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Antioxidant enzyme activity and lipid peroxidation in liver and kidney of rats exposed to microcystin-LR administered intraperitoneally

I. Moreno[a], S. Pichardo[a], A. Jos[a], L. Gómez-Amores[b], A. Mate[b], C.M. Vazquez[b], A.M. Cameán[a,*]

[a]*Área de Toxicología, Facultad de Farmacia, C/Profesor García González, n° 2, 41012 Sevilla, Spain*
[b]*Área de Fisiología, Facultad de Farmacia, C/Profesor García González, n° 2, 41012 Sevilla, Spain*

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Abstract

The effect of acute exposure of intraperitoneal injection of microcystin-LR (MCLR) on antioxidant enzymes and lipid peroxidation has been studied in liver and kidney of rats. Rats were treated with two doses, i.e. 100 and 150 μg of pure MCLR/kg body weight or saline solution. The enzyme activities of glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT) in the liver were significantly decreased in MCLR-treated rats. The decrease of GR activity in the liver was 60%, followed by GSH-Px, SOD and CAT. Similarly, a decrease in the antioxidant enzymes was found in the kidney of MCLR-treated rats, such as GSH-Px (27–31%), GR (22%), SOD (42%) and CAT (25–28%). Concomitantly, significant increases in lipid peroxidation levels were recorded in liver (121 and 196% for 100 and 150 μg/kg, respectively) and kidney (48 and 58% for 100 and 150 μg/kg, respectively) from MCLR-treated rats. In conclusion, acute exposure to MCLR results in a decrease in the antioxidant enzymes and an increase in lipid peroxidation in liver and kidney rats, suggesting the oxidative stress as an important role in the pathogenesis of MCLR-induced toxicity. Antioxidant enzymes were significantly consumed in the liver and a minor decrease was found in kidney, confirming the organ-specific effects of MCLR.

Keywords: Microcystin-LR; Rat; Oxidative stress; Liver; Kidney

1. Introduction

Contamination of natural waters by cyanobacterial blooms is a worldwide problem, causing serious water pollution and public health hazard to humans and livestock (Carmichael, 1994; Falconer, 1994; Park et al., 1998; Oudra et al., 2001). Blooms of cyanobacteria in ponds and water reservoirs have been associated with acute, usually lethal toxicity in various species of domestic animals and wildlife in addition to cases of illness in humans (Falconer, 1999) due to the entrance of the toxins in the aquatic environment after cell lysis. Microcystins (MC) are the most commonly found group of cyanotoxins and more than 70 variants are known (Fastner et al., 2002), many of which are potent hepatotoxins, with microcystin-LR (MCLR) being one of the most abundant and toxic variants in blooms. Acute toxicity of potent MC variants in both mammals and fishes include liver necrosis and haemorrhage, with the lethal dose

* Corresponding author. Address: Bioquimica, Bromatologia, Toxicologia and Medicina Legal, University of Seville, Professor Garcia Gonzalez n 2, 41012 Sevilla, Spain. Tel.: +34 95 455 6762; fax: +34 95 423 3765.

E-mail address: camean@us.es (A.M. Cameán).

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for 50% of the population (LD_{50}) ranging from 50 to >1200 μg/kg (mouse, intraperitoneal injection) (Rinehart et al., 1994). Specifically, the LD_{50} by the intraperitoneal (i.p.) route for MCLR (highly toxic) ranges from 25 to 150 μg/kg body weight in mice (Kuiper-Goodman et al., 1999) and rats were less sensitive than mice to the toxin by oral and i.p. routes (Fawell et al., 1999). In humans, an outbreak of severe hepatitis occurred at a Brazilian haemodialysis centre in Caruaru (Brazil), where 100 patients developed acute liver failure and 50 of these died (Jochimsen et al., 1998). Furthermore, MC act as tumour promoters (Nishiwaki-Matsushima et al., 1992; Harada et al., 1996).

At present, the exact mechanisms by which microcystins induce hepatotoxicity and tumour promotion have not been fully elucidated. It is well recognized that they are potent inhibitors of protein phosphatase 1 and 2A, leading to increased protein phosphorylation, which is directly related to their cytotoxic effects a tumour-promoting activity (Hooser et al., 1989; Carmichael, 1994; Hooser, 2000). Some evidences suggest that oxidative stress may play a significant role in the pathogenesis of microcystin toxicity (Hermansky et al., 1991; Ding et al., 1998a) in mammals, being a component of the pathologic changes brought about by prolonged sublethal exposure to MCLR in rats (Guzman and Solter, 1999).

Increased lipid peroxidation (LPO), measured as malondialdehyde (MDA) production, was observed in rat hepatocytes (Ding et al., 1998b) and hepatic microsomes (Hermansky et al., 1990) following exposure to MC or lyophilised cyanobacterial extracts. Furthermore, the formation of free radical species, possibly derived from oxidative lipid alterations, was demonstrated as a result of in vivo cyanobacterial toxin-induced hepatotoxicity in rats (Towner et al., 2002).

Free radicals or reactive oxygen species (ROS) generated in tissues are effectively scavenged by the antioxidant defense system that constitutes antioxidant enzymes, such as glutathione peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), and when the activity of these antioxidant defense system decreases or ROS production increases, an oxidative stress may occur (Packer, 1995).

Previous studies from our laboratory have shown that i.p. injection of MCLR induced elevated lipid peroxidation in serum and intestine in rats (Moreno et al., 2003), which may be related to changes in the antioxidant defense system in these treated rats. In view of these considerations, the aim of the present study was to evaluate if acute exposure to MCLR in rats modifies the antioxidant defense system and induces LPO in liver and kidney, in order to determine the involvement of ROS formation on MCLR-induced toxicity. To this end, GSH-Px, GR, SOD and CAT activities, as well as MDA levels, as measurement of LPO, were measured in liver and kidney homogenates from rats treated acutely with MCLR.

2. Material and methods

2.1. Cyanobacterial toxin

The cyanobacterial hepatotoxin MCLR was obtained from Calbiochem-Novabiochem (La Jolla, Ca, USA) and test solutions were prepared in saline solution and the correct dosing concentration was confirmed by High Perfomance Liquid Chromatography (HPLC) with diode array detection, using a Varian model 9012 chromatograph equipped with a Varian ProStar model 330 PDA detector.

2.2. Animals

Male Wistar rats weighing about 200 g were obtained from the Seville University breeding colony and kept for a week before using in a well-ventilated room maintained at 23±1 °C, 12 h light/12 h dark cycles, with free access to water and standard pellet diet.

2.3. Acute Intraperitoneal toxicity of MC-LR

Taking into account the values of LD_{50} obtained in mouse by other authors (Fawell et al., 1999), we chose the up and down method designed to estimate the LD_{50} in rats with a limited amount of the purified toxin and a small number of animals (Chan and Hayes, 1994). The dosages of the toxin MCLR were set at 0, 72.0, 87.0, 104.0, 125.0, 150.0 and 180.0 μg/kg as geometric factor = 1.2, consisting of five animals at each dose group. MCLR dissolved in saline solution was also i.p. injected. LD_{50} value was calculated from cumulative mortality observed 48 h after the injection, although the observation period was prolonged until 14 days, using the probit analysis program provided by the Environmental Protection Agency (EPA) (http://www.epa.gov/oppfead1/harmonization).

Once LD_{50} of MCLR was evaluated, rats were divided at random into three groups of 10 animals at each group. In two groups, the animals were administered a single i.p. injection of MCLR (1 ml) at doses of either 100 or 150 μg/kg body weight. The control group was injected the same volume of vehicle, 0.9%, w/v saline solution. All animals received humane care and studies were conducted in accordance with the 'Principles of Laboratory Animal Care' (National Institutes of Health Publication No. 86–23, revised 1985) and approved by the local standard for protecting animal welfare. When animals died (i.e. those administered 150 μg/kg), they were immediately subjected to necropsy. On the other hand, the animals subjected to 100 μg MCLR/kg and those from the control group were euthanatized after 8 h by ether inhalation. To minimize diurnal variations the rats were routinely killed between 17:00 and 18:00 h. Livers and kidneys were quickly removed, immediately washing out the blood with ice-cold 0.9% saline solution, weighted and stored at −70 °C. Homogenates of the tissues were prepared as 1.0 g/10 ml in 250 mM