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SPECIAL REPORT

Curcumin prevents adriamycin nephrotoxicity in rats

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The present study investigated the effect of curcumin on adriamycin (ADR) nephrosis in rats. The results indicate that ADR-induced kidney injury was remarkably prevented by treatment with curcumin. Treatment with curcumin markedly protected against ADR-induced proteinuria, albuminuria, hypoalbuminaemia and hyperlipidaemia. Similarly, curcumin inhibited ADR-induced increase in urinary excretion of N-acetyl-β-D-glucosaminidase (a marker of renal tubular injury), fibronectin and glycosaminoglycan and plasma cholesterol. Curcumin restored renal function in ADR rats, as judged by the increase in GFR. The data also demonstrated that curcumin protected against ADR-induced renal injury by suppressing oxidative stress and increasing kidney glutathione content and glutathione peroxidase activity. In like manner, curcumin abolished ADR-stimulated kidney microsomal and mitochondrial lipid peroxidation. These data suggest that administration of curcumin is a promising approach in the treatment of nephrosis caused by ADR.
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Abbreviations: ADR, adriamycin; CC, curcumin; FN, fibronectin; GAG, glycosaminoglycan; GFR, glomerular filtration rate; NADH, nicotinamide adenine dinucleotide, reduced form; NADPH, nicotinamide adenine dinucleotide phosphate, reduced form; NAG, N-acetyl-β-D-glucosaminidase; ROS, reactive oxygen species; SA, saline

Introduction The clinical use of adriamycin (ADR) is associated with nephrotic syndrome characterized by heavy proteinuria, albuminuria, hypoalbuminaemia and hyperlipidaemia (Bertani *et al*., 1982; Milner *et al*., 1991). Several lines of evidence suggest reactive oxygen species (ROS) as the principal mediator in the development of nephrosis caused by ADR (Mimnaugh *et al*., 1986; Milner *et al*., 1991). The hypothesis was proposed, that if ADR nephrotoxicity is related to free radical formation and lipid peroxidation then antioxidant therapy may protect ADR toxicity in kidney. Curcumin, the yellow curry pigment isolated from turmeric, represents a class of antiinflammatory (Srivastava & Srimal, 1985) antioxidants (Sharma, 1976) reported to be a potent inhibitor of ROS formation. Administration of curcumin has been reported to prevent renal lesions in streptozotocin diabetic rats (Suresh Babu & Srinivasan, 1998). Curcumin has been also shown to provide protection against oxidative stress in a renal cell line (Cohly *et al*., 1998). In addition, curcumin has been reported to reduce ischaemic renal injury (Shoskes, 1998). More recently, curcumin was shown to protect ADR cardiotoxicity in rats (Venkatesan, 1998). However, the protective effect of curcumin against ADR nephrosis has not been addressed to date. Therefore, the present study was undertaken to evaluate the protective effects of curcumin against ADR nephrotoxicity. Our results reveal here that curcumin suppressed ADR nephrotoxicity in rats.

Methods Healthy male Wistar rats (275 ± 10 g) were divided into four groups of six animals each: Group I, control (SA), was injected with 0.1 ml normal saline (0.9% NaCl). Group II received 200 mg kg^{-1} body weight of curcumin (CC) in 1% gum acacia, orally. Nephrosis (Group III, ADR) was induced by a single injection of ADR (7.5 mg kg^{-1} body weight, dissolved in 0.1 ml saline) through the tail vein. The ADR dosing regimen utilized in this study has been employed by other investigator (Bertani *et al*., 1982). Group IV (CC+ADR) received curcumin 7 days before ADR and daily thereafter throughout the study. All animals had free access to food and water *ad libitum*. Body weight, food intake and urine volume were recorded daily throughout the experimental period (30 days).

Collection of blood and urine Thirty days after ADR, blood was collected in test tubes containing potassium-EDTA (1 mg ml^{-1}) and plasma was separated by centrifugation at $2500 \times g$ for 15 min. Both control and treated groups were kept in individual metabolic cages for 24 h urine collection before sacrifice. Urine was centrifuged at $800 \times g$ for 10 min at 25°C to remove cells and particulate material and then dialyzed against phosphate-buffered saline (PBS), pH 7.4. Whole plasma and urine were stored at -70°C and thawed just before use.

Analytical methods Urinary protein, creatinine and albumin, and plasma albumin were measured according to standard methods. Urine was also analysed for N-acetyl-β-D-glucosaminidase (NAG) (Moore & Morris, 1982), fibronectin (FN) (Rennard *et al*., 1980) and glycosaminoglycan (GAG) (uronic acid content) (Benjelloun *et al*., 1993). The glomerular filtration rate (GFR) was determined by insulin clearance according to standard methods. Plasma cholesterol was measured (Parekh & Jung, 1970). Excised kidneys were analysed for lipid peroxides (Ohkawa *et al*., 1979), glutathione (Moron *et al*., 1979), glutathione peroxidase (Rotruck *et al*., 1973) and total lipids (Folsch *et al*., 1957).

In vitro *lipid peroxidation* In a separate study, microsomal and mitochondrial lipid peroxidation were assayed essentially following the method of Mimnaugh *et al*. (1986). A total of 48 rats were used to isolate microsomes and mitochondria. Kidneys from four rats were pooled to get sufficient amount of microsomal or mitochondrial fractions for these experiments and they were repeated six times. Both the microsomal

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and mitochondrial studies were done immediately. Kidney microsomes or mitochondria (1 mg protein ml^{-1}) were incubated in the absence or presence of 100 μM ADR in KCl-Tris-HCl buffer. Curcumin (1, 10 or 100 μM) was added directly to the incubation mixtures. The reaction was initiated using an NADPH-generating system (microsomes) or NADH (mitochondria). The reaction was terminated after 60 min by adding ice-cold trichloroacetic acid, and lipid peroxidation products were estimated (Mimnaugh *et al*., 1986).

Statistical analysis Results are mean $\pm$ s.d. of six rats. One way analysis of variance (ANOVA) with *post hoc* Bonferroni was used to find statistical significance.

Results Intravenous ADR caused a decrease in body weight gain, however, curcumin treatment significantly ($P<0.001$) attenuated ADR-induced decrease in body weight. As shown in Table 1, ADR rats had elevated urine protein, albumin and NAG levels that were markedly decreased by curcumin. ADR rats exhibited an increase in urinary FN and GAG excretion. Interestingly, ADR rats treated with curcumin had urinary FN and GAG levels that were similar to control groups. ADR-induced reduction in GFR, measured as an index of renal function, was significantly ($P<0.001$) suppressed by curcumin. ADR-induced increase in plasma cholesterol, and a decrease in albumin levels were remarkably attenuated by curcumin. In kidneys exposed to ADR there was a significant increase in lipid peroxides and total lipids while the same was attenuated by curcumin. Similarly, ADR-induced decrease in glutathione content and glutathione peroxidase activity of kidney tissue was suppressed by curcumin. Treatment of microsomes or mitochondria with ADR resulted in a significant ($P<0.001$) increase of lipid peroxidation (Figure 1), as measured by the formation of thiobarbituric acid reactive substances. Interestingly, curcumin inhibited ADR-induced microsomal and mitochondrial lipid peroxidation in a dose-dependent manner.

Table 1 Beneficial effect of curcumin on ADR nephrosis

Parameters	*SA*	*CC*	*ADR*	*CC+ADR*
Body weight (g)	380 ± 21.1	373 ± 28.2	316 ± 18.8#	368 ± 25.3
Urine				
Protein (mg mg^{-1} creatinine)	0.48 ± 0.13	0.45 ± 0.1	52.83 ± 9.21*	2.94 ± 1.17
Albumin (mg mg^{-1} creatinine)	0.37 ± 0.40	0.29 ± 0.1	11.34 ± 2.70*	0.64 ± 0.23
NAG (nmol mg^{-1} creatinine)	152.7 ± 25.5	145.6 ± 18.7	489.8 ± 42.3*	182.9 ± 52.9
Fibronectin (ng 24 h^{-1})	149.9 ± 20.8	153.5 ± 22.0	550.5 ± 50.8*	185.0 ± 52.4
GAG (μg uronic acid 24 h^{-1})	23.2 ± 3.9	21.5 ± 3.2	80.5 ± 6.8*	24.3 ± 5.2
GFR (ml min^{-1} g^{-1})	1.07 ± 0.09	1.01 ± 0.1	0.63 ± 0.08#	0.97 ± 0.13
Plasma				
Albumin (g dl^{-1})	30.5 ± 3.8	31.9 ± 4.7	14.6 ± 3.4#	28.2 ± 2.4
Cholesterol (mg dl^{-1})	62.8 ± 5.2	61.8 ± 6.2	109.1 ± 12.3*	70.7 ± 14.6
Kidney				
Lipid peroxides†	1.81 ± 0.55	1.75 ± 0.45	6.82 ± 1.27*	2.31 ± 1.05
Glutathione (μg mg^{-1} protein)	3.68 ± 0.46	3.84 ± 0.66	0.77 ± 0.29#	3.38 ± 0.44
GPx§	35.0 ± 5.2	36.0 ± 5.8	6.51 ± 2.3#	31.4 ± 4.9
Total lipids (mg g^{-1} tissue)	28.7 ± 2.1	29.3 ± 4.4	57.3 ± 6.0*	32.2 ± 4.9

ADR: adriamycin; CC: curcumin; GAG: glycosaminoglycan; GFR: glomerular filtration rate; GPx: glutathione peroxidase; NAG: N-acetyl-β-D-glucosaminidase; SA: saline. †nmol mg^{-1} protein; §nmol glutathione oxidized min^{-1} mg^{-1} protein; Results are mean ± s.d. of six observations. *Significantly ($P<0.001$) higher than all groups; #significantly ($P<0.001$) lower than all groups.

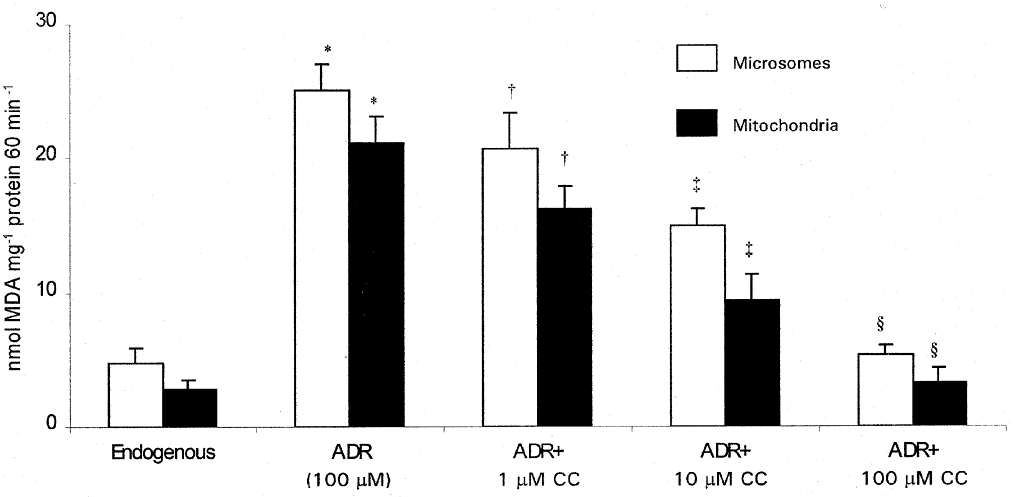

Figure 1 Dose-dependent protective effect of curcumin against ADR-induced microsomal and mitochondrial lipid peroxidation. Microsomes or mitochondria were incubated with NADPH-generating system or NADH, respectively in the absence or presence of 100 μM ADR and lipid peroxidation was measured by the thiobarbituric acid method. Curcumin (1, 10 or 100 μM) was added to incubation mixtures before adding NADPH-generating system or NADH. Data are mean ± s.d. of six observations. *Significantly ($P<0.001$) higher than all groups; †significantly ($P<0.05$) lower than ADR; ‡significantly ($P<0.01$) lower than ADR; §significantly ($P<0.001$) lower than ADR, but not significant compared to controls.

Discussion The main objective of this study was to investigate the hypothesis that curcumin has a protective effect against the development of ADR renal injury. The data presented in this study reveal that curcumin is a potent inhibitor of ADR nephrosis. Experiments performed to examine the mechanism by which curcumin was exerting its protective effect on ADR nephrosis revealed that antioxidant, antiinflammatory, membrane stabilizing and hypolipidemic properties may constitute an important part of its therapeutic effects. First, curcumin treatment prevented ADR-induced decrease in body weight gain. This provides evidence that curcumin directly protects ADR general toxicity. Second, curcumin suppressed renal toxicity by blocking oxidative injury in the kidney because it prevented ADR-induced rise in lipid peroxides. Similarly, curcumin suppressed ADR-stimulated lipid peroxidation in kidney microsomes and mitochondria in a dose-dependent manner. Curcumin treatment also increased the glutathione content and glutathione peroxidase activity of kidney tissues in ADR rats. This increase in both the nonenzymatic and enzymatic antioxidants may play significant role in the mechanism of the protective effect of curcumin (Sharma, 1976). Curcumin has been shown to inhibit hydrogen peroxide induced oxidative injury in a renal cell line (Cohly *et al*., 1998). Curcumin is also protective against ADR cardiac injury (Venkatesan, 1998). These findings support that curcumin suppresses oxidative injury in a wide variety of tissues including heart and kidney. Third, the observation that curcumin treatment was accompanied by significant reduction in urinary excretion of GAG in ADR rats suggests that this treatment may influence the integrity of the glomerular basement membrane. Because the basement membrane is subject to damage in ADR nephrosis (Raats *et al*., 1997), curcumin treatment would retard the abnormal passage of high molecular weight macromolecules from the blood to the urinary space. In addition, curcumin might play a critical role in suppressing ROS mediated destruction of basement membrane and proteinuria (Raats *et al*., 1997). In this respect, it is interesting to note that ADR has been demonstrated to cause increased urinary excretion of GAG and loss of glomerular GAG in rats (Benjelloun *et al*., 1993). Fourth, the renoprotective effect of curcumin is also evident by a remarkable improvement of renal function, as judged by the restoration of GFR in ADR rats. It is also interesting to note that urinary FN levels (Soose *et al*., 1991) are modulated in curcumin-treated ADR rats. Fifth, the prevention of ADR-induced increases in the urinary levels of NAG supports the idea that curcumin exhibits potent antiinflammatory (Shoskes, 1998) and membrane stabilizing properties (Srivastava & Srimal, 1985) and thus prevents heavy proteinuria and exposure to proinflammatory cytokines that induce the damage of the tubular epithelial cells in ADR rats. Finally, the hypolipidaemic property of curcumin could also contribute to its beneficial effects on ADR renal injury. Excessive production and accumulation of lipids can have a devastating effect on renal structure and function (Hutchison, 1993). Our results showing decreased plasma and kidney lipids in curcumin-treated ADR rats are consistent with the hypolipidaemic action of curcumin. Recently, the hypolipidaemic property of curcumin in streptozotocin diabetic rats was reported (Suresh Babu & Srinivasan, 1997).

In summary, the present findings demonstrate that curcumin has multiple therapeutic activities that are beneficial in the kidney and thus curcumin is a promising candidate to block ADR nephrosis. Further studies are in progress.

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