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Effects of dietary selenium on the oxidative stress and pathological changes in tilapia (*Oreochromis niloticus*) exposed to a microcystin-producing cyanobacterial water bloom

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ABSTRACT

The present study investigates the role of selenium (Se) supplementation (as sodium selenite) on the oxidative stress and histopathological changes induced by cyanobacterial cells containing microcystins (MCs) in tilapia fish (*Oreochromis niloticus*). Variation in lipid peroxidation (LPO) levels and carbonyl groups content, reduced glutathione/oxidized glutathione (GSH/GSSG) ratio, and catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), glutathione peroxidase (GPx) and glutathione *S*-transferase (GST) activities in liver and kidney of tilapia fish exposed to a single oral dose of 120 μg MC-LR/fish and sacrificed in 24 h, were investigated in the absence and presence of 1.5, 3.0 and 6.0 μg Se/g diet. Results showed a protective role of Se depending on the dose and the biomarker considered. Thus, the lower Se dose made CAT, liver GR and kidney SOD converged to basal values, whereas LPO and liver SOD and GST needed the higher dose. Kidney GR, however, was not protected at any Se dose. Moreover, Se has also shown to have a pro-oxidant effect with increased kidney LPO values and liver and kidney GPx activities in MC-free fish. The microscopic study revealed tissue alterations induced by cyanobacterial cells in the liver, kidney, heart and gastrointestinal tract that were ameliorated by the highest Se dose assayed. The level of Se supplementation must be therefore carefully selected to provide beneficial effects and to avoid potential negative consequences.

1. Introduction

Microcystins (MCs) are a family of potent hepatotoxins and tumor promoters, produced by several cyanobacteria genera, primarily *Microcystis*, that often forms blooms in eutrophic freshwater ecosystems, representing a threat to aquatic organisms and human beings (Ueno et al., 1996). Over 70 MCs have been successively isolated and identified, among which MC-LR and MC-RR are the most commonly occurring toxic microcystins. Fish are frequently exposed to MCs directly and passively, which consequently causes toxic effects and fish kills (Malbrouck and Kestemont, 2006; Atencio et al., 2008).

MCs have been characterized as potent inhibitors of serine and threonine phosphatases, affecting intracellular signalling, cell growth, and differentiation processes (Runnegar et al., 1995; Toivola and Eriksson, 1999). Oxidative stress induced by MCs exposure is considered to be involved in the development of MCs toxicity in different experimental models (Ding et al., 1998; Wiegand et al.,

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1999; Nong et al., 2007), including fish after oral exposure to *Microcystis*, in natural conditions (Qiu et al., 2007; Li et al., 2007) and also under laboratory conditions (Li et al., 2005; Jos et al., 2005; Cazenave et al., 2006a; Prieto et al., 2007). MC-LR and MC-RR cause oxidative stress and lipid peroxidation (LPO) increase in tilapias (*Oreochromis* sp.) (Prieto et al., 2006) and cyanobacterial water blooms induce changes in cellular glutathione (GSH) levels in different fish species (Blaha et al., 2004; Li et al., 2003). The detoxification of MC-LR in the liver occurs by GSH conjugation via the action of glutathione *S*-transferase (Pflugmacher et al., 1998; Takenaka, 2001).

Oxidative stress occurs when reactive oxygen species (ROS) overwhelm the cellular defences and damage proteins, membranes, and DNA (Kelly et al., 1998). ROS generated in tissues are effectively scavenged by the antioxidant defence systems that in aquatic organisms comprise specific antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and both water-soluble [vitamin C, reduced glutathione (GSH), carotenes] and fat-soluble (vitamin A, vitamin E) low-molecular weight free radical scavengers.

The activity of the extracellular and cytosolic forms of glutathione peroxidase (GPx) to produce oxidized glutathione (GSSG) and water is dependent upon the essential trace element selenium (Maier and Knight, 1994). Selenium (Se) appears to play a dichotomous role in living organisms because it is both a nutrient and a toxicant. Selenium protects against oxidative stress by being a part of GPx constituted by four subunits and each subunit contains one Se atom (Batcioglu et al., 2002), and it is also a component of deiodinase and thioredoxin reductase, which is involved in DNA synthesis, oxidative stress defence, and protein repair (Arnér and Holmgren, 2000). It is also well known that Se has potent cytotoxic effects by reacting with sulphydryl groups to produce biologically active ROS (Spallholz et al., 2004). As a nutrient, the dietary Se requirement for fish is 0.1–0.5 μg/g dry weight, and its beneficial effects are firmly established. On the other hand, at dietary concentrations of only 7–30 times of those required (>3 μg/g), Se becomes toxic (Lemly, 1997). Thus, homeostatic regulation of optimal levels of Se appears critical for protecting tissues from H_2O_2-induced oxidative damage and for maintaining overall health (Palchaudhuri et al., 2001).

Miller et al. (2007) studied the effects of dietary Se (as sodium selenite) on antioxidant defence mechanisms of rainbow trout (*Oncorhynchus mykiss*) and found that the acute Se exposure decreased liver LPO but the subchronic exposure did not alter LPO or antioxidants (GPx, GSH). Orun et al. (2005) reported decreased LPO levels in the liver and other organs, in rainbow trout (*O. mykiss*) exposed for 72 h to 2–6 mg/L sodium selenite. Lin and Shiau (2007) studied how a dietary supplementation of 0.8 and 1.6 μg Se/g of diet for 8 weeks affected hepatic thiobarbituric acid reactive substances (TBARS) in *Epinephelus malabaricus*. A higher GPx activity in fish fed Se-supplemented diets was observed in several fish species, such as rainbow trout (Hilton et al., 1980), Atlantic salmon (Bell et al., 1987).

The protective role of Se against the oxidative stress induced by MCs in fish has not been previously investigated. There is only a study involving mice carried out by Gehringer et al. (2003a) that investigated the effect of Se supplementation, as sodium selenite (1.5 μg Se/mouse/day) on microcystin hepatotoxicity, before an intraperitoneal injection of a sub-lethal dose of MC-LR and repeated sub-lethal doses of MC-LR.

The aim of this study was to investigate the role of Se pre-treatment on the toxicity induced by cyanobacterial cells containing MC-LR in tilapias (*Oreochromis niloticus*) exposed by oral route. Activities of CAT, SOD, GPx, and GR, as well as LPO and protein oxidation, in the liver and kidney of fish were evaluated. Moreover, reduced and oxidized glutathione ratio (GSH/GSSG) and changes in glutathione *S*-transferase were also determined. Finally, the reversion of the main pathological changes induced by cyanobacterial cells in the liver, kidney, heart and gastrointestinal tract of tilapias by selenium was studied.

2. Material and methods

2.1. Chemicals

Selenium as sodium selenite pentahydrate was obtained from Merck (Darmstadt, Germany). All other chemicals were obtained from Sigma–Aldrich (Madrid, Spain). Microcystin standards (MC-LR, MC-RR, MC-YR) were supplied by Cyanobiotech (Berlin, Germany) with a purity of 99, 98, and 95%, respectively.

2.2. Experimental setup and acclimation of fish

Sixty-four male *O. niloticus* (Nile tilapia, Perciformes: Cichlidae), average weight 50 ± 8 g, and length of 12 ± 1 cm, were obtained from a fish hatchery in Córdoba. Fish were transferred to the laboratory where they were held in 8 glass aquaria (eight individuals/aquarium) with 96 L of freshwater with continuous system of water filtration and aeration (Eheim Liberty 150 Bio-Espumador cartridges, Deizisau, Germany), and a 12:12 light/dark photoperiod. Temperature was maintained at 21 ± 2 °C and dissolved oxygen values at 7.0 ± 0.5 mg/L. Mean values for additional parameters of water quality were: pH 7.6 ± 0.3, conductivity 287 μS/cm, Ca^{2+} 0.60 mM and Mg^{2+} 0.3 mM. Fish were fed with commercial fish food (Dibaq, Segovia, Spain) containing 6% lipids, 31% proteins, 37% carbohydrates, 2.5% fiber, 1.5% total phosphorus, 12% ash, 200 mg α-tocopherol/kg, 1700 international unit (IU) vitamin D3/kg, and 10,000 IU vitamin A/kg feed. The amount of commercial food administered per fish was 0.3 g/day. Fish were acclimatized for 15 days before the beginning of the experiments.

2.3. Collection of Microcystis water bloom and determination of MCs

MCs from a *Microcystis* water bloom from the Guadiana River (Mértola, Portugal) were extracted from dried cell material using the method of Moreno et al. (2004). The lyophilised cells (50 mg) were extracted three times with 10 mL of 0.1 M acetic acid and 20 mL of a mixture of methanol:chloroform (1:1 v/v). The cell suspension was sonicated in an ultrasound bath for 15 min, stirred for