SELECTIVE HEPATIC TOXICITY AND PROTEIN PHOSPHATASE INHIBITION OF MICROCYSTIN IN THE LITTLE SKATE *RAJA ERINACEA*

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Microcystins (mcyst) are cyclic heptapeptides that are potent hepatotoxins. They are produced by a variety of cyanobacteria in both freshwater and marine environments. Their mode of action is the specific inhibition of ser/thr protein phosphatases (PP) 1 and 2A. Animal or human exposure to water contaminated with mcyst results in death, with the liver being the target organ (Jochimsen, E.M. et al., *New England J. Med.* 338:873-878, 1998; Pouria, S. et al., *Lancet* 352:21-26, 1998). Previously we have shown that the little skate, *Raja erinacea* is very sensitive to the toxic effects of mcyst (Runnegar, M. et al., *MDIBL Bull.* 36:77-80, 1997; and 37:9-12, 1998). Here we report that, as in mammals, mcyst is liver specific, and we characterize the hepatic lesion caused by mcyst. We also examine the persistence of mcyst in the circulation of the skate.

Injection of mcyst in the tail vein was used to determine the in vivo toxicity of mcyst to skates, blood samples were collected before dosing and as indicated. After sacrifice, the liver, kidneys and rectal glands were taken for histology and determination of PP activity. The presence of toxic mcyst in plasma was determined by the quantitation of PP inhibition from diluted plasma and comparison with a mcyst standard.

Varying concentrations of mcyst were added to suspensions of skate hepatocytes or to shark rectal gland cells grown in culture (Cantiello, H.F. et al., *Am. J. Physiol.* 272:C466-C475, 1997) Incubations were carried out at 15^0C. Ser/thr PP activity of cells lysates or tissue extracts was determined as in Runnegar, M. et al., (*J. Pharmacol. Exp. Ther.* 273: 545-543, 1995).

When mcyst was given in vivo to skates by i.v. injection, there were dose dependent histolological changes (Table 1). Lower doses led to limited inflammatory changes, while at higher doses hepatocyte necrosis was seen. As in mice, histological damage of liver was only found when there was profound PP inhibition which was unchanged over a period of 7 days. At the higher doses there was some decrease in PP activity of kidney, but this change was not statistically significant. Histologically the kidneys showed some vacuolization of the proximal tubules, but this was sporadic and did not correlate with the hepatic damage. The histology and PP activity of rectal glands of all skates were unchanged.

As shown in Figure 1, seven days after dosing with 0.125-0.500 mg mcyst/kg, PP inhibiting activity of mcyst could still be detected in skate plasma. Figure 2 shows the percentage of dose present in plasma at 24 hr. At higher doses of mcyst a greater percentage of the dose was still present in the circulation, suggesting that the extensive liver damage seen in these animals results in impaired clearance of the peptide from plasma to the liver.

As in other species the liver is the target organ for in vivo mcyst toxicity. This specificity is reflected in vitro: PP activity of hepatocytes was inhibited by one hr exposure to 1 μM mcyst. In contrast, cultured shark rectal gland cells were insensitive to mcyst: incubations for 24 hr with 10 μM mcyst resulted in no significant inhibition of PP activity or detachment of cells. Calyculin A, a cell permeant PP inhibitor of equivalent potency to mcyst, caused profound PP inhibition (to 11% of controls) of rectal gland cultures with cell detachment reflecting the cytoskeletal changes that accompany PP inhibition (Runnegar, M. et al., *J. Pharmacol. Exp. Ther.* 273: 545-543, 1995).

Table 1 Toxicity of mcyst in skates 24 hr after dosing.

Mcyst Dose mg/kg body weight	PP activity per mg protein at 24 hr as % of Control		Histological findings in liver
	Liver	Kidney	
0.032	92±29	159±26	not done
0.063	15±7*	126±42	not done
0.125	1±1*	88±9	mild liver injury with portal and periportal inflammatory changes
0.250	1±1*	76±9	moderate liver injury with more pronounced portal and periportal inflammatory changes, some hepatocyte necrosis
0.500	1±1*	82±21	severe liver injury with widespread inflammatory changes and extensive hepatocyte necrosis

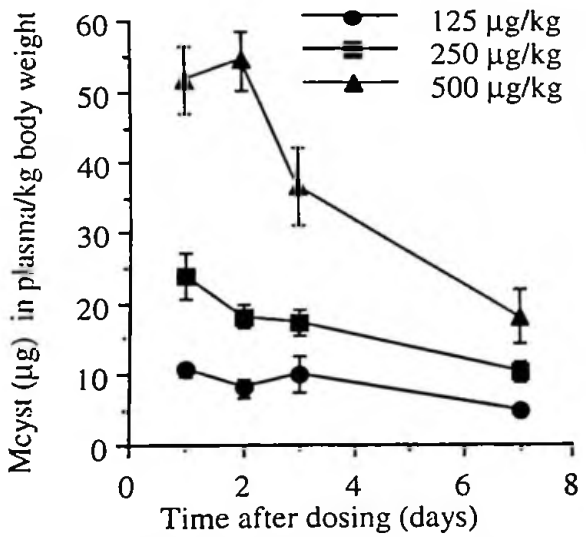

Figure 1. Time and dose dependence of mcyst persistence in plasma.

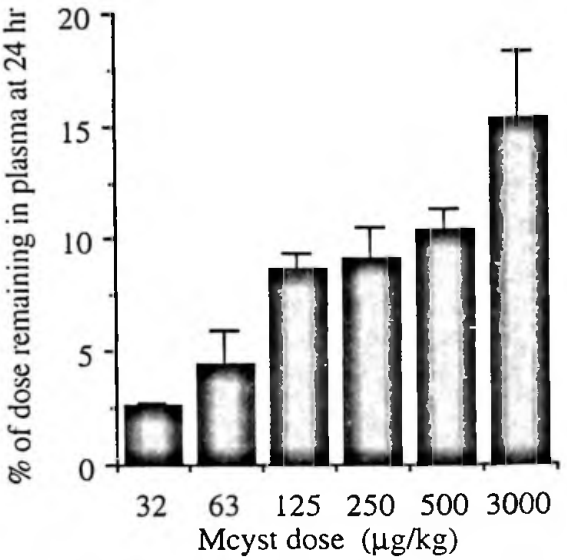

Figure 2. Mcyst remaining in plasma 24 hr after dosing as percentage of administered dose. Mcyst levels were assayed using PP inhibition.

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