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Pathological modifications following sub-chronic exposure of medaka fish (*Oryzias latipes*) to microcystin-LR

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ABSTRACT

Microcystins (MCs) are toxic monocyclic heptapeptides produced by many cyanobacteria. MCs, especially MC-LR, cause toxic effects in animals and are a recognized potent cause of environmental stress and health hazard in aquatic ecosystems when heavy blooms of cyanobacteria appear. Consequently, one of the major problems is the chronic exposure of fish to cyanotoxins in their natural environment. The present experiment involving chronic exposure confirmed initial findings on acute exposure to MC contamination: exacerbated physiological stress and tissue damage in several tissues of exposed medaka fish. The gonads were affected specifically. In female gonads the modifications included reduction of the vitellus storage, lysis of the gonadosomatic tissue and disruption of the relationships between the follicular cells and the oocytes. In the males, spermatogenesis appeared to be disrupted. This is the first report showing that a cyanotoxin can affect reproductive function, and so can impact on fish reproduction and thus fish stocks.

1. Introduction

Cyanobacteria are photosynthetic organisms that sometimes undergo phases of massive proliferation and reach a considerable biomass, under certain trophic and climatic conditions. The development of cyanobacterial blooms is a worldwide phenomenon increased by global changes, and more particularly climatic modifications, such as increased temperature, and eutrophication of the aquatic ecosystems as a result of anthropogenic activities [1].

In addition to the effects on aquatic ecosystems functioning (increased turbidity, loss of biodiversity, accumulation of biomass, disturbance of dissolved oxygen distribution, etc.), as well as the resulting unpleasant visual and olfactory effects, these developments can also have an impact on animal and human populations as a result of the ability of the cyanobacteria to synthesize toxins known as cyanotoxins (reviewed by [2]). The resulting deterioration of the water quality and other symptomatic changes are regarded as undesirable and potentially detrimental for the various uses of water. The most indexed genera of cyanobacteria known to produce toxins are *Anabaena*, *Aphanizomenon*, *Cylindrospermopsis*, *Lyngbya*, *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* [3]. There are two groups of cyanotoxins:

(I) Hepatotoxins (target: the digestive system) mainly consisting of microcystins (MCs), nodularins and cylindrospermopsins.
(II) Neurotoxins (target: the nervous system) including anatoxins and saxitoxins.

Animal and human intoxications are characterized by respiratory difficulties, dermatitis, gastro-enteritis and even death [4].

MCs are hepatotoxic cyclic heptapeptides which possess among 80 described variants which differ by amino-acids in positions 2 and 4, and by the methylation state of amino-acids in positions 3 and 7 [5]. The MC-LR variant (with leucine and arginine as the variable amino acids) is one of the most toxic and most often studied. Due to their structure and amino-acid composition microcystins are hydrophilic and spatially large molecules apparently incapable of crossing cell membranes via passive diffusion. They require active transport from the blood through cell membranes via specific transporters: multispecific organic anion transporting polypeptides (OATPs) present in several tissues [6,7].

MCs specifically inhibit cytoplasmic and nuclear serine/threonine protein phosphatases, especially PP1 and PP2A by linking covalently with cys-273 on PP1 and cys-266 on PP2A of their catalytic sub-unit [8–10]. This inhibition induces hyperphosphorylation of regulated proteins and causes deregulation of

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numerous cellular processes such as cytoskeleton organization, signalling pathways, metabolism and cell cycle.

Acute intoxications occurred in both animals and human beings. For example, in 1996 in Brazil, 2000 cases of human intoxication leading to 88 deaths were shown to be associated with the use of water contaminated by these hepatotoxins for haemodialysis [11,12]. Organotropism of MCs for liver and gastrointestinal tract has been demonstrated in rodents and fish after acute intoxication via intra-peritoneal injection or gavage with MC-LR. Histopathological studies in both fish and mammals revealed serious lesions of the liver: disorganization of the cytoskeleton, reduction of the glycogen content, hepatic haemorrhage, necrosis or apoptosis [13–26]. However, MCs also induce lesions in other organs such as intestine, spleen, kidney, muscles, lungs or gills: diffuse necrosis and degenerative changes [15,16,25,27–30], plus glomerulopathy in the kidney [31]. MCs also have an impact on larval development, inducing delay of hatching and teratogenesis after microinjection of MC-LR in vitellus at different embryo-larval stages [32,33].

So far, most of the laboratory studies using rodents or aquatic species (fish, amphibians) have been conducted following acute or sub-acute exposure to these toxins (MCs), and therefore may be not representative of the environmental situation. A few experiments have explored the chronic effects of MCs on organisms, in a few cases on fish. They have revealed growth inhibition, severe organ damage, such as necrosis and haemorrhage, decrease of embryo survival, susceptibility to ectoparasitic infestations and increased mortality [27,34–38]. Epidemiology studies have also shown an increase in primary liver cancers in urban populations consuming water contaminated by MC-producing cyanobacteria, suggesting that these toxins may be tumorigenic [39–43]. In addition, MCs have been shown to behave as tumor promoters when rodents were exposed to low doses [44]. Chronic exposure to low concentrations of MCs in drinking water may therefore constitute a serious hazard to public health, contributing to promoting cancer in humans. However, the molecular mechanisms by which MCs cause susceptibility to carcinogenesis are not understood.

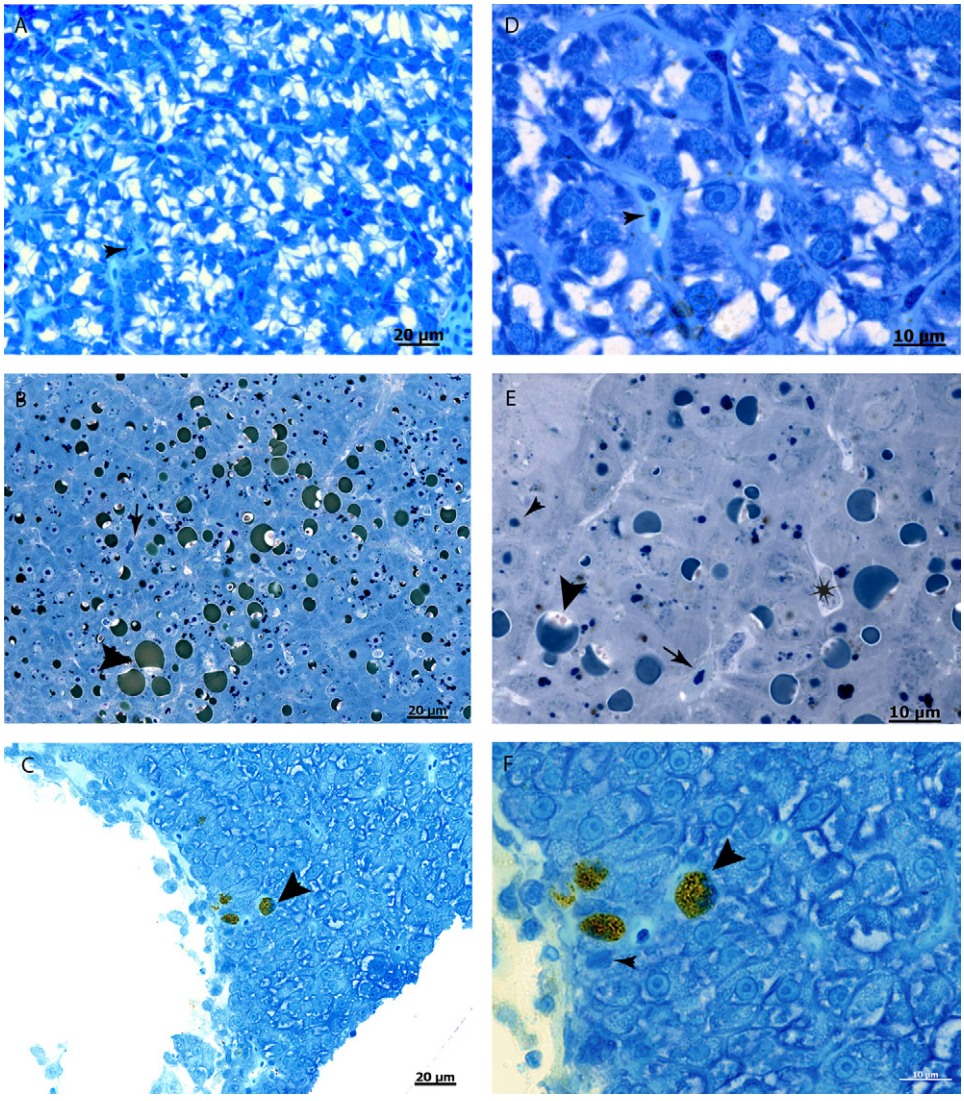

Fig. 1. Semi-thin section of adult medaka liver, toluidine blue staining with or without additional osmification and anti-microcystin labelling (AD4G2 or MC10E7) with hydroxyperoxidase. (A, D) Control. Hepatocytes are densely interconnected and Diss spaces (A, D, ➤) are clearly visible. (B, E) Treated (balneation, 30 days with microcystin-LR 5 µg L^{-1}). Hepatocytes are disorganized, Diss spaces are reduced (B, E, ➤). At high magnification, hepatocytes display several lytic areas (E, ✶). Cytoplasmic storages have disappeared, and some pycnotic nuclei can be observed (E, ➤). After osmification, an accumulation of lipid material with a clear lenticular space that possesses purple spots in the center is revealed in some treated hepatocytes (B, E, ➤), that is not seen in non-osmificated tissues (C, F). (C, F) Immunolocalisation in liver of treated fish revealed labelled cells identified as macrophages (C, F, ➤) near degenerated cells (F, ➤).