observed effects	Reference
ICR mice	♂, 5 weeks, 27.6±1.3 g	**intratracheal admin.** 50-200 μg.kg^{-1} MC-LR	until death	MC-LR accumulation caused bleeding in liver	**Ito et al. (2001)**
ICR mice	♂, 5 weeks, 27.6±1.3 g	**intratracheal admin.** LD (100 μg.kg^{-1} MC-LR)	5, 10, 20, 30, 45, 60, 90, 120 min	MC-LR accumulation caused bleeding in liver after 90 min.	**Ito et al. (2001)**
ICR mice	♂, 5 weeks, 27.6±1.3 g	**intratracheal admin.** sublethal (50 μg.kg^{-1} MC-LR)	7, 24, 48 h, 3, 4, 7,10, 14 d	after 2 weeks, small amounts of MC-LR were still present in epithelial cells in the gastrointestinal mucosa	**Ito et al. (2001)**
Balb/c mice	♂, 6-8 weeks	**inhalation exposure** 260-265 MC-LR.m^{-3}	7 d 0.5, 1 and 2 h	microscopic lesions in the nasal cavity of mid- and high-dose groups, necrosis of respiratory epithelium, neutrophilic inflammation, degeneration, necrosis and atrophy of the olfactory epithelium, no-adverse-effect dose 3 μg.kg^{-1} b.w.≈20 ng.cm^{-2} nasal epithelium.	**Benson et al. (2005)**
VAF/plus CD-1 Mice	♂, 20-27 g	**i.v. *via* tail vein** 35 μg.kg^{-1} [^{3}H]-MC-LR	6, 12 h, 1,2,3,4,5,6 d pe	at 60 min, liver contained urine, feces contained 67±4% of dose, during 6 days no change, 23.7±1.7% of dose was excreted (9.2±1.0% in urine, 14.5±1.1% in feces)	**Robinson et al. (1991)**

a.l. = ad libitum, ACP = acid phosphatase, ALP = alkaline phosphatase, ALT = alanine amino transferase, AST = aspartate aminotransferase, CAT = catalase, GIT = gastrointestinal tract, GPX = glutathione peroxidase, GR = glutathione reductase, GSH = glutathione synthetase, GST = glutathione-S-transferase, HSP-70 = heat shock protein 70, LDH = lactate dehydrogenase, MCE = microcystin extract, MDT = mean time to death, pe = post exposure, ROS = reactive oxygen species, SDH = succinate dehydrogenase, SOD = superoxide dismutase, SoDH = sorbitol dehydrogenase, γ-GT = γ-glutamyl transpeptidase

SOUHRN

Vliv microcystinu, toxinu sinic, na laboratorní hlodavce *in vivo*

Sezonní výskyt vodních květů sinic se v poslední době stal celosvětově diskutovaným tématem a to nejen díky zhoršení hydrochemických parametrů vodního prostředí v důsledku rozkladu biomasy, ale také v souvislosti s produkcí cyanotoxinů, které jsou nebezpečné pro zvířata i lidi. Je dokonce zaznamenáno několik případů úmrtí savců v důsledku požití cyanotoxinů, zejména nejběžněji se vyskytujícího toxinu, microcystinu-LR (MC-LR). Cílem této studie bylo stručně shrnout dopady microcystinů (MCs) na laboratorní hlodavce (myši a krysy), kteří byli použiti v kontrolovaných podmínkách jako modelová zvířata pro vyšší savce včetně člověka.

Ve většině experimentů byl MC aplikován intraperitoneálně, protože oproti orálnímu nebo intratracheálnímu použití bylo možné lépe sledovat nepříznivý vliv na jednotlivé orgány. Lze konstatovat, že bez ohledu na způsob podávání MC hlodavcům vyvolal tento toxin vážné poškození různých orgánů, prioritně jater, kde byly zaznamenány krevní výrony a jaterní tumory, dále poškození trávicího traktu, ledvin, varlat a nadvarlat, plic, zhoršení parametrů krevní plazmy a negativní dopad na potomstvo. Kromě výše zmíněných histologických nálezů byl potvrzen dopad na biochemické ukazatele, jako jsou enzymy: GST, CAT, GR, GPX, SOD, AST, ALT, γ-GT, protein fosfatáza, SDH, a LDH nebo stresové proteiny jako HSP-70 a další parametry: GSH, celkový bilirubin, močovinový dusík, a kreatin.

I když dávky MC použité během experimentů byly mnohem vyšší než ty, přirozeně se nacházející v biomase sinic a ve vodním prostředí v průběhu vegetačního období, i tak je tento cyanotoxin stále potenciální hrozbou pro savce. Jelikož jsou uvedené výsledky založené především na intraperitoneálním podávání microcystinu, ke kterému v přirozených podmínkách nedochází, při dalších experimentech by měl být kladen důraz především na orální, tracheální a transdermální aplikace.

microcystin, myši, krysy, intraperitoneální, orální, intratracheální aplikace

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REFERENCES

BENSON, J. M., HUTT, J. A., REIN, K., BOGGS, S. E., BARR, E. B. and FLEMING, L. E., 2005: The toxicity of microcystin LR in mice following 7 days of inhalation exposure. *Toxicon*, 45, 6: 691–698.

BOARU, D. A., DRAGOS, N., WELKER, M., BAUER, A., NICOARA, A. and SCHIRMER, K., 2006: Toxic potential of microcystin-containing cyanobacterial extracts from three Romanian freshwaters. *Toxicon*, 47, 8: 925–932.

BOTHA, N., VAN DE VENTER, M., DOWNING, T. G., SHEPHARD, E. G. and GEHRINGER, M. M., 2004: The effect of intraperitoneally administered microcystin-LR on the gastrointestinal tract of Balb/c mice. *Toxicon*, 43, 3: 251–254.

BU, Y., LI, X., ZHANG, B., CHUNG, I. and LEE, J., 2006: Microcystins cause embryonic toxicity in mice. *Toxicon*, 48, 8: 966–972.

CARBIS, C. R., SIMONS, J. A., MITCHELL, G. F., ANDERSON, J. W. and MCCAULEY, I., 1994: A biochemical profile for predicting the chronic exposure of sheep to *Micricystis aeruginosa*, an hepatotoxic species of blue-green alga. *Res. Vet. Sci.*, 57: 310–316.

CARMICHAEL, W. W., 1989: Freshwater cyanobacteria (blue-green algae) toxins. In *Natural Toxins: Characterization, Pharmacology and Therapeutics.* Owenby CL and Odell GV (eds.) Pergamon Press, Oxford, pp. 3–16.

CARMICHAEL, W. W., 2001: Health effects of toxin-producing cyanobacteria: "The Cyanohabs". *Hum. Ecol. Risk Assesov.*, 7: 1393–1407.

CHEN, T., WANG, Q., CUI, J., YANG, W., SHI, Q., HUA, Z., JI, J. and SHEN, P., 2005: Induction of apoptosis in mouse liver by microcystin-LR. A combined transcriptomic, proteomic, and simulation strategy. *Molecular and Cellular Proteomics*, 4.7: 958–974.

CHERNOFF, N., HUNTER III, E. S., HALL, L. L., ROSEN, M. B., BROWNIE, C. F., MALARKEY, D., MARR, M. and HERKOVITS, J., 2002: Lack of teratogenicity of Microcystin-LR in the mouse and toad. *Journal of Applied Toxicology*, 22, 1: 13–17.

CLARK, S. P., DAVIS, M. A., RYAN, T. P., SEARFOSS, G. H. and HOOSER, S. B., 2007: Hepatic gene expression changes in mice associated with prolonged sublethal microcystin exposure. *Toxicologic Pathology*, 35: 594–605.

DAWSON, R. M., 1998: The toxicology of microcystins. *Toxicon*, 36, 7: 953–962.

DEMIR, N., 2007: Changes in the phytoplankton community of a costal, hyposaline lake in western Anatolia, Turkey. *Limnology*, 8, 3: 337–342.

DING, X., LI, X., DUAN, H.., CHUNG, I. and LEE, J., 2006: Toxic effects of *Microcystis* cell extracts on the reproductive system of male mice. *Toxicon*, 48, 8: 973–979.

DONE, S. H. and BAIN, M., 1993: Hepatic necrosis in sheep associated with ingestion of blue-green algae. *Vet. Rec.*; 133: 600.

FAWELL, J. K., MITCHELL, R. E., EVERETT, D. J. and HILL, R. E., 1999: The toxicity of cyanobacterial toxins in the mouse: I Microcystin-LR. *Human and Experimental Toxicology* 18, 162–167.

FITZERALD, S. D. and POPPENGA, R. H., 1993: Toxicosis due to mycrocystin hepatotoxins in three Holstein heifers. *J. Vet. Diagn. Invest.*; 5: 651–653.

FONSECA, B. M. and BICUDO, C. E .D., 2008: Fytoplankton seasonal variation in a shallow stratified eutrophic reservoir (Garcas Pond, Brazil). *Hydrobiologica*, 600: 267–282.

FRAZIER, K., 1998: Microcystin toxicosis in cattle due to overgrowth of blue-green algae. *Vet. Hum. Toxicol.*, 40: 23–24.

GEHRINGER, M. M., SHEPHARD, E. G., DOWNING, T. G., WIEGAND, C. and NEILAN, B. A., 2004: An investigation into the detoxification of microcystin-LR by the glutathione pathway in Balb/c mice. *The International Journal of Biochemistry and Cell Biology*, 36: 931–941.

GUPTA, N., PANT, S. C., VIJAYARAGHAVAN, R. and RAO, P. V .L., 2003: Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. *Toxicology*, 188: 285–296.

GUZMAN, R. E. and SOLTER, P. F., 2002: Characterization of sublethal microcystin-LR exposure in mice. *Veterinary Pathology* 39: 17–26.

GUZMAN, R. E., SOLTER, P. F. and RUNNEGAR, M. T., 2003: Inhibition of nuclear protein phosphatase activity in mouse hepatocytes by the cyanobacterial toxin microcystin-LR. *Toxicon*, 41, 7: 773–781.

HERMANSKY, S. J., MARKIN, R. S., FOWLER, E. H. and STOHS, S. J., 1993: Hepatic ultrastructural changes induced by the toxin microcystin-LR (MCLR) in mice. *J. Environ. Pathol. Toxicol. Oncol.*, 12: 101–106.

HERMANSKY, S. J., STOHS, S. J., MARKIN, R. S. and MURRAY, W. J., 1990: Hepatic lipid peroxidation, sulfhydryl status, and toxicity of the blue-green algal toxin microcystin-LR in mice. *Journal of Toxicology and Environmental Health*, 31: 71–91.

HERMANSKY, S. J., MARKIN, R. S., FOWLER, E. H. and STOHS, S. J., 1993: Hepatic ultrastructural changes induced by the toxin microcystin-LR (MCLR) in mice. *Journal of Environmental Pathology, Toxicology and Oncology* 12, 2: 101–106.

HOOSER, S. B., BEASLEY, V. R., LOVELL, R. A., CARMICHAEL, W. W. and HASCHEK, W. M., 1989: Toxicity of microcystin LR, a cyclic heptapeptide hepatotoxin from Micrycystis aeruginosa, to rats and mice. *Vet. Pathol.* 26: 246–252.

ITO, E., KONDO, F. and HARADA, K. I., 2001: Intratracheal administration of microcystin-LR, and its distribution. *Toxicon*, 39: 265–271.

ITO, E., KONDO, F. and HARADA, K. I., 2000: First report on the distribution of orally administered microcystin-LR in mouse tissue using an immunostaining method. *Toxicon*, 38: 37–48.

ITO, E., KONDO, F. and HARADA, K., 1997: Hepatic necrosis in aged mice by oral administration of microcystin-LR. *Toxicon*, 35, 2: 231–239.

JAYARAJ, R., ANAND, T. and RAO, P. V. L., 2006: Activity and gene expression profile of certain antioxidant enzymes to microcystin-LR induced oxidative stress in mice. *Toxicology*, 220, 2–3: 136–146.

JOCHIMSEN, E. M., CARMICHAEL, W. W., AN, J. S., CARDO, D. M., COOKSON, S. T., HOLMES, C. E., ANTUNES, M. B., DE MELO FILHO, D. A., LYRA, T. M., BARRETO, V. S., AZEVEDO, S. M. and JARVIS, W. R., 1998: Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil. *N. Engl. J. Med.*, 338: 873–878.

LIN, J. and CHU, F. S., 1994: Kinetics of distribution of microcystin LR in serum and liver cytosol of mice: An immunochemical analysis. *Journal of Agricultural and Food Chemistry* 42, 4: 1035–1040

LOPEZ RODAS, V. and COSTAS, E., 1999: Preference of mice to consume Microcystis aeruginosa (toxin-producing cyanobacteria): A possible explanation for numerous fatalities of livestock and wildlife. *Research in Veterinary Science* 67: 107–110.

LOVELL, R. A., SCHAEFFER, D. J., HOOSER, S. B., HASCHEK, W. M., DAHLEM, A. M., CARMICHAEL, W. W. and BEASLEY, V. R., (1989). Toxicity of intraperitoneal doses of microcystin-LR in two strains of male mice. *Journal of Environmental Pathology, Toxicology and Oncology* 9 (3), pp. 221–238.

MIURA, G. A., ROBINSON, N. A., LAWRENCE, W. B. and PACE, J. G., 1991: Hepatotoxicity of microcystin-LR in fed and fasted rats. *Toxicon*, 29, 3: 337–346.

MORENO, I. M., MATE, A., REPETTO, G., VÁZQUEZ, C. M. and CARMEÁN, A. M., 2003: Influence of microcystin-LR on the activity of membrane enzymes in rat intestinal mucosa. *Journal of Physiology and Biochemistry*, 59, 4: 293–300.

NISHIWAKI, R., OHTA, T., SUEOKA, E, SUGANUMA, M., HARADA, K., WATANABE, M. F. and FUJIKI, H., 1994: Two significant aspects of microcystin-LR: Specific binding and liver specificity. *Cancer Letters* 83, 283–289.

NISHIWAKI-MATSUSHIMA, R., OHTA, T., NISHIWAKI, S., SUGANUMA, M., KOHYAMA, K., ISHIKAWA, T., CARMICHAEL, W. W. and FUJIKI, H., 1992: Liver tumor promotion by the cyanobacterial cyclic peptide toxin microcystin-LR. *Journal of Cancer Research and Clinical Oncology*, 118, 6: 420–424.

PICANÇO, M. R., SOARES, R. M., CAGIDO, V. R., AZEVEDO, S. M. F. O., ROCCO, P. R. M. and ZIN, W. A., 2004: Toxicity of a cyanobacterial extract containing microcystins to mouse lungs. *Brazilian Journal of Medical and Biological Research*, 37: 1225–1229.

RAO, P. V. L., GUPTA, N., JAYARAJ, R., BHASKAR, A. S. B. and JATAV, P. C., 2005: Age-dependent effects on biochemical variables and toxicity induced by cyclic peptide toxin microcystin-LR

in mice. *Comparative Biochemistry and Physiology – Part C, Toxicology and Pharmacology,* 140, 1: 11–19.

ROBINSON, N. A., MIURA, G. A., MATSON, C. F., DINTERMAN, R. E. and PACE, J. G., 1989: Characterization of chemically tritiated microcystin-LR and its distribution in mice. *Toxicon,* 27, 9: 1035–1042.

ROBINSON, N. A., PACE, J. G., MATSON, C. F., MIURA, G. A. and LAWRENCE, W. B., 1991: Tissue distribution, excretion and hepatic biotransformation of microcystin-LR in mice. Journal of Pharmacology and Experimental Therapeutics, 256, 1: 176–182.

SIVONEN, K. and JONES, G., 1999: Cyanobacterial toxins. In *Toxic Cyanobacteria in Waters,* CHORUS, I., and BARTRAM, J. (eds). St. Edmundsbury Press: Suffolk, Great Britain, 41–111.

SOARES, R. M., CAGIDO, V. R., FERRARO, R. B., MEYER-FERNANDES, J. R., ROCCO, P. R. M., ZIN, W. A. and AZEVEDO, S. M. F. O., 2007: Effects of microcystin-LR on mouse lungs. *Toxicon,* 50, 3: 330–338.

UENO, Y., MAKITA, Y., NAGATA, S., TSUTSUMI, T., YOSHIDA, F., TAMURA, S. I., SEKIJIMA, M., TASHIRO, F., HARADA, T. and YOSHIDA, T., 1999: No chronic oral toxicity of a low dose of microcystin-LR, a cyanobacterial hepatotoxin, in female BALB/c mice. *Environmental Toxicology,* 14: 45–55.

VAN HALDEREN, A., HARDING, W. R., WESSELS, J. C., SCHNEIDER, D. J., HEINE, E. W. P., VAN DER MERWE, J. and FOURIE, J. M., 1995: Cyanobacterial (blue-green algae) poisoning of livestock in the western Cape Province of South Africa. *J. S. Af. Vet. Assoc.,* 66: 260–264.

WENG, D., LU, Y., WEI, Y., LIU, Y. and SHEN, P., 2007: The role of ROS in microcystin-LR-induced hepatocyte apoptosis and liver injury in mice. *Toxicology,* 232, 1–2: 15–23.

YOSHIDA, T., MAKITA, Y., NAGATA, S., TSUTSUMI, T., YOSHIDA, F., SEKIJIMA, M., TAMURA, S. and UENO, Y., 1997: Acute oral toxicity of microcystin-LR, a cyanobacterial hepatotoxin, in mice. *Natural Toxins* 5, 3: 91–95.

YOSHIDA, T., TSUTSUMI, T., NAGATA, S., YOSHIDA, F., MAITA, K., HARADA, T. and UENO, Y., 2001: Quantitative analysis of intralobular distribution of microcystin-LR in the mouse liver. *Journal of Toxicologic Pathology,* 14: 205–212.

YU, S., 1995: Primary prevention of hepatocellular carcinoma. *J. Gastroenterol.,* 10: 674–682.

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