Brazilian Journal of Medical and Biological Research (1999) 32: 985-988
ISSN 0100-879X Short Communication

Effects of microcystin-LR in isolated perfused rat kidney

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Abstract

Microcystin is a hepatotoxic peptide which inhibits protein phosphatase types 1 and 2A. The objective of the present study was to evaluate the physiopathologic effects of microcystin-LR in isolated perfused rat kidney. Adult Wistar rats (N = 5) of both sexes (240-280 g) were utilized. Microcystin-LR (1 µg/ml) was perfused over a period of 120 min, during which samples of urine and perfusate were collected at 10-min intervals to determine the levels of inulin, sodium, potassium and osmolality. We observed a significant increase in urinary flow with a peak effect at 90 min (control (C) = 0.20 ± 0.01 and treated (T) = 0.32 ± 0.01 ml g^{-1} min^{-1}, $P<0.05$). At 90 min there was a significant increase in perfusate pressure (C = 129.7 ± 4.81 and T = 175.0 ± 1.15 mmHg) and glomerular filtration rate (C = 0.66 ± 0.07 and T = 1.10 ± 0.04 ml g^{-1} min^{-1}) and there was a significant reduction in fractional sodium tubular transport at 120 min (C = 78.6 ± 0.98 and T = 73.9 ± 0.95%). Histopathologic analysis of the perfused kidneys showed protein material in the urinary space, suggestive of renal toxicity. These data demonstrate renal vascular, glomerular and urinary effects of microcystin-LR, indicating that microcystin acts directly on the kidney by probable inhibition of protein phosphatases.

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Research supported by CAPES and CNPq.

Received April 14, 1998
Accepted March 29, 1999

Key words
• Microcystin-LR
• Kidney
• Perfused kidney

Members of the cyanobacterial genera *Microcystis*, *Oscillatoria* and *Anabaena* produce cyclic peptides, denoted microcystins, which are potent hepatotoxins. These substances are responsible for the death of fish, birds, wild animals and agricultural livestock in many countries where freshwaters contain toxic cyanobacterial blooms, and the adverse effects of these toxins on human health have been recognized (1,2).

Microcystin-LR (MCLR) is a highly hepatotoxic heptapeptide in mice and rats, which consists of two variable L-amino acids, i.e., leucine and arginine, three D-amino acids and the two unusual amino acids, N-methyldehydroalanine and 3-amino-9-methoxy-10-phenyl-2,6,8-trimethyl-deca-4(E),6(E)-dienoic acid (Adda) (3,4). MCLR has been shown to inhibit significantly the catalytic subunits of only two specific types of protein phosphatases, protein phosphatase 1 (pp-1c) and protein phosphatase 2A (pp-2Ac) (5-8). The purpose of the present study was to evaluate and compare the physiopathologic effects of microcystin-LR (Sigma Chemical Co., Saint Louis, MO, USA; molecular mass

995.2) from *Microcystis aeruginosa* on the isolated perfused kidney without blood cells.

Adult Wistar rats of both sexes weighing 240-280 g were anesthetized with sodium pentobarbital (50 mg/kg body weight *ip*). Twenty percent mannitol was administered intravenously just before the right ureter was cannulated with polyethylene (PE50) tubing. The right renal artery was cannulated with a 19-gauge blunt disposable needle passed through the superior mesenteric artery as described by Bahlmann et al. (9), Nishiitsutji-Uwo et al. (10) and Ross (11). The excised right kidney was perfused without interruption of kidney flow at 37°C with Krebs-Henseleit buffer containing 6 g/l bovine serum albumin (BSA fraction V, Sigma). The perfusion solution was dialyzed for 48 h at 4°C in 1.5 l of Krebs to reduce its contamination, and its pH was adjusted to 7.4. The total perfusate used per experiment was 100 ml and contained: 147 mmol/l Na^+, 5 mmol/l K^+, 2.5 mmol/l Ca^{2+}, 2 mmol/l Mg^{2+}, 110 mmol/l Cl^-, 2.5 mmol/l HCO_3^-, 1 mmol/l SO_4^{2-}, 1 mmol/l PO_4^{3-}, 0.075 g urea, 0.075 g inulin and 0.15 g glucose. The perfusion system was based on Bowman's technique (12) as modified in our laboratory by the addition of an artificial lung to improve oxygenation and of a 1.2-µm millipore filter (13). The flow rate of perfusion was maintained at 25-35 ml/min per kidney. The preparation was allowed to equilibrate for 15-20 min before the study period, which lasted 120 min. Perfusion pressure and resistance were measured at 5-min intervals. Samples of the perfusate and urine were collected every 10 min for the determination of sodium, potassium, inulin and osmolality and the results were averaged every 30 min. Sodium and potassium concentrations were determined by flame photometry (Flame Photometer Model 445) and inulin was determined according to Walson et al. (14). Fractional sodium tubular transport (%TNa^+) was calculated by the method of Pitts (15). The osmolality of the samples was measured with an Advanced Instrument osmometer (Needham Heights, MA, USA). After renal perfusion, the kidneys were evaluated histologically by light microscopy. The data are reported as mean ± SEM for three periods of 10 min each at 30, 60, 90 and 120 min. In each group, the first 30 min of perfusion were considered to be an internal control. The perfusion pressure (PP), urinary flow (UF), glomerular filtration rate (GFR) and %TNa^+ remained stable in the control preparations throughout the 120-min perfusion. The data were analyzed by the Student *t*-test, with the level of significance set at $P<0.05$.

Microcystins induce severe hepatic hemorrhage leading to shock (16). Gross macroscopic examination suggests that all the visceral organs are affected and the animal perhaps succumbs to multiple organ failure. However, except for the liver, gross histological examination of other visceral organs does not show any consistent abnormalities (17). Some conspicuous effects of microcystin-LR on carp kidney have been reported by Raberg et al. (18). These changes were apparently more severe than the relatively mild effects on kidney observed in mice (19-21). In addition, Kotak et al. (22) demonstrated kidney lesions in the fish (*Oncorhynchus mykiss*), consisting of coagulative tubular necrosis with dilation of Bowman's space. Bhattacharya et al. (23) showed a dose- and time-dependent elevation in plasma urea and creatinine levels with a concomitant decrease in total protein and albumin levels in rats. In view of these diverse observations, we have examined the potential nephrotoxicity of microcystin-LR in isolated perfused rat kidney, which permits the study of direct effects of toxin in the absence of systemic effects.

The infusion of 1 µg/ml microcystin-LR, which corresponds to 1 µM, after 30 min of an internal control caused a significant increase in perfusion pressure at 90 min (control (C) = 129.7 ± 4.81 and treated (T) = 175.0 ± 1.15 mmHg, $P<0.05$). Urinary flow was also significantly increased with a maxi-

mal effect at 90 min (C = 0.20 ± 0.01 and T = 0.32 ± 0.01 ml g^{-1} min^{-1}, P<0.05). GFR also increased at 90 min (C = 0.66 ± 0.07 and T = 1.10 ± 0.04 ml g^{-1} min^{-1}, P<0.05). $\%TNa^+$ was 78.6 ± 0.98 during the first 30 min of the internal control period and decreased significantly to 73.9 ± 0.95 (P<0.05) at 120 min of perfusion after administration of the toxin (Table 1).

Table 1 - Effects of microcystin-LR on perfused rat kidney.

The kidneys were perfused with 1 µM microcystin-LR (Mcyst) in modified Krebs-Henseleit solution and control conditions. Data are reported as mean ± SEM for 5 rats. *P<0.05 compared to control (Student *t*-test). PP, Perfusate pressure; GFR, glomerular filtration rate; UF, urinary flow; $\%TNa^+$, fractional sodium tubular transport.

Time	PP (mmHg)	GFR (ml g^{-1} min^{-1})	UF (ml g^{-1} min^{-1})	$\%TNa^+$
30 min				
Control	118.3 ± 0.01	0.73 ± 0.06	0.15 ± 0.01	81.3 ± 0.16
Basal Mcyst	129.7 ± 4.81	0.66 ± 0.07	0.20 ± 0.01	78.6 ± 0.98
60 min				
Control	119.0 ± 0.58	0.72 ± 0.03	0.16 ± 0.01	82.0 ± 0.46
Mcyst	160.7 ± 8.69*	1.03 ± 0.05*	0.28 ± 0.02*	78.8 ± 1.14
90 min				
Control	116.9 ± 0.10	0.74 ± 0.05	0.16 ± 0.01	81.0 ± 0.44
Mcyst	175.0 ± 1.15*	1.10 ± 0.04*	0.32 ± 0.01*	75.9 ± 0.678*
120 min				
Control	115.6 ± 1.69	0.74 ± 0.05	0.16 ± 0.01	80.6 ± 0.32
Mcyst	175.3 ± 1.45*	1.03 ± 0.03*	0.31 ± 0.02*	73.9 ± 0.95*

Histological evaluation of kidneys treated with microcystin-LR showed an intense amount of protein in the urinary spaces. The increase in perfusion pressure was followed by a significant increase in glomerular filtration rate. The time course of the effect of microcystin-LR on perfusion pressure coincided with that for glomerular filtration rate, suggesting that the latter effect is followed by an increase in perfusion pressure. This increase in perfusion pressure was probably responsible for the increase in glomerular pressure and, consequently, the increase in urinary flow. If microcystin-LR had a direct effect on the glomeruli we would expect a lesion resulting in an increase of glomerular filtration rate with a further increase in urinary flow rate. Sodium excretion depends on the relationship between glomerular filtration rate and sodium reabsorption rate. In the present experiments, the fractional sodium tubular transport was reduced in the microcystin-LR-treated group, this effect probably occurring in the proximal renal tubules. An important component of the glomerulus is the mesangium. Mesangial cells exhibit phagocytic activity and secrete prostaglandins and most of them also contain myofilaments and can contract in response to a variety of stimuli. Since mesangial cells are located in close contact with glomerular capillaries, they may influence the glomerular filtration rate by regulating blood flow through the capillaries (24). The intense amount of protein material was observed in the glomeruli of the toxin-treated group, probably because of toxic effects of the microcystin-LR on the glomeruli and tubules or due to an increase in vascular permeability due to endothelial injury. The present observations are the first demonstration of a direct specific effect of microcystin-LR, with alterations of renal functional parameters in the isolated perfused rat kidney in the absence of systemic effects. These renal effects are probably caused by inhibition of protein phosphatases.

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