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Age-dependent effects on biochemical variables and toxicity induced by cyclic peptide toxin microcystin-LR in mice

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Abstract

Microcystins are naturally occurring hepatotoxins produced by certain strains of *Microcystis aeruginosa* and microcystin-LR is the most toxic among the 60 microcystin variants isolated so far. These toxins have been implicated in both human and livestock mortality. In the present study we evaluated the age-dependent hepatotoxic effects of microcystin-LR (MC-LR) in mice after intraperitoneal and oral route of exposure. For acute toxicity studies by intraperitoneal route, 1 LD_{50} dose of MC-LR (43.0 μg/kg) was administered to 6- to 36-week-old mice. Results showed that time to death in toxin treated animals decreased with age of mice. In comparison to control mice, treated animals of all age groups showed significant increases in liver body mass index and increases in serum enzymes (lactate dehydrogenase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, sorbitol dehydrogenase). For acute oral toxicity studies, 1 LD_{50} of microcystin-LR containing extracts (3.5 g of MCE/kg) was administered to 6- and 36-week-old mice. The effects on biochemical variables were similar to intraperitoneal route of exposure. Significant age-dependent effects that were observed in microcystin treated animals by intraperitoneal and oral routes of exposure include: time to death, hepatic lipid peroxidation, glutathione depletion and DNA fragmentation. The age-dependent effects observed in some of the biochemical variables may be due to difference in the amount of microcystin-LR up take and also the age-dependent ability to detoxify the toxin in mice.

Keywords: Cyanobacterial toxin; Microcystin-LR; Hepatotoxin; Age effects; *Microcystis aeruginosa*; DNA damage; Oral toxicity

1. Introduction

Toxin producing cyanobacteria pose a worldwide threat to humans and animals due to their wide spread occurrence in both drinking and recreational waters (Hitzfeld et al., 2000; Rao, 2004). The toxins of freshwater cyanobacteria are classified into two groups, neurotoxins and hepatotoxins, which include cyclic-peptide microcystins and nodularin. Microcystins and related polypeptides are selectively hepatotoxic in fish, birds and mammals. The consequence of acute poisoning by these compounds is rapid disorganization of the hepatic architecture, break down of sinusoidal structures and, in mammals, pooling of blood in the liver (Dawson, 1998). Human illnesses attributed to cyanobacterial toxins can be categorised into gastroenteritis and related diseases, allergic and irritation reactions, and liver diseases (Bell and Codd, 1994; Chorus et al., 2000). Microcystins and nodularin have been found to be potent inhibitors of protein phosphatase type 1 and 2A as well as skin and liver tumour promoters in laboratory animals (Nishiwaki-Matsushima et al., 1992; Falconer and Humpage, 1996) as well as aquatic fauna (Malbrouck et al., 2003; Pinho et al., 2003). Microcystins are also suspected to be involved with promotion of primary liver cancer in humans exposed to long-term doses of these peptide toxins through drinking water (Chorus et al., 2000). The bioaccumulation of cyanotoxins by aquatic animals including fish, mollusc and zooplankton has been reported (Amorin and Vasconcelos, 1999) which indicates

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that oral consumption by animal tissues containing cyanotoxins is possible and can lead to human toxicity. Tragic death of 60 haemodialysis patients was reported in Brazil due to the presence of cyanobacterial toxins in the water supply used in haemodialysis unit (Azevedo et al., 2002). The provisional guideline value set by WHO for MC-LR in potable waters is 1.0 μg/L (WHO, 1999).

Over 60 variants of microcystin have been reported so far conforming to the generalized format: Cyclo(D-Ala[1]-X[2]-D-MeAsp[3]-Y[4]-Adda-Arg[5]-D-Glu[6]-Mdha[7]-), where X and Y are variable amino acids, D-MeAsp is D-erythro-β-methylaspartic acid, Adda is (2*S*,3*S*,8*S*,9*S*)-3-amino-9-methoxy-2-6-8-trimethyl 10-phenyldeca-4-6-dienoic acid and Mdha is *N*-methyldehydroalanine. The unusual amino acid Adda is essential for expression of biological activity. The XY variable amino acids for MC-LR are leucine (L), arginine (R).

Previous studies with various rodent and non-rodent species have indicated that mice are more suitable animal model for studying microcystin induced toxic effects because of the higher sensitivity of this species to the effects of the toxin (Fawell et al., 1999). There was only one report on the effect of microcystin on different age groups of mice (Ito et al., 1997). The objectives of the present study are (a) evaluation of age-dependent (6–36 weeks old) biochemical effects of purified microcystin-LR in mice by intraperitoneal route and (b) comparison of acute toxic effects in two age groups (6- and 36-week-old mice) by oral route of exposure with cell free extracts of MC-LR producing strain *Microcystis aeruginosa* (PCC 7806).

2. Materials and methods

2.1. Chemicals

The cyanobacterial toxin microcystin-LR was obtained from Alexis Biochemicals (Switzerland). All other chemicals were obtained from Sigma Chemical (St Louis, MO, USA) unless otherwise specified.

2.2. Animals

Swiss albino male mice (*Mus musculus*) from establishment's animal facility were used. The animals were maintained on the basis of date of birth and different age groups of animals (6, 12, 18, 24, 30 and 36 weeks old) were used. The mass range of animals used are given in parenthesis: 6 weeks (24–26 g), 12 weeks (31–34 g), 18 weeks (34–38 g), 24 weeks (37–42 g), 30 weeks (42–45 g) and 36 weeks (43–46 g). Animals were housed in polypropylene cages with dust-free rice husk as bedding material and provided with pellet food (Amrut Laboratory Feeds, Maharashtra, India) and water ad libitum.

2.3. Preparation of microcystin containing extracts (MCE)

Microcystin containing extracts (MCE) were prepared from laboratory cultures of *M. aeruginosa*. The axenic cultures of *M. aeruginosa* (PCC 7806) was obtained from Pasteur Culture Collection, Institute Pasteur, France. The cyanobacterial cells were grown in MA medium (Ichimura, 1979) in a 15 L bioreactor (NBS Bioflo, 3000, New Brunswick, USA). The microcystin-LR containing extracts (MCE) from cultured cells were prepared as described earlier (Nidhi et al., 2001). Briefly, the lyophilised biomass was sonicated, centrifuged at 30,000×*g*, the supernatant was passed through 0.22 μm Millipore filter and lyophilised. This extract is termed as MC-LR containing extracts. Microcystin-LR content in the MCE was determined by HPLC according to the method of Harada et al. (1988) after comparison with authentic standard.

2.4. Experimental protocol

To evaluate the possible effects of age on microcystin induced toxicity, experiments were conducted with mice

Table 1
Effect of intraperitoneal administration of 1.0 LD_{50} MC-LR (43 μg/kg body mass) on serum enzymes in different age groups of mice

Age in weeks	Group	LDH (IU/L)	AST (IU/L)	ALT (IU/L)	γ-GT (U/L)	SDH (U/mL)
6	Control	630.7±64.7[a]	28.3±5.7[a]	30.3±2.6[a]	3.9±0.1[a]	122.5±5.1[a]
	Treated	1390.5±142.3[a]	158.8±20.0[b]	360.1±23.7[c]	38.9±2.5[b]	270.0±17.7[b]
12	Control	640.0±140.4[a]	30.7±1.9[a]	34.8±1.4[a]	4.6±0.7[a]	118.6±10.3[a]
	Treated	2890.1±372.3[b]	250.9±20.9[c]	320.2±50.4[bc]	40.8±6.6[b]	251.2±36.2[b]
18	Control	738.0±137.4[a]	36.4±2.2[a]	31.2±0.8[a]	4.1±0.4[a]	121.0±2.3[a]
	Treated	2910.0±349.4[b]	336.2±31.5[d]	242.0±41.9[bc]	45.0±9.1[b]	297.1±14.7[b]
24	Control	661.5±68.7[a]	36.2±3.3[a]	34.2±1.3[a]	4.6±0.2[a]	134.4±9.6[a]
	Treated	3084.0±178.0[b]	177.8±19.0[b]	203.4±27.2[b]	44.3±3.7[b]	346.0±31.3[b]
30	Control	614.4±123.3[a]	30.6±2.4[a]	30.4±7.9[a]	4.7±1.0[a]	121.2±22.4[a]
	Treated	3616.0±234.3[b]	327.4±15.4[d]	300.1±30.8[bc]	51.0±3.7[b]	261.6±46.2[b]
36	Control	542.3±42.3[a]	29.2±0.5[a]	28.7±2.2[a]	4.2±0.5[a]	116.0±25.2[a]
	Treated	4499.0±451.3[c]	286.6±16.2[cd]	347.1±12.2[c]	50.3±2.5[b]	335.4±12.6[b]

Values are mean±S.E. of six animals per group. In each column, means followed by same superscript(s) are not significantly different at $p<0.05$ by Student–Newman–Keuls multiple comparison test.