Cyanohepatotoxins influence on the neuroendocrine and immune systems in fish – a short review

Anna Sierosławska, Anna Rymuszka
The John Paul II Catholic University of Lublin, Department of Physiology and Ecotoxicology, Poland

Correspondence to: Anna Sierosławska PhD, The John Paul II Catholic University of Lublin, Department of Physiology and Ecotoxicology, ul. Norwida 4, 20-061, Lublin, Poland.
PHONE/FAX: +480-815 367 089, e-mail: ansie@kul.lublin.pl

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Abstract

Cyanotoxins are the metabolites of cyanobacteria, belonging to different chemical groups and of diverse mechanisms of toxicity. Generally, they are divided into hepatotoxins, neurotoxins and dermatotoxins/irritant toxins. There is a growing evidence, that besides the above mentioned toxicity, exposure to cyanotoxins may also induce other effects, among others the disruption of neuroendocrine and immune systems.

The purpose of that paper is to sum up the current information obtained from the literature and from our own studies about the influence of cyanohepatotoxins on neuroendocrine and immune systems of fish. From the presented data it appears, that microcystins, nodularin and cylindrospermopsin, except for their hepatotoxic activity, are potent to exert such effects as HPI axis activation resulting in physiological and behavioural changes, disturbances in thyroid hormones release/metabolism, as well as impairment of immune responses in fish. However the studies in that area are still incomplete and many questions remain to be answered, especially what consequences for fish population health status it brings.

Article outline:
1. Microcystins and nodularin; 2. Cylindrospermopsin

REVIEW

Introduction

Cyanotoxins are the metabolites of cyanobacteria, belonging to different chemical groups and of diverse mechanisms of toxicity. Generally, according to the target organ or system, they are divided into hepatotoxins, neurotoxins and dermatotoxins/irritant toxins (Briand *et al.* 2003). That division does not include many other toxic effects triggered by the cyanobacteria products. There is growing evidence, that prolonged exposure to cyanotoxins may entail cellular and molecular damage promoting tumours and cancers. It may also evoke reproductive dysfunctions and teratogenic effects or induce the disruption of endocrine and immune system (Hudnell, 2008). The latter ones are often difficult to be detected as the observed effects may be indirect, and neurological or endocrinal disorders may evoke immunological disturbances and inversely.

Although a lot of data concerning the toxicity of cyanotoxins exist in the literature, consequences of multidirectional impact of the toxins are still not well understood. The purpose of that paper was to sum up the current information obtained from the literature and from our own studies concerning

Abbreviations & units

MCs	microcystins
Nod	nodularin
CYN	cylindrospermopsin
HPI axis	hypothalamic-pituitary- interrenal axis
T_3 and T_4	$_t$riiodothyronine and thyroxine
ROS	reactive oxygen species

the influence of cyanohepatotoxins on neuroendocrine and immune systems of fish.

1. Microcystins and nodularin

MCs are monocyclic heptapeptides with over 75 different forms, produced by various species of cyanobacteria, primarily *Microcystis*. Nod, a product of *Nodularia spumigena*, is a pentapeptide of similar structure and toxicity to MCs. The LD_{50} of the most toxic form among MCs, MC-LR, is 50 μg kg^{-1} and of Nod is 30 μg kg^{-1} after intraperitoneal injection to mouse (Briand *et al.* 2003).

The target organ of the toxins is the liver, where they inhibit serine/threonine protein phosphatase 1 and 2A , however wide range of other negative effects after acute and chronic exposure has been noticed (Hudnell, 2008). Similarly to many other waterborne toxicants, MCs are capable of inducing a classical stress response in fish. Such stress symptoms as elevated cortisol plasma levels, dose-dependent changes in behaviour, such as irregular swimming, disorientation or increased startle response, as well as abnormal ventilation rates have been described (Bury *et al.* 1996; Ernst *et al.* 2007; Li *et al.* 2008). Moreover, in the reports cited above, hyperglycemia, being also a hallmark of stress response, was seen. Similarly, in our own study, after five-day-exposure of carp (*Cyprinus carpio* L.) to cyanobacterial extract containing environmentally relevant MC-LR concentration (12 μg L^{-1}), among other biochemical changes in blood plasma, also increase in glucose levels was seen, however only 24 h after the end of exposure (data not published). MC-LR has been proven to induce behavioural changes in fish at concentrations even below the WHO guideline of 1 μg L^{-1}. The low concentrations of the toxin caused increased motility in the daytime, interpreted as an escape reaction, and along with the rise of the toxin concentrations, reduction of swimming activity, as well as the phase shifting in circadian activity patterns in two fish species, zebrafish (*Danio rerio*) and sunbleak *Leucaspius delineatus* (Baganz *et al.* 2004).

The observed behavioural changes in fish rise questions, if they are a result only of stress response or maybe they are the neurotoxic effects of hepatotoxins, as it is known that MCs and Nod are capable to cross the blood-brain barrier. Moreover, MCs tend to accumulate in the brain and for Nod this organ is one of the primary targets for accumulation (Cazenave *et al.* 2005 and references therein). It has been documented, that MCs are transported into the murine whole brain cells with the use of multi-specific organic anion transporting peptides, being also expressed in different types of vertebrate cells, including amphibian blood-brain-barrier, epithelial cells of the blood-cerebrospinal-fluid-barrier or membrane of human neurons and leucocytes (Cazenave *et al.* 2005; Huber *et al.* 2007; Feurstein *et al.* 2009), as well as in different fish cells (Di Giulio & Hinton, 2008). The toxins, present in the cytosol and nuclei of brain cells, induced loss of cytoskeleton integrity, protein phosphatase inhibition and reduction of cell viability. The neurotoxic potency of MCs could explain the wide range of neurological disorders in the Caruaru patients, such as dizziness, tinnitus, hearing loss or visual impairment of not well understood causations (Pouria *et al.* 1998), as well as the pathological changes in the brain observed in progeny of mice treated with a toxin-containing extract of *Microcystis aeruginosa* (Falconer *et al.* 1988).

It has been also found, that injection of MCs at the doses of 150 and 600 μg per kg body weight can disturb thyroid functions in crucian carp (*Carassius auratus*), manifested as decrease of thyroid hormones levels in plasma (Li *et al.* 2008). While after application of lower dose of the toxin, T_4 level was relatively stable, with parallel significant decrease of T_3 level, the higher used dose caused severe drop of both T_4 and T_3 levels. There may be a few potential reasons of the observed effects. The authors suggest, that the declines in T_3 concentrations partially may be the result of MCs-induced hepatic damage and, as a consequence, the reduction of hepatic deiodination of T_4. Moreover, handling stress, as well as dexamethasone (glucocorticoid analogue) injection, also occurred to be able to acutely disturb iodothyronine deiodinases activity in fish (Walpita *et al.* 2007), so the observed changes could be in some extend an outcome of simultaneously observed elevated cortisol levels. Additionally, at higher used dose of the toxin, disruption of the thyroidal synthesis and/or secretion of T_4 should be considered. The latter results suggest that high MCs doses are capable to induce hypothyroidism in fish, however it is not clear if the toxins act directly or observed effects are the lethal stress consequences (Li *et al.* 2008).

Impact of stress hormones on physiological responses differs, depending on duration of exposure and may have permissive, preparative, stimulatory or suppressive functions (Esch *et al.* 2002; Pruett, 2003). Chronic stress may lead to maladaptive responses, resulting in varied consequences, including immunological disorders. There is growing evidence, that cyanohepatotoxins may be able to impair immune functions in fish, both after *in vivo* and *in vitro* intoxication (Rymuszka *et al.* 2008). The mechanisms of that phenomena are not always fully understood, as there are no studies proving toxins ability to enter the immunocompetent cells. However, there is evidence that the toxins can induce many disturbances in immune cells activity and immune responses, so it may be supposed, that the observed effects arise from complex and multidirectional, direct or/and indirect

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impact of cyanohepatotoxins, such as toxins-induced stress responses, ROS overproduction or protein phosphatase inhibition in the immune cells.

Significant decrease in lymphocyte and neutrophil myelocyte counts in blood after oral and intraperitoneal exposure of carp and silver carp (*Hypophthalmichthys molitrix* Val.*)* on MCs, with concomitant decrease of phagocyte activity has been described by Palikova *et al.* (1998). The experimental data indicate that MCs and Nod have a capacity to modulate phagocytic cells functions at different steps. Increased migration of mammalian polymorphonuclear leucocytes, enhancement of spontaneous adherence, as well as changes in ingestion, intracellular killing and ROS production after cell incubation with the toxins were described (Hermández *et al.* 2000; Kujbida *et al.* 2008). MC-LR was also found to stimulate phagocytes at a concentration of 5 μg ml^{-1} in Murray cod (*Maccullochella peelii*) by both increasing the numbers of cells engaged in phagocytosis and the number of beads engulfed per cell (Harford, 2004). In our own studies stimulation of respiratory burst activity at 5 μg ml^{-1} MC-LR and potentation of zymozan particle phagocytosis at 1 and 5 μg ml^{-1} in rainbow trout (*Oncorhynchus mykiss*) phagocytes was observed, while incubation with higher toxin concentrations (10 and 20 μg ml^{-1}) decreased ROS production (Sierosławska *et al.* 2007). Observed stimulatory effects of MCs may be explained by their ability to increase the release of the pro-inflammatory cytokines, IL-1β and TNF-α, which are known to upregulate Fc-receptor-mediated phagocytosis (Harford, 2004 and references therein). The possibility that MCs may induce activation or suppression of the innate immune responses was confirmed by Wei *et al.* (2008) studies on zebrafish cells, in which elevated expressions of immune-related genes, including interferon regulatory factor 7, lectin or mannose binding-like lectin genes and decreased expression of complement genes were found.

Moreover, there are some reports on down-regulation of lymphocyte functions by the toxins in vertebrates. Yea *et al.* (2000, 2001) described dose-dependent inhibition of polyclonal antibody response and lymphoproliferation, probably mediated through decreased IL-2 mRNA stability, after *in vitro* treatment of mouse cells with MCs and Nod. A series of studies performed by Lankoff *et al.* (2004a; 2004b) also demonstrated *in vitro* suppressive effects of MC-LR on the proliferative response of lymphocytes isolated from human and chicken peripheral blood. Moreover, the toxin decreased IL-2 production, while the level of IL-6 was increased. Similar inhibitory activity of MC-LR on lymphocyte proliferation was also observed in our own studies on rainbow trout cells (Rymuszka *et al.* 2007). On the contrary, the study by Harford (2004) showed that MC-LR did not significantly affected PHA-stimulated responses of Murray cod lymphocytes in the concentration range 0.05-5 μg ml^{-1}. The observed suppressive effects may be in some extend due to MC-LR mediated apoptosis and ultrastructural alterations, which were observed in fish and mouse lymphocytes (Teneva *et al.* 2005; Wei *et al.* 2008).

2. Cylindrospermopsin

CYN is known to be produced by *Cylindrospermopsis raciborskii*, *Aphanizomenon ovalisporum*, *Umezakia natans* and *Raphidiopsis curvata*. It is an alkaloid acting mainly as an inhibitor of proteins synthesis, with the LD_{50} after intraperitoneal administration to mice of 2100 μg kg^{-1} (Briand *et al.* 2003).

No studies on neuroendocrine effects of CYN in fish were found. However, taking under consideration, that the toxin inhibits protein synthesis, any cells actively secreting proteins or undergoing hypertrophy may be affected (Falconer, 2005). The only available paper on the endocrine influence of CYN revealed, that the toxin has the potential to change the ratio of progesterone:estrogen serum concentrations, and, in the consequence, it may have effects on the establishment and maintenance of pregnancy in humans (Young *et al.* 2008).

The studies on CYN potential immunotoxic effects in fish are restricted to the report indicating, that acute exposure to the toxin can modulate Murray cod head kidney cell responses, such as phagocytic activity and phagocytic index and, in lesser extend, mitogenesis (Harford, 2004). Similarly, there were only a few studies on CYN impact on mammals immune cells. Massive necrosis of lymphocytes in the cortical layer of the thymus after toxin-containing extract administration to mice were described (Terao *et al.* 1994). Furthermore, in mice repeatedly exposed to CYN, lymphophagocytosis in the spleen and thymus, being an indication of an immunotoxic response, occurred (Shaw *et al.* 2000). The observed toxin effects on murine thymus and spleen were however considered by Seawright *et al.* (1999) to be the normal response of the immune system to the stress and not specific to CYN.

Conclusion

The goal of our paper was to highlight the possible, but yet not sufficiently studied connections between intoxication by cyanohepatotoxins and some disturbances in the neuroendocrine and immune cells activity.

Fish losses observed during cyanobacterial blooms are usually connected with oxygen depletion or direct toxin poisoning. However, the toxin concentrations which are needed to induce fish kills by direct toxicity to the target organs/tissues are relatively high. The other factors resulting in fish mortality or fitness reduction observed during and after the blooms may be also involved. Thus we might speculate the possible links between fish health status and cyanohepatotoxin-induced neuroendocrine and immune changes, and, as a result, decreased ability of an organism to sustain

homeostasis against pathogens or xenobiotics. It is well established, that many environmental stressors, likely also cyanotoxins, induce immunosuppression and augment the susceptibility of fish to pathogens, which triggers the outbreak of infectious diseases. Toxin-induced stress reactions, working by activating HPI axis, are probably responsible for at least a part of described above effects. However, except for that indirect mechanisms, there is some evidence on direct effects of the toxins on immune cell functions, which however requires further research. Moreover, toxin-induced non specific activation of immune system may be involved in the development of acute or chronic illness, associated with a complex of neurologic symptoms, as was observed in the patients exposed to the *Microcystis* bloom (Hudnell, 2008). Also more studies are needed on neuroendocrine interactions, especially in the context of the potential cyanotoxin-mediated changes in thyroid functions.

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