Sex Differences in Susceptibility to Microcystin-LR Toxicity Following Oral Administration in CD-1 Mice

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1 ABSTRACT

Microcystin-LR (MCLR) is a potent hepatotoxin produced during cyanobacterial blooms in freshwater sources. The toxicity of MCLR has been previously characterized in mice, but most of the earlier studies were conducted in a single sex, most studies were conducted by the intraperitoneal route of administration and, in some cases, the purity of MCLR was questionable. In order to better characterize the toxicity of MCLR in mice using a human-relevant route of administration, groups of 10 male and female CD-1 mice were administered MCLR via oral gavage at dose levels of 0, 3000, and 5000 µg/kg/day for up to 7 days. There were two early deaths in females at 5000 µg/kg/day after a single dose, which resulted in the high-dose level being reduced to 4000 µg/kg/day on Day 2. Thereafter, mortality was limited to a 3000 µg/kg/day male mouse on Day 4 and a 5000/4000 µg/kg/day female mouse on Day 7. MCLR-related changes in clinical chemistry parameters at termination included significant elevations in AST (up to 4.1X in males and 13.0X in females) and ALT (up to 10.7X in males and 24.8X in females) compared to the respective controls and were more pronounced in female mice. Histopathological findings in early death animals included marked hemorrhage and necrosis in the liver. Histopathological findings in mice that survived to scheduled euthanasia included dose-dependent centrilobular hepatic necrosis, hepatocellular degeneration, and hepatocellular hypertrophy. To the authors' knowledge, this is the first published report of a greater sensitivity to MCLR-induced toxicity in female mice compared to male mice following oral MCLR exposure.

2 METHODS AND MATERIALS

All in vivo study procedures were approved by the Testing Facility's Institutional Animal Care and Use Committee. MCLR was supplied by Beagle Bioproducts Inc. Male and female CD-1 mice (Charles River Laboratories, Raleigh, NC) were 7 weeks of age at the initiation of dosing. Animals were individually housed in cages equipped with an automatic watering valve and provided PMI Nutrition International Certified Rodent Chow No. 5CR4 (14% protein) ad libitum.

Groups of 10 male and female mice were administered MCLR at 0, 3000, or 5000 µg/kg/day by oral gavage. Due to mortality observed after the first dose, the high-dose level was reduced from 5000 to 4000 µg/kg/day on Day 2. General health/mortality and moribundity checks were conducted twice daily, cage side observations were conducted daily at 1 to 3 hours post-dose, and detailed clinical observations were conducted on the day of randomization (Day -4), on Day 1, and on Day 8. Body weights were recorded on Days -4, 1, and 8.

Blood was collected from the vena cava under isoflurane anesthesia on Day 8, processed to serum, and clinical chemistry parameters were evaluated. Study animals were subjected to a complete necropsy examination, which included evaluation of the carcass and musculoskeletal system, all external surfaces and orifices, cranial cavity and external surfaces of the brain, and thoracic, abdominal, and pelvic cavities with their associated organs and tissues. Representative samples of the epididymis, prostate gland, seminal vesicles, kidney, colon, liver, ovaries, small intestine (jejunum), testes, and uterus were collected from all animals, fixed, and processed to slide for histopathologic evaluation.

3 RESULTS (Continued)

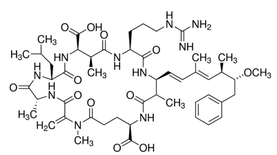
MCLR-related mortality was observed following the first (and only) dose at 5000 µg/kg/day, with 2 females found dead on Days 1 or 2. Consequently, the high dose was reduced to 4000 µg/kg/day on Day 2. On Day 4, a 3000 µg/kg/day male was found dead and on Day 7, a 5000/4000 µg/kg/day female was euthanized moribund. The cause of death was attributed to marked hemorrhage and hepatocellular necrosis.

Observed clinical signs were minimal. Males given MCLR at 5000/4000 µg/kg/day had a decrease in mean body weight gain and a Day 8 mean body weight that was ~7% lower than that of controls. There were no clear MCLR-related effects on body weight or body weight gain at 3000 µg/kg/day or in females at 5000/4000 µg/kg/day.

There were MCLR-related elevations in AST (up to 4.1X in males and 13.0X in females), ALT (up to 10.7X in males and 24.8X in females), ALP (up to 2.9X in males and 2.2X in females), bilirubin (up to 4.1X in males and 2.3X in females), and, in females, cholesterol (up to 1.5X), compared to the controls. The changes were generally dose related in males but not in females, due in part to the observed variability.

Test article-related gross pathology findings were limited to the pale discoloration of the liver in 1 female at 3000 µg/kg/day and in 2 males at 5000/4000 µg/kg/day that correlated with hepatocellular degeneration and necrosis microscopically. MCLR administration resulted in dose-dependent minimal to mild hepatocellular hypertrophy in the liver at ≥ 3000 µg/kg/day in both sexes, minimal to moderate degeneration and minimal to marked necrosis of hepatocytes at ≥ 3000 µg/kg/day in both sexes, and mild hemorrhage in males at 5000/4000 µg/kg/day and marked hemorrhage in early death animals. In general, the histopathologic findings were of a greater severity in early death animals.

3 RESULTS



Structure of MCLR

Table 1. Fold changes Relative to Control on Day 8 for Selected Clinical Chemistry Parameters in CD-1 Mice Administered MCLR

Sex	Dose Level (µg/kg/day)	AST (U/L)	ALT (U/L)	ALP (U/L)	Total bilirubin (mg/dL)	Cholesterol (mg/dL)
Males	3000	1.9*	3.5**	1.5	1.4	1.3**
	5000/4000	4.1***	10.7***	2.9**	4.1***	1.1
Females	3000	13.0**	24.8**	2.2***	2.3*	1.4**
	5000/4000	12.1*	13.5*	1.8*	1.6***	1.5**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table 2. Summary of Histopathology Findings in Liver

Finding	Males			Females		
Dose Level (µg/kg/day)	0	3000	5000/4000	0	3000	5000/4000
Hepatocellular hypertrophy	(0)	(7)	(10)	(0)	(2)	(6)
Minimal	-	6	1	-	2	3
Mild	-	1	9	-	0	3
Degeneration, hepatocellular	(0)	(5)	(10)	(0)	(4)	(7)
Minimal	-	4	2	-	2	0
Mild	-	1	8	-	2	6
Moderate	-	0	0	-	0	1*
Necrosis, hepatocellular	(0)	(6)	(7)	(0)	(3)	(5)
Minimal	-	5	2	-	1	2
Mild	-	0	3	-	0	0
Moderate	-	0	2	-	2	1*
Marked	-	1*	0	-	0	2*
Hemorrhage	(0)	(1)	(2)	(0)	(0)	(2)
Minimal	-	0	0	-	-	0
Mild	-	0	2	-	-	0
Marked	-	1*	0	-	-	2*

* Early death

4 CONCLUSIONS

Others have reported nephrotoxicity, gastrointestinal toxicity, and reproductive tract toxicity following oral or intraperitoneal administration of MCLR. In this study, there were no meaningful histopathologic findings in the kidneys, epididymis, prostate, seminal vesicles, testes, ovaries, uterus, small intestine, or colon, nor were there any meaningful clinical chemistry findings indicative of target organs other than the liver.

The oral LD50 for MCLR has been variously reported as 3000 µg/kg, 5000 µg/kg, and 10,000 µg/kg. However, most of the earlier studies were conducted in a single sex, the strains that were used differed from study to study and, in some cases, the purity of the MCLR was likely questionable. The results of the current study indicate that for CD-1 mice, the oral LD50 is > 5000 µg/kg, and that female mice are more sensitive than male mice.

A plausible explanation for the observed sex difference in mortality following oral administration of MCLR is the difference in glutathione S-transferase (GST) activity and glutathione (GSH) levels in male and female CD-1 mice. The principal means of MCLR detoxification is via the formation of GSH conjugates, which in rodents can occur spontaneously in the presence of GSH or can be catalyzed by GSTs. Female CD-1 mice have been shown to have both lower constitutive GST activity and lower levels of GSH than male CD-1 mice, which would predispose CD-1 female mice to MCLR-related hepatotoxicity following an oral dose.