Review paper

Immunotoxic potential of cyanotoxins on the immune system of fish

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Abstract

Toxigenic cyanophyta (Cyanobacteria) are found in eutrophied waters in many countries around the world. Many species of Cyanobacteria produce a wide range of toxins which during the decomposition of blooms are often the main cause of increased number of diseases and higher mortality among aquatic animals. Several studies have shown that cyanotoxins (e.g. microcystins), are able to modulate the activity of immune cells of vertebrates. However, the effect of cyanotoxins as natural water contaminant on immune system of fish still needs elucidation.

The objective of this review is to summarise and evaluate data on immunotoxicity of cyanotoxins to fish available in the literature and results from our studies.

Key words: *Cyanobacteria, microcystin-LR, anatoxin-a, immunotoxicity.*

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Introduction

Cyanobacteria are the dominant phytoplanctonic group in eutrophic surface waters. Contamination by cyanobacterial blooms is a worldwide problem relevant to the freshwater and marine environment but also to the practice of fish farming causing serious water pollution. Cyanobacterial water blooms (approximately 50%) are toxic and may exert negative impact on water quality and in consequence may lead to potential hazards to wildlife, livestock and also human health [1]. Several cyanobacterial genera, especially *Microcystis*, *Anabaena*, *Oscillatoria* (*Planktothrix*), *Aphanizomenon* and *Lyngbya* are capable of producing a variety of toxic secondary metabolites – cyanotoxin. These toxins are groupped according to their toxicological properties into four major groups: hepatotoxins (e.g. microcystins, nodularin) neurotoxins [anatoxin-a, homoanatoxin-a, anatoxin-a(s), saxitoxins] cytotoxins (cylindrospermopsin), irritants and gastrointestinal toxins (aplysiatoxin, lyngbyatoxin, lipopolysaccharide endotoxins) [2].

Immunotoxicity of microcystins (MCs)

From among different forms of toxin produced by *Cyanobacteria*, the most common are microcystins (MCs), synthesised by some freshwater cyanobacteria like *Microcystis*, *Planktothrix*, *Anabaena*, which often dominate in a water bloom [3, 4]. Microcystins are a group (over 70) of cyclic hepatopeptides. Among microcystins, the most common, and also the most extensively studied, are microcystin-LR (MC-LR), microcystin-RR (MC-RR) and microcystin-YR (MC-YR). The acute toxicity (LD_{50}, *i.p.* mouse) of the individual MC congeners ranging between 50 (MC-LR) and 600 (MC-RR) μg/kg b.w. [5]. They cause severe liver damage by morphological and functional changes in hepatocytes and are potent tumor promoters. At the biochemical level, microcystins are inhibitors of serine/threonine protein phosphatases 1 and 2A (PP1 and PP2A) [6, 7].

Microcystins have been reported to induce pathological changes among salmonids and cyprinids. Moreover, differences in sensitivity have been shown in various fish species. Toxic effects are found not only in the liver but also in the kidney, gastrointestinal tract and gills [8-11]. There are a few studies which suggest a possible role of cyanobacterial toxins in fish illness and mortality [12, 13]. Fish, similar to other aquatic animals can accumulate microcystins [14, 15].

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Incidental observations of adverse effects suggested that microcystins may affect the immune system in fish, but mechanisms of microcystin immunotoxicity is not well understood [16, 18, 19]. We studied the *in vitro* influence of microcystin-LR on the viability of lymphocytes and phagocytes isolated from rainbow trout (*Oncorhynchus mykiss*). Lymphocytes isolated from fish pronephros were exposed to MC-LR concentrations of 1, 5 and 10 µg/ml medium at 18°C for 4, 24, 48, 72, 96 and 120 h. Percentage of cytotoxicity was determined after phagocytes incubation with MC-LR at the concentrations of 1, 5, 10 and 20 µg/ml for 2, 4 and 24 h. Time-and dose-dependent effects of microcystin-LR on the immune cells viability were shown. After application of the toxin at a concentration of 1 µg/ml no significant effects compared to the control were observed. The viability of the studied cells exposed to the higher concentration of toxin for longer time was significantly decreased [22, 23].

The mechanism of MC-LR cytotoxicity is not clear. Lactate dehydrogenase (LDH) release is commonly used as a marker for lethal cell injury. For example, Navratil et al. [24] and Vajcova et al. [16] reported that microcystin and a crude extract of toxic *Cyanobacteria* were able to increase of plasmatic enzyme activity-LDH. Recently, some evidence suggested that oxidative stress is also involved in the toxicity of microcystins to different cells. Jos et al. [17] showed that MC-LR induced oxidative stress in tilapia (*Oreochromis sp.*) exposed to repeated doses of toxin. Moreover, the activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) in liver and kidney were significantly elevated after repeated intoxication with MC-LR. A similar study was performed on common carp (*Cyprinus carpio L.*) hepatocytes by Li et al. [11]. These results suggested that the toxicity of microcystin-LR caused the increase of reactive oxygen species (ROS) contents.

Moreover, in our study we compared the mitogenic response of lymphocytes isolated from blood, pronephros and spleen exposed to different concentrations (1, 5, 10, 20 and 40 µg/ml) of MC-LR. A significant suppression of T and B cell proliferation was seen after administration of the toxin at the highest concentration used in the experiment (40 µg/ml). Lower concentrations of microcystin-LR 5, 10 and 20 µg/ml had no statistically significant influence on the cell proliferation. When the cells were treated with the lowest used concentration, 1 µg/ml a statistically significant increase of T and B cell in response to the mitogens was seen in comparison to the control [22].

Similar results were obtained in reference to the metabolic activity measured as respiratory burst activity. Statistically significant decrease of these parameter was seen after application of the toxin at the highest used concentrations (10 and 20 µg/ml), while the two lower doses (1 and 5 µg/ml) caused the stimulation of the superoxide anion production in the triggered cells. Moreover, we studied the phagocytic cell ability measured as zymozan particle phagocytosis. This parameter was elevated only in the presence of MC-LR at the dose of 5 µg/ml [23]. On the contrary, Wright et al. [18] investigated the *in vitro* effects of MCs on phagocytosis and lymphoproliferation of pronephros cells from Murray cod. The authors applying lower concentrations (0.05 and 0.5 µg/ml) found no changes in lymphocyte number and proliferation. Palikova et al. [19] described the influence of MC-LR on immunological indices of juvenile carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*). This study has demonstrated that effects depends on the administration route and the fish species. In silver carp the decrease of myelocytes (neutrophilic myelocytes and metamyelocytes) brought a major decrease of phagocytic activity, while this decrease occurred in T cytotoxic and B lymphocyte count after oral administration of bloom biomass in common carp. In chronic studies using the early life stages of the carp, Palikova et al. [20] found a significant decrease of phagocytic activity after exposure to the extract of *Cyanobacteria* containing microcystin LR, YR and RR. These results suggest the modulatory effects of microcystin-LR on lymphocytes and other piscine immune cell populations such as phagocytes.

Immunotoxicity of anatoxin-a (Antx-a)

The most common cyanobacterial neurotoxin is a low-molecular-weight alkaloid, anatoxin-a. This is postsynaptic, cholinergic neuromuscular blocking agents. Anatoxin-a is produced by several cyanobacterial genera: *Anabaena*, *Microcystis*, *Planktothrix*, *Aphanizomenon*, *Cylindrospermum* and *Nostoc*. The lethal dose LD_{50} for mice or rat in different forms of application is between 150 and 250 µg/kg [2, 5]. Although anatoxin-a is not stable in the water and relatively little is known about its toxicity for aquatic ecosystems, it is assumed that exposure to this toxin may be detrimental to fish health. Oberemm [21] reported that 400 µg/l of Antx-a altered temporarily the heart rate in zebrafish. Osswald et al. [25] studied the possibility of accumulation of this toxin in juvenile fish *Cyprinus carpio*. This study clearly show that blooms containing anatoxin-a may be lethal and the toxin may accumulate in the fish body.

There have been no report of the influence of anatoxin-a on fish immune system. We studied the cytotoxic and apoptotic/necrotic effects of pure anatoxin-a on fish lymphocytes isolated from carp (*Cyprinus carpio*) as well as their effects on the proliferative ability of T and B cells. Anatoxin-a did not show cytotoxic effects on both lymphocyte subpopulations. On the other hand, a significant suppression of T and B cell proliferation was seen after administration of the toxin at all the concentrations used in the experiment (from 0,01 to 1 µg/ml). Moreover, we noticed that Antx-a induces apoptosis but not necrosis in fish lymphocytes. Our studies suggested that this cyanotoxin initiates apoptosis, which causes the inhibition of the T and

B-cell proliferation (unpublished). However, further research is needed to determine the effects of a anatoxin-a on fish immune system and fish health.

Conclusions

Since the immunomodulative effects on fish of cyanotoxin resulting in higher susceptibility to diseases are not known, the information presented in this review could be useful to evaluate the potential risk caused by cyanobacteria for fish health.

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