Artykuł przeglądowy *Review*

Harmful cyanotoxins: hepatotoxic effects of microcystin in mammalian animals

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Summary

Cyanobacterial blooms, often observed in eutrophic water reservoirs, produce toxic metabolites known as cyanotoxins that affect animal health. There are five groups of cyanotoxins classified on the basis of their toxic action: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins. Microcystin (MC) is a very common and well described hepatotoxin produced by various genera, such as Microcystis, Anabaena, Planktothrix, Anabenopsis, Hapalosiphon and Nostoc. It acts as an inhibitor of serine/threonine protein phosphatase 1 (PP1) and 2A (PP2A), inducing hyperphosphorylation of cell proteins and a variety of toxic changes in hepatocytes often leading to liver insufficiency and death caused by hypovolemic shock. Since the reports on MC toxicity are on the increase this cyanotoxin should be treated as an important environmental factor affecting human and animal health. A brief overview of existing literature on the intake, mechanism of action, and hepatotoxic effects on mammalian animals is presented in this paper.

Keywords: microcystin, cyanotoxin

Cyanobacteria (also known as blue-green algae) are a group of prokaryotic organisms living in freshwater and marine environment (5). These gram-negative microorganisms have the ability to form massive blooms in eutrophic surface water. Many species of cyanobacteria produce variety of toxic metabolites, harmful if consumed with contaminated water. Some cases of acute intoxication induced by cyanobacterial toxic products have been reported in humans and animals (6). Some reports on poisoning of cattle, dogs and birds and wildlife animals are present in the literature (8). It is believed that drinking or direct contact with water contaminated with cyanotoxins can induce serious diseases such as hepatoenteritis, gastroenteritis, dermatitis, allergic diseases and symptomatic pneumonia (2, 28). Cyanobacterial toxins are classified into five groups according to their biological effect: hepatotoxins, neurotoxins, cytotoxins, dermatotoxins and irritant toxins. Microcystin (MC) is most widespread and well characterised hepatotoxin produced by various genera of cyanobacteria: *Microcystis*, *Anabaena*, *Planktothrix*, *Hapalosiphon*, *Anabenopsis* and *Nostoc* (5). Although a lot of data concerning the occurrence and influence of this toxic metabolite exist in the literature, consequences to animal health are still not entirely known. The purpose of this review is to present the current state of knowledge about influence of microcystin on the liver and isolated hepatocytes and related cell lines of mammalian animals.

Microcystin is most diversed and very common toxin of over 60 structural types (the well known types are: MC-LR, MC-RR, MC-YR). All variants are cyclic heptapeptides consisting of seven amino acids, including a few D-amino acids and two unusual amino acids such as methyledehydroalanine (MDHA) and hydrophobic b-amino acid, 3-amino-9methoxy-2-6,8--trimethyl-10-phenyldeca-4,6-dienoic acid (ADDA) (3). It is released to the aquatic environment by cyanobacteria cell lysis (19) and depending on the administration route has different bioavailability and toxicity in vivo. The LD_{50} of purified MC-LR is 50 μg kg^{-1} after intraperitoneal injection and 10, 9 mg/kg after oral administration to mouse (29). Toxicity of microcystin is age-dependent and associated with physiological condition of the small intestine, including level of permeability of the capillaries in the villi and degree of the surface epithelial cell exfoliation (18). Some part of the toxin is absorbed via the stomach but most molecules are carried by via bile acid transporters of the intestinal cells of the ileum (14).

MC passes the intestinal barrier but its majority remains in the intestinal tract and is excreted in faeces. The toxin enters the liver by the portal vein via a carrier mediated transport system due to its specific

hydrophobic and pH-dependent properties (11). The uptake to hepatocytes is temperature-dependent. It is estimated that about 50-70% of MC accumulate in the liver after intraperitoneal, intravenous or intra-tracheal administration. Some amounts of the cyanotoxin can be also detected in the intestine, but not in other organs (26).

Microcystin induces suppression of serine/threonine protein phosphatase 1 (PP1) and 2A (PP2A) activities in the liver cells (23). Protein phosphatases are enzymes that remove the phosphate groups from amino acids in proteins and together with protein kinases – enzymes adding the phosphate groups to amino acids, they regulate cell metabolism (25). Microcystin-induced toxic changes in hepatocytes are associated with a lack of balance between phosphatases and kinases.

Microcystin-induced imbalance of protein phosphorylation starts the disruption of hepatocyte cytoskeleton giving a typical shape to the cells. The cytoskeletal components of the liver cells are polimeric intermediate filaments and microfilaments which lose their subunits and dissociate when exposed to the cyanotoxin. The cytoskeleton shrinks and the hepatocytes break contact with other liver cells and sinusoidal capillaries (7). These changes lead to the hepatic haemorrhage, liver insufficiency and finally death of an organism because of hypovolemic shock (29). Microcystin-LR was described to promote the collapse of actin filaments in primary rat hepatocytes leading to rounding, blebbing (cytoskeletal disruption), loss of microvilli and separation of cultured hepatocytes. The cells lose adhesion to each other and begin to detach from the surface (13). Toxic effects of MC-LR were noted in the liver of rabbit. Frangez et al. (16) found that subchronic exposure with cyanobacterial lyophilizate containing MC-LR and MC-RR induced liver injury characterized by fatty infiltration and periportal fibrosis.

Microcystin changes in the activity of hepatic main enzymes. Gupta et al. (17) noted a distinct augmentation of the alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma-glutamyl transpeptidase (gamma-GT) at mean time of death (MTD) 30 min. after intraperitoneal administration of microcystin MC-LR, MC-RR and MC-YR to mice. Augmented hepatic enzyme levels was caused of hepatocyte damage induced by cyanotoxins. Moreover, increased liver body weight index was observed as a consequence of blood pooling. MC-LR evoked more significant increase of AST level in comparison to MC-RR and MC-YR. On the other hand long time of exposure even at low concentrations can induce inhibition of ALT levels. Solter et al. (27) found that subchronic exposure (28 days) to microcystin-LR after intraperitoneal injection diminished ALT synthesis in rat hepatocytes in a dose-dependent manner. A significant inhibition of glucosidase (beta-D glucuronidase and N-acetyl-glucosaminidase) activity and synthesis of proteolytic enzymes (cathepsins D and L, arginine aminopeptidase, dipeptidase II, dipeptidase IV) was observed after exposure of mouse hepatocytes to microcystin-YR (20, 21).

Protein phosphatases play an important role as enzymes in protein phosphorylation controlling glycogen and glucose metabolism. Microcystin, as a potent PP1 and PP2A inhibitor was described to affect these biochemical processes. MC-LR, by suppressory action towards protein phosphatases, increases activity of glucose-6-phosphatase (enzyme essential for release of glucose into the bloodstream from glycogen breakdown) in rat hepatocytes *in vitro* (10).

Microcystin-induced changes can be observed in hepatic glutathione (GSH) activity. Treatment of primary rat hepatocytes with microcystin cyanobacterial extract and pure MC-LR leads to a dose-dependent increase of intracellular GSH level. Furthermore, inhibitors of GSH synthesis cause depletion of its level and, as a consequence, increase hepatocyte susceptibility to MCE (4). On the other hand, the *in vivo* studies performed by Gupta et al. (17) revealed that GSH level was significantly reduced in mice administered intraperitoneally with MC-LR and MC-RR.

Microcystin-LR is an effective inducer of rapid hepatocyte apoptosis in mammals. Isolated rat liver cells showed apoptotic changes within 2 minutes after exposure to MC-LR at 16 μmol/l (15). McDermott et al. (24) observed that hepatocytes exposed to microcystin MC-LR appeared slightly shrunken with small cytoplasmic protrusions and blebs. Higher concentrations of the toxin induced cell swelling and dissolution of organelles. DNA fragmentation and mitochondrial changes were associated with chromatin condensation. MC-LR induced hepatocyte apoptosis could be mediated by caspase (15). It is also speculated that apoptosis of hepatocytes is associated with microcystin-induced radical oxygen species (ROS) formation (12). Bouaicha et al. (4) observed that MC-LR significantly increased the ROS level in rat hepatocytes at 10 ng/ml. ROS production induced by the toxin could be also one of the major mechanisms of its genotoxic action and tumor initiation in the liver (30). Microcystin-induced ROS cause damage at the DNA level accompanied by protein phosphatases inhibition eventually promoting proliferation of neoplastic hepatocyte. A time- and dose-dependent formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG)- a marker of DNA oxidative damage, was observed in primary rat hepatocytes *in vitro* after exposure to MC-LR. Induction of 8-oxodG was also demonstrated in rat liver *in vivo* after intraperitoneal administration of the toxin (22). Ito et al. (18) noted liver neoplastic nodules up to 5 mm in diameter 28 weeks after intraperitoneal injection of microcystin-LR to mice. Neoplastic nodules were formed without using an initiator after a series of injections of the toxin at sublethal doses. On the other hand no nodule formation

or chronic injuries were observed in the liver when the toxin was administered orally at a higher dose. This finding suggests that the route of exposure plays an important role in tumour initiation. The exposure of hepatocytes to MC-LR evokes an altered morphology of their nuclei being of irregular shape and condensed (18).

Recent studies on the influence of microcystins have been performed using various hepatic cell lines. MC--LR toxicity was determined in vitro on permanent hepatic cell line-rat Reuber H35 hepatoma cells (H-4--II-E) (9). H-4-II-E cells were sensitive, particularly to higher concentration of MC-LR. The toxin also induced toxic changes in immortalized hepatocytes derived from male Wistar rats (1).

This review presented the existing information on hepatotoxic effects of microcystin-LR. The results obtained by many authors show that microcystin is a highly toxic agent and also a potent tumor inducer that could be dangerous to humans, domestic pets and could create loses in livestock when drinking contaminated surface water. High sensitivity of mammalian liver to this cyanotoxin and possible health hazard suggest that a strict monitoring of water reservoirs used for farming practice and recreational purposes should be introduced.

References

1. *Battle T., Touchard C., Moulsdale H. J., Dowsett B., Stacey G. N.*: New cell substrates for in vitro evaluation of microcystin hepato-cytotoxicity. Toxicol. in vitro 1997, 11, 557-567.
2. *Bell S. G., Codd G. A.*: Cyanobacterial toxins and human health. Rev. Med. Microbiol. 1994, 5, 256-264.
3. *Botes D. P., Wessels P. L., Kruger H., Runnegar M. T. C., Sanitkarn S., Smith R. J., Barna J. C. J., Williams D. H.*: Structural studies on cyanoginosins-LR, YR, YA and YM, peptide toxins from Microcystis aeruginosa. J. Chem. Soc., Perkin Trans. 1985, 1, 2311.
4. *Bouaicha N., Maatouk I.*: Microcystin-LR and nodularin induce intracellular glutathione alteration, reactive oxygen species production and lipid peroxidation in primary cultured rat hepatocytes. Toxicol. Lett. 2004, 148, 53-63.
5. *Carmichael W. W.*: Cyanobacteria secondary metabolites – The cyanotoxins. J. Appl. Bacteriol. 1992, 72, 445-459.
6. *Carmichael W. W.*: Diseases related to freshwater blue-green algal toxins and control measures, [in:] Falconer I. (Ed.): Algal Toxins in Seafood and Drinking Water. Academic Press, London 1993, 187-209.
7. *Carmichael W. W.*: The toxins of cyanobacteria. Sci. Am. 1994, 270, 64-70.
8. *Carmichael W. W. Schwartz L. D.*: Preventing livestock deaths from blue--green algae poisoning. Farm. Bull. 2275, US Dept. of Agriculture; Washington, DC, USA 1984.
9. *Chong M. W. K., Gu K. D., Lam P. K. S, Yang M., Fong W. F.*: Study on the toxicity of microcystin-LR on cultured cells. Chemosphere 2000, 41, 143-147.
10. *Claeyssens S., Chedeville A., Lavoinne A.*: Inhibition of protein phosphatases activates glucose-6-phosphatase in isolated rat hepatocytes. FEBS Lett. 1993, 315, 7-10.
11. *De Maagd P. G. J., Hendriks A. J., Seinen W., Sijm D. T. H. M.*: pH-dependent hydrophobicity of the cyanobacteria toxin microcystin-LR. Water Res. 1999, 33, 677-680.
12. *Ding W. X., Shen H. M., Ong C. N.*: Critical role of reactive oxygen species and mitochondrial permeability transition in microcystin-induced rapid apoptosis in rat hepatocytes. Hepatology 2000, 32, 547-555.
13. *Eriksson J. E., Toivola D., Meriluoto J. A. O., Karaki H., Han Y., Hartshorne D.*: Hepatocyte deformation induced by cyanobacterial toxins reflects inhibition of protein phosphatases. Biochem. Biophys. Res. Commun. 1990, 173, 1347-1353.
14. *Falconer I. R., Dornbusch M., Moran G., Yelung S. K.*: Effect of cyanobacterial (blue-green algal) toxins from Microcystis aeruginosa on isolated enterocytes from chicken small intestine. Toxicon 1992, 30, 790-793.
15. *Fladmark K. E., Brustugun O. T., Hovland R., Boe R., Gjertsen B. T., Zhivotovsky B., Doskeland S. O.*: Ultrarapid caspase-3 dependent apoptosis induction by serine/threonine phosphatase inhibitors. Cell Death Differ. 1999, 6, 1099-1108.
16. *Frangez R., Kosec M., Sedmak B., Beravs K., Demsar F., Juntes P., Pogacnik M., Suput D.*: Subchronic liver injuries caused by microcystins. Pflugers Arch. – Eur. J. Physiol. 2000, 440 (Suppl), R103-R104.
17. *Gupta N., Pant S. C., Vijayaraghavan R., Lakshmana R. P. V.*: Comparative toxicity evaluation of cyanobacterial cyclic peptide toxin microcystin variants (LR, RR, YR) in mice. Toxicology 2003, 188, 285-296.
18. *Ito E., Kondo F., Terao K., Harada K. I.*: Neoplastic nodular formation in mouse liver induced by repeated intraperitoneal injections of microcystin--LR, Toxicon 1997, 35, 1453-1457.
19. *Lahti K., Rapala J., Färdig M., Niemelä M., Sivonen K.*: Persistence of cyanobacterial hepatotoxin, microcystin-LR in particulate material and dissolved in Lake water, Water Res. 1997, 31, 1005-1012.
20. *Lankoff A., Kołątaj A.*: Influence of microcystine-YR and nodularin on the activity of some glucosidases in mouse liver. Toxicology 2000, 146, 177-185.
21. *Lankoff A., Kołątaj A.*: Influence of microcystin and nodularin on the activity of some proteolytic enzymes in mouse liver. Toxicon 2001, 39, 419-423.
22. *Maatouk I., Bouaicha N., Plessis M. J., Perin F.*: Detection by 32P-postlabelling of 8-oxo-7,8-dihydro-2-deoxyguanosine in DNA as biomarker of microcystin-LR- and nodularin-induced DNA damage in vitro in primary cultured rat hepatocytes and in vivo in rat liver. Mut. Res. 2004, 564, 9-20.
23. *MacKintosh C., Beattie K. A., Klumpp S., Cohen P., Codd G. A.*: Cyanobacterial microcystin-LR is a potent and specific inhibitor of protein phosphatases 1 and 2A from both mammals and higher plants. FEBS Lett. 1990, 264, 187-192.
24. *McDermott C. M., Nho W. C., Howard W., Holton B.*: The cyanobacterial toxin, microcystin-LR, can induce apoptosis in a variety of cell types. Toxicon 1998, 36, 1981-1996.
25. *Millward T. A., Zolnierowicz S., Hemmings B. A.*: Regulation of protein kinase cascades by protein phosphatase 2A. TIBS 1999, 24, 186-191.
26. *Robinson N. A., Pace J. G., Matson C. F., Miura G. A., Lawrence W. B.*: Tissue distribution, excretion and hepatic biotransformation of microcystin--LR in mice. J. Pharmacol. Exp. Therap. 1991, 256, 176-182.
27. *Solter P., Liu Z., Guzman R.*: Decreased hepatic ALT synthesis is an outcome of subchronic microcystin-LR toxicity. Toxicol. Appl. Pharmacol. 2000, 164, 216-220.
28. *Turner P. C., Gammie A. J., Hollinrake K., Codd G. A.*: Pneumonia associated with contact with cyanobacteria. Brit. Med. J. 1990, 300, 1440-1441.
29. *Yoshida T., Makita Y., Nagata S., Tsusumi T., Yoshida F., Sekijima M., Tamura S., Ueno Y.*: Acute oral toxicity of microcystin-LR, a potent cyanobacterial hepatotoxin in mice. Nat. Toxins 1997, 5, 91-95.
30. *Žegura B., Lah T. T., Filipic M.*: The role of reactive oxygen species in microcystin-LR-induced DNA damage. Toxicology 2004, 200, 59-68.

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