Research Article

Antioxidant Effect of Curcumin Against Microcystin-LR-Induced Renal Oxidative Damage in Balb/c Mice

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Abstract

Purpose: To investigate the effect of curcumin on microcystin-LR (MC-LR)- induced renal oxidative damage in Balb/c mice.

Methods: 40 male Balb/c mice were assigned randomly to 4 groups each having 10 mice. One group served as normal (saline treated) while another group was used as curcumin control. The third group was given MC-LR and used as toxin control. The fourth group was pre-treated with curcumin (300 mg/kg body wt) given orally once daily for 14 days before interperitoneal injection (i.p) of MC-LR (75 µg/kg body wt). Biochemical assays including serum creatinine, blood urea nitrogen (BUN), urinary glucose, gamma-glutamyl transferase (GGT) and catalase (CAT) levels were measured. Renal biochemical tests such as protein carbonyl contents and DNA-protein cross-links, glutathione peroxidase (GSH-Px) activity, glutathione (GSH) and lipid hydroperoxide (LOOH) were evaluated.

Results: Serum creatinine, BUN, urinary glucose, GGT increased in mice treated with MC-LR, while creatinine clearance decreased compared to controls (p < 0.001), indicating occurrence of tubular damage. There was increased protein carbonyl content and DNA-protein cross-links in the kidney homogenates of these mice. Curcumin administration significantly reversed these effects and attenuated the MC-LR-induced reduction in the activities of CAT, GPH-Px, GSH as well as the MC-LR-induced increases in plasma and kidney lipid hydroperoxide.

Conclusion: These results indicate that curcumin possesses natural antioxidant properties that renders it a potent protective agent against renal oxidative damage mediated by microcystin-LR.

Keywords: Antioxidants, Curcumin, Microcystin, Renal oxidative damage

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INTRODUCTION

Contamination of aquatic ecosystems as a consequence of human activities is a well-established fact [1]. One direct effect of pollution processes is the production of cyanobacterial blooms, some of which are characterized by the elaboration of toxins [2]. One of such toxins is MC-LR, produced principally by *Microcystis aeruginosa* (*M. aeruginosa*). It is a potent hexapeptide hepatotoxin known to induce severe intrahepatic haemorrhaging necrosis and apoptosis [3,4]. MC-LR specifically inhibits serine/threonine protein phosphatases (PP1 and 2A), resulting in the disruption of many important cellular processes [5]. The mechanism of MC-LR- induced nephrotoxicity is not completely understood. However, studies have implicated ROS particularly superoxide anion in the pathophysiology of MC-LR-induced nephropathy [6].

Recently, a number of several natural antioxidants have been evaluated for their chemopreventive effects in various pathological states [7]. Curcumin (diferuloyl methane) is a phenolic phytochemical compound responsible for the yellow colour of turmeric (*Curcuma longa* Linn), a common ingredient in South Asia cookery and medical concoctions. It was defended as a non-vitamin active antioxidant that has a high oxygen-radical scavenging and quenching capacity [8].

It is very useful in living tissues for reduction of the risk of adverse oxidative reactions that are produced by hydroxyl radicals and peroxides. In fact, little or no attention has been paid to the use of naturally occurring substances with potent antioxidant properties to protect against nephrotoxic damage induced by MC-LR. In the light of this, we have explored the possible protective role of curcumin as a natural antioxidant substance on MC-LR-induced oxidative stress. Specifically, therefore, this study was designed to investigate the role of curcumin pre-treatment in protection against MC-LR-induced renal oxidative damage in Balb/c mice.

EXPERIMENTAL

Chemicals

Curcumin (*Corma longa*, Linn) was purchased from Sigma Chemicals (USA). All other reagents used were of analytical grade purity and were supplied by Sigma Chemicals, USA, unless otherwise specified.

Animals

Male Balb/c mice (6-7 weeks old, average body wt.30 g) were used in this study. They were maintained on standard laboratory diet and tap water *ad libitum* throughout the experiment. The animals were housed in stainless metal cages under a 12 h light cycle and room temperature. The experiment was approved by the Ethics Committee for Animal Experimentation of the University of Yarmouk/ Jordan (Ethics No. Y/J-1771/2008). The procedures involving animals and their care conformed to the standard guideline for the use of laboratory animals [9].

Toxin extraction

M. aeruginosa cells were collected from selected sites of Al-Gohr area in Jordan twice a month. Isolated cells were cultivated in a culture medium as recommended by Lehtimaki [10]. Microcystin was extracted from the freeze-dried cells using the method of Mazur and Plinski [11]. The LD_{50} of the toxin extract was determined according to the up-down method described by Fawell et al. [12].

Toxicological studies

Forty mice were divided randomly into 4 groups (10 mice each). Group 1 was the control group in which the mice received neither toxins nor curcumin supplementation. Group 2 received curcumin supplementation (300 mg/kg body wt/day) orally for 14 days

prior to sacrifice. Group 3 mice received the same dose of curcumin for 14 days followed by a single i.p. dose of MC-LR (75 µg/kg body wt). Mice in group 4 received a single i.p. dose of toxin (75 µg/kg body wt) only. Mice in all the groups were sacrificed on the 15th day by cervical dislocation. Prior to sacrifice, 24 h urine free of food and feces was collected from each group into ice-cold graduated cylinders for the determination of protein, creatinine, glucose and gamma-glutamyl transferase activity.

Mice were sacrificed by cervical dislocation and the kidneys were excised and washed in ice-cold saline. These were homogenized in ice-cold 0.1M Tris-HCl buffer (pH 7.4) using Ultra homogenizer. The homogenates were first centrifuged at 10,000 xg for 15 min and the supernatants were further centrifuged at 100,000 xg for 1h. Supernatant (cytosolic fraction) was recovered and the protein concentration was determined, aliquoted and used for the determination of enzymatic activities. Blood was obtained by cardiac puncture technique into centrifuge tubes and allowed to clot, after which serum was recovered by centrifugation for 10 min at 3000 xg. Heparinised blood samples were centrifuged at 1500 xg for 10 min to obtain plasma.

Blood urea nitrogen (BUN), creatinine, and urinary glucose were determined using commercial kits. Gamma-glutamyl transferase was determined by the method of Clarkson and Thompson [13] while catalase (CAT) and superoxide dismutase (SOD) activities were measured according to the previously described method [14]. Superoxide dismutase (SOD) activity was determined by measuring the inhibition of autoxidation of epinephrine at pH 10.2 and 30°C. Glutathione peroxidase (GSH-Px) activity was determined in kidney homogenates by measuring the oxidation of NADPH at 340 nm, pH 7.0 and 37°C [15]. Glutathione (GSH) was determined in the 10,000 xg supernatant fraction of the kidney homogenate according to the method of

Misra [16]. Lipid peroxidation was measured as lipid hydroperoxide (LOOH) in both plasma and kidney by the method of Gunzler and Flohe [17]. Protein carbonyl contents were determined as previously described [18]. Protein content of all samples was determined by the method of Biuret using bovine serum albumin as a standard while DNA-protein cross-links were assayed by the method of Zhikovich and Costa [19].

Statistical analysis

All values were expressed as mean ± S.E.M. The statistical significance of differences between groups was assessed using one-way analysis of variance (ANOVA). The Mann-Whitney U test was used to compare the differences between two groups. A value of $p < 0.05$ was considered significant. Statistical analysis was performed using SPSS 16.0 for Windows software (SPSS Inc, Chicago, IL, USA).

RESULTS

Table 1 shows that the liver weight of MC-LR-treated mice increased as compared to control group or curcumin group mice. Serum creatinine and BUN levels significantly ($p < 0.05$) increased in mice after MC-LR treatment while creatinine clearance decreased compared to controls. Treatment of mice with curcumin before and simultaneously with MC-LR prevented the MC-LR-induced increase in BUN and serum creatinine ($p<0.001$). Furthermore, curcumin ameliorated the MC-LR mediated decrease in the creatinine clearance. It has also been revealed that mice treated with MC-LR revealed high levels of urinary protein, glucose, gamma-glutamyl transferase activity and protein carbonyl contents ($p < 0.001$) in comparison with control group.

Furthermore, treatment of mice with MC-LR decreased the activities of CAT, GSH-Px, SOD and GSH, but on administration of curcumin significantly reversed the MC-LR-induced decreases (Table 2). The MC-LR

treated mice showed a significant increase in the contents of DNA-protein cross-links when compared to control mice. On administration of curcumin (300 mg/kg/day) for 14 days, a dramatic reduction in DNA-protein cross-links was noticed.

Table 3 shows the effects of curcumin on MC-LR-induced renal and plasma lipid hydroxyperoxide in the presence and absence of exogenous oxidants (1mM $FeSO_4$, 1mM ascorbate, 0.2mM H_2O_2). Lipid peroxidation induced by MC-LR in the presence of oxidants was more pronounced than in their absence ($p < 0.001$). Curcumin reduced the MC-LR- mediated increase in LOOH formation in mice plasma and kidney

Table 1: Effect of curcumin supplementation on liver weight and some clinical parameters in Balb/c mice given a single dose of MC-LR

Parameter	Control	MC-LR	Curcumin	MC-LR + curcumin
Liver weight (g)	1.52 ± 0.03	2.88 ± 0.06*	1.49 ± 0.02	1.71 ± 0.03
Urinary protein (mg/24h)	18.6 ± 0.9	31.2 ± 1.61*	16.6 ± 2.1	22.4 ± 2.1**
Glucose (mg/24h)	8.4 ± 1.1	48.6 ± 2.1*	6.3 ± 1.1	10.6 ± 1.8**
Creatinine clearance (ml/min/100g)	0.52 ± 0.003	0.08 ± 0.001*	0.51 ± 0.004	0.46 ± 0.003**
Creatinine (mg/dl)	0.52 ± 0.003	2.41 ± 0.010*	0.38 ± 0.001	0.83 ± 0.008
BUN (mg/dl)	31.8 ± 3.2	84.8 ± 9.1*	30.6 ± 3.1	44.2 ± 3.8
GGT (U/mg)	118 ± 11.4	786 ± 31.8*	116 ± 14.2	381 ± 11.6**
Protein carbonyl (nmol/mg protein)	0.66 ± 0.006	1.84 ± 0.04*	0.61 ± 0.008	0.82 ± 0.01

*All values are mean ± S.E.M (n = 10);. *significantly different from control and/or curcumin group ($p \leq 0.001$); **significantly different from MC-LR group ($p \leq 0.001$).*

Table 2: Effect of curcumin on the levels of glutathione and antioxidant enzymes of Balb/c mice treated with MC-LR

Animal group	GSH (nmol/mg)	SOD (U/mg)	CAT (U/mg)	GSH-Px (U/mg)	DNA-protein cross-links (%)
Control	4.6 ± 0.4	48.8 ± 1.7	28.1 ± 3.1	1.8 ± 0.1	0.9
MC-LR (75 µg/kg)	1.2 ± 0.1*	36.6 ± 6.0	16.2 ± 2.1*	0.9 ± 0.2*	9.7
Curcumin (300 mg/kg/day)	4.4 ± 0.1	48.1 ± 3.3	30.8 ± 2.8	2.0 ± 0.4	0.7
MC-LR + curcumin	3.1 ± 0.2**	44.4 ± 6.2	23.8 ± 3.3**	1.5 ± 0.2**	2.7

*Results are mean ± S.E.M; *significantly different from control group ($p \leq 0.001$); **significantly different from MC-LR group ($p \leq 0.01$).*

Table 3: Effect of curcumin on lipid hydroperoxide formation in plasma and kidney of Balb/c mice treated with MC-LR

Animal group	LOOH in plasma (nmol/mg protein)		LOOH in kidney (nmol/mg protein)	
	Absence of oxidants	*Presence of oxidants*	*Absence of oxidants*	*Presence of oxidants*
Control	210 ± 33.7	301 ± 38.8*	225 ± 36.6	336 ± 40.6*
MC-LR (75µg/kg)	808 ± 48.6**	998 ± 51.8*	886 ± 48.9**	1220 ± 71.8*
Curcumin (300 mg/kg/day)	212 ± 39.6	313 ± 50.6**	227 ± 34.2	356 ± 43.6**
MC-LR + curcumin	401 ± 42.8	681 ± 55.1*	428 ± 51.5	666 ± 50.6*

*Values are expressed as mean ± S.E.M; *significantly different from absence of oxidants ($p \leq 0.001$); **significantly different from control ($p \leq 0.001$). Assays were performed in the absence and presence of oxidants (1 mM $FeSO_4$, 1 mM ascorbate, 0.2 mM H_2O_2)*

in the absence of oxidants, but had no effect on MC-LR- induced formation of LOOH in the presence of oxidants in both plasma and kidney (Table 3).

DISCUSSION

M. aeruginosa dominates the cyanobacterial communities of King Talal Reservoir during the warmer season (specifically, from June to October). The major cyanobacterial species found in the samples used in this study was *M. aeruginosa*. Phosphate inhibitory activity revealed the bioactivity of the toxin while spectrophotometric analysis confirmed that the extracted toxin was indeed MC-LR [3]. It has been found that nephrotoxicity is a major complication of MC-LR administration [20].

The results of the present study indicate that MC-LR administration (i.p.) brought about a significant increase in BUN and serum creatinine while creatinine clearance was significantly decreased ($p < 0.001$). MC-LR-treated mice showed increased liver weight and this may be due to massive intrahepatic hemorrhage and pooling of blood in the liver. Curcumin treatment provided marked protective effect and improvement on these parameters.

The apparent increase in urinary protein and glucose as well as urine output indicates proximal tubular dysfunction [21]. Tubular damage was further confirmed by increased urinary excretion of brush border marker gamma-glutamyl transferase suggesting a direct toxic injury. These results may be a consequence of the oxidative stress potential of MC-LR, as demonstrated by significant elevation in lipid peroxidation and decreases in GSH-Px, CAT and SOD activities in the mice kidneys.

Curcumin treatment significantly attenuated the MC-LR-mediated increase in urinary protein and glucose, BUN, serum creatinine and decrease in creatinine clearance as well as the activity of gamma-glutamyl transferase. This effect may be related to the antioxidant properties of curcumin since it has been found that ROS may be involved in the impairment of glomerular filtration rate [22]. Furthermore, curcumin prevented depletion of GSH, GSH-Px, and CAT activity induced by MC-LR treatment. The apparent protective effect might be due to the ability of curcumin to neutralize the increase in free radicals caused by MC-LR [23]. Nishinaka et al. have reported that curcumin acts as a potent antioxidant [24]. It has also been previously reported that curcumin has a protective role against adriamycin-induced renal injury [25] and ferric nitrilotriacetate-induced oxidative renal damage in rats [26]. Thus, the preventive effect of curcumin on the MC-LR-induced reduction in the activity of GSH-Px, CAT and SOD could contribute to the restoration of markers of renal tubular injury.

CONCLUSION

The present study provides evidence that co-administration of curcumin with MC-LR attenuates the increase in lipid peroxidative damage; restores antioxidant status as well as markers of renal injury and urinary excretory indices. The present findings demonstrate that curcumin possesses significant therapeutic effects and is a promising candidate for chemoprevention of MC-LR- induced renal damage

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