Review paper

The immunotoxic and nephrotoxic influence of cyanotoxins to vertebrates

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Abstract

Cyanotoxins, various toxins produced by cyanobacteria, have different biological properties and organ specificity. They are classified as hepatotoxins, neurotoxins, cytotoxins and toxins referred as dermatotoxins. Microcystins (MCs) and nodularin are hepatotoxins, but some authors proved their nephrotoxic and immunotoxic influence. The purpose of that work was to present the results of our studies and the state of the current knowledge about potential nephrotoxic and immunotoxic influence of cyanotoxins on vertebrate animals.

Key words: *cyanotoxins, immunotoxicity, nephrotoxicity.*

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Introduction

Toxic algae blooms are observed in many water bodies in Poland (e.g. Dobczycki Lake, Goczałkowicki Reservoir, Sulejówek Lake, Zemborzycki Reservoir, Baltic Sea) and constitute the worldwide and multifaceted research problem, having essential health implications for people and animals [1-3]. The cases of acute and chronic poisoning of aquatic organisms, including fish, farmed and domestic animals e.g. dogs, horses, cattle, birds living in the wild were reported. There are also data on intoxication of people being in contact with recreational or drinking water contaminated with cyanotoxins [4]. These substances have a various chemical structure (peptides, alkaloids) and the multidirectional mechanism of toxic action and are grouped into hepatotoxins, neurotoxins, dermatotoxin, cytotoxins, and toxins triggering other effects [5].

Long-term exposure to subclinical doses, which does not induce visible symptoms of poisoning can cause changes on the cellular level and can induce systemic dysfunctions. It is worthwhile noticing that sometimes organ changes are correlated with the decrease of the resistance to pathogens as the result of dysfunction of sensitive mechanisms of immunohomeostasis.

Nephrotoxic effects of cyanotoxins

Kidneys are the organ particularly exposed to the toxic action of xenobiotics. It is a consequence of its excretory functions and intensive blood flow through that tissue. The kidney is a complex organ where toxic changes can be observed, because many nephrotoxicants including drugs, chemicals and natural toxins and their metabolites are filtrated. The proximal tubular cells of the kidney very often can concentrate many nephrotoxins and hence are prone to the harmful effects of toxins.

There is a lot of data in the literature showing the influence of microcystins on the renal system of vertebrates (Table 1). Nephrotoxic effects of chronic administration relatively low doses (10 μg/kg i.p.) of microcystin-LR (MC–LR) and microcystin-YR (MC–YR) were studied by Milutinović et al. [20, 21]. Authors described many pathological changes in the kidneys of rats treated with MCs for 8 months. Degenerative changes in the kidneys were observed such as collapsed tufts of glomerular capillaries, the enlarged diameter of renal corpuscles, the widened Bowman's space and proximal and distal convoluted tubules, thickened Bowman's capsule in some renal corpuscles. Moreover, interstitial tissue was occasionally infiltrated by lymphocytes and appeared oedematous. Authors noted that the kidneys were far more affected than the liver. The cytoskeleton abnormalities and the DNA damage suggested, that the mechanisms underlying the chronic nephrotoxicity are similar at the cellular level to the mechanisms of the acute hepatoxicity of microcystins.

In the series of experiments performed by Nobre et al. [15, 18, 22, 23] it was shown that microcystin-LR can affect

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Table 1. Effects of cyanotoxins on renal morphology and physology

Species, type of cells	Cyanotoxins	Doses	Reported effects	Reference
rat, mouse	MC-LR	rat *i.p.* 160 μg/kg b.w., mice 100 μg/kg b.w.	increased weight of kidneys, moderate vacuolation of proximal tubular epithelium with mild tubular dilatation	Hooser et al. [6]
common carp	MC-LR	*i.p.* 250-300 μg/kg b.w.	degenerative changes in the tubules and glomeruli and interstitial tissue in kidneys	Rabergh et al. [7]
rat renal epithelial cells (ATCC, CRL 1571)	pure MC-LR	*in vitro* 1, 100, 200 μM	affected the structural organisation of microtubule, intermediate filaments, actin filaments	Wickstrom et al. [8]
rat renal epithelial cells (ATCC, CRL 1571)	pure MC-LR	*in vitro* 10, 100, 200 μM	plasma membrane blebbing, loss of cell-to-cell contact, clumping and rounding of cells, cytoplasmic vacuolization, redistribution of cytoplasmic organelles, nuclear changes characteristic for apoptosis	Khan et al. [9]
rat, kidney cells	pure MC-LR	*in vitro* 133 μM	changes in microtubules and microfilaments	Khan et al. [10]
rainbow trout	cyanobacterial extract with MC-LR	*i.p.* 1000 μg/kg b.w.	renal lesions coagulative tubular necrosis and dilation in Bowman's space	Kotak et al. [11]
rat	*Microcystis aeruginosa* PCC 7806 lyophilized cell extract	*i.p.* 32.7, 65.4, 130.8 mg/kg b.w.	elevated plasma urea and creatinine levels followed by hematuria, proteinuria and bilirubinuria, decreased activity of kidney lactate dehydrogenase and glutamic oxaloacetic transaminase, a dose- and time-dependent elevation in plasma urea and creatinine levels with a concomitant decrease in total protein and albumin levels	Bhattacharya et al. [12]
hamster kidney cells (BHK-21)	cell-free extract from *Microcystis aeruginosa* and purified MC-LR	*in vitro* 25, 50, 100, 150 μg/ml	DNA damage	Rao et al. [13]
silver carp	pure MC-LR	*i.p.* 250 μg/kg b.w.	dystrophic and necrobiotic alterations of kidney tubuli	Vajcova et al. [14]
perfused rat kidney model	MC-LR	1 μM	change of the kidney functional parameters: perfusion pressure, renal vascular resistance, glomerular filtration rate, urinary flow	Norbe et al. [15]
rainbow trout	MC-LR from *Microcystis aeruginosa* (PCC 7806)	*per os* an equivalent of MC-LR 400 μg/kg b.w.	damage of renal proximal tubular: increased vacuolation of individual tubular epithelial cells, apoptosis, cell shedding	Fischer and Dietrich [16]
swine	MC-LR from *Microcystis aeruginosa*	*i.v.* 25, 72 μg/kg b.w.	decrease of renal perfusion	Beasley et al. [17]
perfused rat kidney model	MC-LR	1 μM	impaired renal function, probably causing vascular and glomerular lesions	Norbe et al. [18]
monkey kidney cells	pure Antx-a and extracts from *Anabena flosaqae*	1-10 μg/ml	condensed chromation, nuclear fragmentation, formation of apoptotic bodies	Rao et al. [19]
rat	MC-LR and MC-YR from *Microcystis aeruginosa*	*i.p.* 10 μg/kg b.w. MC-LR or 10 μg/kg b.w. MC-YR	histopathological changes, collapsed glomeruli with thickened basal membranes, enlarged tubules with eosinophilic deposits and accumulations of cytoplasm and actine filaments in epithelial tubule cells	Milutinovic et al. [20, 21]

Table 1. Effects of cyanotoxins on renal morphology and physology – continue

Species, type of cells	Cyanotoxins	Doses	Reported effects	Reference
perfused rat kidney model	MC-LR	1 µg/ml	affected renal physiology by altering vascular, glomerular and urinary parameters, provoke intestinal secretion of water and electrolytes (sodium, potassium, chloride)	Nobre et al. [22, 23]
tilapia fish	MC-LR from lyophilized cyanobacterial cells	*per os* lyophilized cyanobacterial cells 60.0 µg MC-LR/fish/day	increased enzymatic activities of acid and alkaline phosphatases, kidney lesions, dilation of Bowman's space and necrotic epithelial cells with pyknotic nuclei in the tubules	Molina et al. [24]
tilapia fish	MC-LR from natural blooms of *Microcystis aeruginosa*	*per os* 60.0 µg MC-LR/fish/day	increased lipid peroxidation (LPO), catalase (CAT) and glutathione peroxidase (GPx) activity, enhanced production of glutathione disulfide (GSSG)	Jos et al. [25]
rat	pure MC-LR	*i.p.* 100, 150 µg/kg b.w.	decreased activity of antioxidant enzymes, increased lipid peroxidation	Moreno et al. [26]
tilapia fish	pure MC-LR, MC-RR	*i.p.* 500 µg/kg b.w. MC-LR or MC-RR	increased enzymatic activities of superoxide dismutase (SOD), catalase, glutathione peroxidase, level of lipid peroxidation	Prieto et al. [27]
mouse	pure MC-LR	*i.p.* 38.3, 76.62 µg/kg b.w.	no changes phosphatase activity, a lack of MC-LR protein phosphatase adduct	Jayara and Rao [28]
mouse	crude cyanobacterial extract	*per os* drinking water with 1.77, 2.32, 6.12 µg/l MC-LR	no changes in the kidney histology	Carvalho et al. [29]
silver carp	toxic *Microcystis* blooms with MCs	natural food uptake in lakes	dilation of Bowman's capsule, increased and proliferation of lysosomes, increased of the enzymatic activities of superoxide dismutase and catalase	Li et al. [30]
rat	MC-LR	from 5 to 50 nM/mg protein	decreased mitochondrial membrane potential, induce and inhibition of ATPase and ATP synthase activities	La-Salete et al. [31]
mouse	pure MC-LR	*i.p.* 10, 25, 40, 50 µg MC-LR/kg b.w. *per os* 2 and 4 mg/kg b.w.	DNA damage	Gaudin et al. [32]
mouse	MC-LR from natural blooms of *Microcystis aeruginosa*	*i.p.* 25 µg/kg b.w.	a lack of histopathological alterations in kidney, increased in lipid peroxidation level	Andrinolo et al. [33]
tilapia fish	MCs from a *Microcystis* water bloom	*per os* 120 mg/kg b.w. MC-LR	increased lipid peroxidation level, the enzymatic activities of superoxide dismutase decrease catalase activity, degenerative changes in the kidney glomerular atrophy, glomerulopathy of mesangial cells, intraglomerular hyalinization, and loss of capillary vessels, a thickening of the basal membranes, collapsed fenestrated capillaries	Atencio et al. [34]
monkey kidney cell line, Vero-E6	MC-LR from *Microcystis aeruginosa* extracts and pure MC-LR	*in vitro* from 1.4 to 175 µM	decreased cell viability	Dias et al. [35]

Table 2. Effects of cyanotoxins on immune cells and organs

Species, type of cells, organs	Cyanotoxins	Doses	Reported effects	Reference
common carp, silver carp, blood	pure MC-LR, biomass of blue-green algae with MC-LR	*i.p.* 400 µg/kg b.w. *per os* 3, 300, 600, 1200 µg/kg b.w.	decrease of total leucocyte count, leukocrit, phagocytic activity	Palikova et al. [37]
human, PMNs leucocytes	MC-LR nodularin	from 0.01 to 1 nM	modulation of the early and late adherence of PMNs	Hernandez et al. [38]
rabbit, macrophages	MC-LR	*in vitro* 0.1, 0.3, 1 µg/ml	stimulation of the synthesis of TNF-α and IL-1β	Rocha et al. [39]
mouse, spleen and thymus	nodularin	*in vitro* from 0.01 to 50 µM	inhibition of the lymphoproliferative response to ConA	Yea et al. [40]
human, blood	extract of cyanobacterial bloom containing MC-LR	*in vitro* 250, 500, 750, 1000 nM	modulation of the morphological changes characteristic for apoptosis	Mankiewicz et al. [41]
mouse, spleen and thymus	MC-LR, MC-YR, nodularin	*in vitro* 0.1, 1, 10, 50 µM *i.p.* 1, 10, 50 µg/kg b.w.	inhibition of the polyclonal antibody response and lymphoproliferation to ConA and LPS, decrease of IL-2 mRNA stability, T-cell dependent AFC responses	Yea et al. [42]
rat, thymus	extracts from *Anabena flosaquae*, pure Antx-a	*in vitro* 10-50 µg/ml 1-10 µg/ml	cytotoxicity and apoptosis in thymocytes, generation of ROS	Rao et al. [19]
mouse, spleen, thymus	bloom extract with MCs	*i.p.* 4.97, 9.94, 19.88 µ/kg b.w.	inhibition of the phagocytosis, lymphoproliferative response to LPS but not ConA	Shen et al. [43]
human, blood lymphocyte	pure MC-LR	*in vitro* 1, 10, 25 µg/ml	DNA damage in lymphocytes	Lankoff et al. [44]
human and chicken blood lymphocyte	pure MC-LR	*in vitro* 1, 10, 25 µg/ml	decreased proliferation of lymphocytes, modulation of the production of IL2 and IL-4, increased apoptosis and necrosis of the cells	Lankoff et al. [45]
murray cod, head kidney	pure MC-LR	*in vitro* 0.05, 0.5, 5 µg/ml	no action on the granulocyte or lymphocyte number and the lymphoproliferation index, enhancement of phagocytosis	Wright et al. [46]
common carp, blood	crude	*in vivo* 1, 3 and 13 µg/l	decreased leukocyte and phagocytic activity	Palikova et al. [47]
mouse, peritoneal macrophages	pure MC-LR	*in vitro* 1, 10, 100, 1000 nmol/l	inhibition of iNOS mRNA level down regulated the proinflammatory cytokines IL-1β and TNF-α, decrease of GM-CSF and INF-γ mRNA levels	Chen et al. [48]
mouse, spleen	purified extract from of cyanobacteria	*i.p.* 7, 14, 24, 36 µg/kg bw	decrease of TNF-α, IL-1β, IL-2 and IL-4 levels	Shi et al. [49]
mouse, spleen	pure MC-LR, pure Antx-a	*in vitro* 7.5 µg/ml 0.1 µg/ml	decrease of cell viability of splenocytes, apoptosis of B cells, AnTx-a affect B and T lymphocytes	Teneva et al. [50]
mouse, peritoneal macrophages	pure MC-LR	*in vitro* 1, 10, 100, 1000 nmol/l	decreased expression of IL1-β, TNF-α, GM-CSF, and IFN-γ	Chen et al. [51]
human, blood	pure MC-LR	*in vitro* 10 µg/l	increase of PMN apoptosis, mild changes in ROS production and phagocytosis, not affected cytokine production	Goncalves et al. [52]
human, blood	MC-LR, [Asp3]-MC-LR	*in vitro* 0.1, 1, 10, 10, 1000 nM	enhancement of neutrophil migration, increased phagocytosis	Kujbida et al. [53]

Table 2. Effects of cyanotoxins on immune cells and organs – continue

Species, type of cells, organs	Cyanotoxins	Doses	Reported effects	Reference
crucian carp, kidney, spleen	pure MC-LR, MC-RR	*in vitro* 1, 5,10, nM	apoptosis in lymphocytes	Zhang et al. [54]
rainbow trout, blood, pronephros, spleen	pure MC-LR	*in vitro* 1, 5, 10, 20, 40 µg/ml	decreased number of lymphocytes, modulation the proliferation of lymphocytes	Rymuszka et al. [55]
rainbow trout, blood, pronephros, spleen	pure MC-LR	*in vitro* 1, 5,10, 20, µg/ml	decrease of viability of phagocytic cells, modulation of RBA and phagocytic activity	Sieroslawska et al. [56]
human, red blood	pure MC-LR, MC-LA, MC-YR	*in vitro* 1 and 1000 nM	increased IL-8, CINC-2αβ and extracellular ROS levels	Kujbida et al. [57]
grass carp, pronephros, spleen	pure MC-LR	*i.p.* 50 µg/kg b.w.	apoptosis in spleen leucocytes	Wei et al. [58]

renal physiology. By using perfused rat kidney model authors showed an intense amount of proteinaceous material in urinary spaces following perfusion with MC-LR. Further studies confirmed that microcystin-LR promoted renal changes, such as altered vascular, glomerular and urinary parameters. MC-LR induced activation of phospholipase A_2 (PLA_2) and cyclooxygenase and this mechanism was similar to the mechanisms inducing hepatotoxic changes. Moreover, it was demonstrated that microcystin-LR stimulated macrophages to release the inflammatory mediators capable of promoting nephrotoxicity in the isolated perfused rat kidney. Authors examined the supernatant of macrophages stimulated *in vivo* by microcystin–LR and showed the presence of proinflammatory agents capable of provoking secretion of water and electrolytes (sodium, potassium and chloride). The observed collapsed filaments and other morphological changes support thesis that MCs could trigger apoptotic processes in the exposed kidney cells. That confirms the hypothesis that *in vivo* MCs induce cytoskeletal alterations and nuclear changes in different cells typical for appoptosis and/or necrosis [6, 13, 14, 26, 31, 35].

Rao et al. [19] suggests, that the observed cytotoxic effects leading to apoptosis were induced by generation of reactive oxygen species and caspase activation of cyanobacterial neurotoxins-anatoxin a in non-neuronal cells [19]. The results of this study showed that anatoxin-containing cell-free extracts from *Anabena flos aquae* and purified anatoxin-a induced concentration dependent cytotoxicity and apoptosis in African green monkey kidney cells (Vero). The authors observed morphological changes typical for apoptosis as plasma membrane blebbing, cell shrinkage, condensed chromatin, nuclear fragmentation and formation of DNA-containing apoptotic bodies. Several comparative studies have shown that microcystins develop the same cytotoxic response [8-10].

It was revealed by the immunostaining that the injected conjugates can accumulate in the kidneys [36]. It might be thus speculated that in the conditions of chronic exposure to MC-LR accumulation of its metabolites in the kidneys and changes in their physiology may occure. Kotak et al. [11] indicated that kidney tubular epithelial cells in fish were affected after acute exposure by interperitoneal injection of MC-LR 400 µg/kg and 1000 µg/kg. Similarly, Radbergh et al. [7] have shown degenerative changes in the tubular epithelial cells, glomeruli and interstitial tissue in kidneys of carp intraperitoneally exposed to MC-LR with the LD_{50} ranging from 80 to between 300 and 550 µg/kg. The studies also indicated that fish can tolerate higher doses of the toxin and have a longer survival period compared to mice. Probably the uptake of microcystin to the kidney may be dependent on body temperature.

Fisher and Dietrich [16] found microcystin-induced alterations in kidney tissues of carp when *Microcystis aeruginosa* (PCC 7806) amounting to an equivalent of 400 µg MC-LR/kg bw were directly administered to the fish stomach. In the kidney degenerative changes were observed in the renal proximal tubules, a segment known

for its high capacity of active protein and peptide reabsorption. Moreover, the studies on the mechanism of cell toxicity showed that cyanotoxins induce oxidative stress in tissues of vertebrates and the potential alterations of the antioxidant status [25, 27, 30, 34].

Immunotoxic effects of cyanotoxins

Many substances present in the aquatic environment at relatively low concentrations demonstrate toxic action on the immune cells and organs of fish and higher vertebrates. Immune system, together with other systems e.g. nervous and endocrine, takes part in regulating homeostasis, so cyanotoxins, which have multidirectional nature of the action can also induce directly or indirectly dysfunctions of the immune system. The immunotoxic effects of cyanotoxins are summarised in Table 2. Immunosuppression was also confirmed in our study, which determined the influence of microcystin-LR and anatoxin-a to the basic functions of the fish immune cells [56, 57, 60].

The obtained findings showed the inhibition of the viability of lymphocytes and phagocytes isolated from rainbow trout by the toxin in the time and concentration dependent manner. Microcystin-LR suppressed the examined functions of immune cells (metabolic activity of phagocytes, proliferative response of lymphocytes) more distinctively compared to anatoxin-a (in press). Moreover, we noted that phagocytes are more sensitive to microcystin-LR than lymphocytes. It is interesting, that extracts containing the cyanotoxins are more immunotoxic, than the pure form toxins (unpublished).

Moreover, other authors describe the decrease of the total number of white blood cells, including T lymphocytes (particularly cytotoxic T cell), B cells, mielocytes and the lowered value of the phagocytic index after the exposure to microcystins [37, 47, 59].

The mechanism of toxic action of microcystins is the blockade of the activity of protein phosphatases serine (PP1) and threonine (PP2) what leads to hyperphosphorylation of plasmic and cellular cytoskeleton proteins. The disorders of the intracellular homeostasis can result in the uncontrolled proliferation and as a consequence induce carcinogenesis [19, 41, 44, 50, 52, 54, 55, 59, 61].

The activation of the phagocytic cells is connected with a sequence of changes in their cytoskeleton. Our studies showed that phagocytes may be the target cells for toxic effects of the microcystin-LR in the fish immune system [57, 60]. The toxin at the concentrations environmentally relevant caused the increased production of reactive forms of oxygen in phagocytes and modulation of the phagocytosis. The disorders of the metabolic activity of these cells can result from the direct effects of the toxin on their cytoskeleton which influence the activation and process of phagocytosis.

Our studies indicated that microcystin-LR is more suppressive to B lymphocytes, than to T cells [56]. Differences in the response of the two lymphocyte populations to the cyanotoxin probably result from the action of cytokines – essential regulatory proteins of the immune system, as well as nervous and endocrine systems. This hypothesis is confirmed by studies carried out on mammalian immune cells. These studies showed that microcystin-LR influenced the production of cytokines: interleukin-2 (IL-2) and interleukin-6 (IL-6) responsible for lymphocyte functioning [45]. Moreover, the influence of this toxin on the expression of cytokines such as: IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, as well as on the nitric oxide synthase activity in phagocytes was observed [39, 42, 48, 49, 51, 52, 58].

In summary, our research and the observations of other authors suggested that cyanotoxins may induce nephrotoxic and immunotoxic effects and changes in physiology of vertebrates.

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