Microcystin-LR Induces Alterations in Heart Muscle

(microcystin-LR / chronic intoxication / heart)

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Abstract. MC-LR belongs to a family of cyanobacterial toxins. MC-LR acts as serine-threonine phosphatase-1 and -2A inhibitor. Chronic intoxication with low doses of this toxin promotes liver tumour formation and induces kidney injury.

The aim of the study was to evaluate whether chronic exposure to relatively low doses of MC-LR has toxic effects on hearts of treated animals. Male adult Wistar rats were treated every second day for 8 months with MC-LR (10μg/kg i. p., N = 5). Control groups were treated either with a vehicle (ethanol and methanol 4 : 1 v/v; N = 5) or with physiologic saline (N = 4). We found that MC-LR could induce enlargement of cardiomyocytes (MC-LR = 20.984μm ± 1.351, vehicle=17.454μm ± 0.518, saline = 15.996μm ± 1.430), loss of cell cross-striations, lower myofibril volume fraction (MC-LR = 0.3657mm^3/mm^3 ± 0.0337, vehicle=0.4716mm^3/mm^3 ± 0.0086, saline = 0.4793 mm^3/mm^3 ± 0.0101), fibrosis (MC-LR = 0.0747mm^3/mm^3 ± 0.01288, vehicle = 0.0275 mm^3/mm^3 ± 0.0076, saline = 0.0309mm^3/mm^3 ± 0.0074) and mononuclear infiltration in the interstitial tissue. The TUNEL staining of the heart sections of rats in all groups showed no apoptotic cells. We may conclude that long-term exposure to relatively low doses of MC-LR represents a considerable risk of injury of the heart.

Introduction

Microcystins (MCs) such as microcystin-LR (MC-LR) belong to the family of more than 60 structurally similar toxins (Sivonen and Jones, 1999). They are produced by some species of freshwater cyanobacteria. MCs act as serine-threonine phosphatase-1 and -2A inhibitors (MacKintosh et al., 1990) and/or increase formation of reactive oxygen species (ROS) (Ding et al., 2001). Consumption of MCs may cause lethal poisoning of livestock, wild life and fish (Codd et al., 1997).

Acute intoxication with high doses of MC-LR causes cytoskeletal alterations, apoptosis and necrosis of hepatocytes resulting in intrahepatic haemorrhage that could be lethal (Hooser, 2000). It was also reported that MC-LR induced myocardial cell damage in the heart muscle after 24 hours (Zhang et al., 2002). In chronic intoxication MC-LR acts as liver-tumour promoter (Nishiwaki-Matsushima et al., 1992; Sekijima et al., 1999) and induces specific injury of rat kidneys (Milutinovic et al., 2002; 2003), but nothing has been described about the chronic effects of MCs on the heart.

We aimed at evaluating whether chronic treatment with relatively low doses of MC-LR in 18-month-old experimental rats could provoke toxic effects on the heart.

Material and Methods

Animals and treatment

We used male Wistar rats weighing from 444 g to 599 g at the beginning of the experiment. The animals were handled following the guidelines in the Slovenian Law for Animal Health Protection and Instructions for Granting Permit for Animal Experimentation for Scientific Purposes. Rats were treated every second day for 8 months with MC-LR (N = 5) in relatively low doses (10 μg MC LR/kg i. p.). The control group was treated with a vehicle (N = 5) (0.8 % ethanol and 0.2 % methanol dissolved in 0.9 % saline) in a volume of 3.7 ml/kg or with pure saline (N = 4). MC-LR was isolated as described before (Sedmak and Kosi, 1997; Sedmak and Elersek, 2005). At the end of experiment the animals were sacrificed in CO_2 anaesthesia.

Staining with haematoxylin and eosin (HE)

Hearts were quickly removed, fixed in buffered 10 % formalin for 24 h and embedded in paraffin. Microtome sections (4 μm) were then cut and stained with HE.

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Abbreviations: HE – haematoxylin-eosin, MC-LR – microcystin-LR, MCs – microcystins, TUNEL – terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labelling.

Evaluation of the volume density of the interstitial tissue and the myofibril volume fraction

Stereological analysis (Weibel, 1979) was performed in the light microscope using Weibel's test system. Volume density of interstitial tissue (Vi) and myofibril volume fraction (Mvf) were estimated as described previously (Zorc et al., 2003). The average value (Vi and Mvf) of each treatment group was then expressed as the average value ± SD. The statistical significance of the differences of the measured parameter between the treatment groups was evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's post hoc analysis ($P < 0.05$).

Measurement of the size of the cardiomyocytes

Three histological sections of the heart ventricles of each animal were performed in the light microscope at an objective magnification of 40x. The diameter of 50 cardiomyocytes was measured by using Zeiss Axioscope software. The average diameter of the cardiomyocytes of each treatment group was then expressed as the average level ± SD. The statistical significance of the differences of the measured parameter between the treatment groups was evaluated by one-way analysis of variance (ANOVA) followed by Scheffe's post hoc analysis ($P < 0.05$).

TUNEL assay

Detection of apoptosis was performed with the terminal deoxynucleotidyl transferase mediated-deoxyuridine triphosphate nick-end labelling (TUNEL) method (Apo Taq plus Peroxidase Kit ONCOR, Gaithersburg, MD) following the manufacturer's instructions as described previously (Zorc et al., 2003).

Results and Discussion

We report for the first time about the toxic effect of chronic treatment with relatively low doses of MC-LR on the hearts of experimental rats. All rats survived the treatment. HE staining revealed that treatment of rats with the vehicle had no effect on the morphology of the heart tissue. In the heart sections of the animals treated with MC-LR some disarray and short runs of myocardial fibres interrupted by connective tissue were observed. An increased volume density of interstitial tissue (MC-LR = 0.0747 mm^3/mm^3 ± 0.01288, vehicle = 0.0275 mm^3/mm^3 ± 0.0076, saline = 0.0309 mm^3/mm^3 ± 0.0074) with few lymphocyte infiltrates were seen in rats treated with MC-LR (Fig. 1). The cardiomyocytes in the MC-LR group were enlarged (MC-LR = 20.984 µm ± 1.351, vehicle = 17.454 µm ± 0.518, saline = 15.996 µm ± 1.430) with enlarged and often bizarre-shaped nuclei. In some cells the loss of cell cross-striations, degenerative muscle fibres with myocytolysis were observed. A reduced myofibril volume fraction was seen in the MC-LR group (0.3657 mm^3/mm^3 ± 0.0337) in comparison with the controls (vehicle = 0.4716 mm^3/mm^3 ± 0.0086, saline = 0.4793 mm^3/mm^3 ± 0.0101). The TUNEL staining of the heart sections of rats treated with MC-LR showed no apoptotic cardiomyocytes. The observed injuries in the heart of MC-LR-treated rats were similar to myocardial changes described in rats that survived a short period of time after intoxication with a high dose of MC-LR (Zhang et al., 2002). These authors also observed damage of cardiomyocytes with disappearing myofibrils and necrosis 24 hours after the injection of MC-LR.

We may conclude that long-term exposure to relatively low doses of MCs represents a considerable risk of heart injury.

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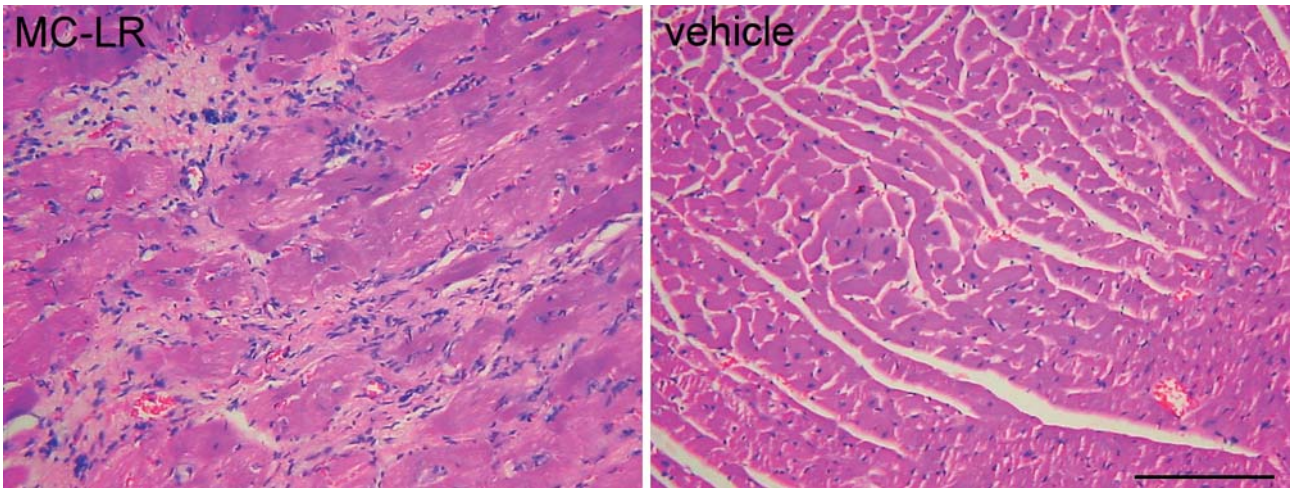

Fig. 1. Heart sections treated with MC-LR and the vehicle. MC-LR section shows fibrosis with a few lymphocytes. Bar = 150 µm.

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