

Carvedilol, A Beta Adrenoceptor Blocker with Antioxidative Potential, Attenuates Cisplatin-induced Nephrotoxicity in Rats

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Abstract: In the present study, kidney damage induced by cisplatin treatment ($7\text{mg kg}^{-1}\text{day}^{-1}$) was characterized by significant increases in kidney weight as a percentage of total body weight, alterations in renal function as serum creatinine and serum BUN levels. On the other hand, there was decrease in serum albumin and calcium levels compared with untreated control animals. In addition, cisplatin induced a significant renal oxidative stress demonstrated by elevated renal MDA, reduction of GSH and reduced activities of renal catalase and glutathione peroxidase (GPx) compared with untreated control animals. Carvedilol-induced attenuation of cisplatin nephrotoxicity ($5\text{mg kg}^{-1}\text{day}^{-1}$) 5 days prior to and 5 days post cisplatin was clearly manifested by the improvement of renal dysfunction. Carvedilol administration also reduced the increased renal MDA level and restored the depleted renal GSH and antioxidant enzymes (catalase and GPx). The renoprotective effect of carvedilol may be attributed to its radical scavenging and antioxidant activities. In conclusion, these findings of the present study strongly suggest the role of oxidative stress in the pathophysiology of cisplatin-induced nephrotoxicity and that carvedilol can be used for the renoprotection of cisplatin-induced nephrotoxicity.

Key words: cisplatin, nephrotoxicity, carvedilol

INTRODUCTION

Cisplatin (CIS) is one of the most effective anticancer drugs administered to treat a variety of cancers such as ovarian, testicular, bladder, head and neck, and uterine cervix carcinomas^[1,2]. High doses of cisplatin are more effective than low doses in ovarian and colorectal cancer^[3,4]. However, high dosage treatment induces nephro- and neurotoxicity. In spite of hydration, hypertonic saline and diuretics to protect against renal complications, a high percentage of treated patients develop from mild to severe renal imbalance characterized by increased serum creatinine levels and uremia, hypomagnesemia, hypokalemia and reduced glomerular filtration rate^[5,6].

The mechanisms of cisplatin nephrotoxicity, the dose-limiting factor in clinical studies, are still not fully understood. However the generation of free oxygen radicals in tubular cells has been proposed as an important pathogenic process^[7]. Various data indicate that cisplatin induces oxidative stress^[8,9] lipid peroxides^[10] and DNA damage^[11,12]. Several antioxidants such as diethyldithiocarbamate^[13], thymoquinone^[14] and lipoic acid^[15] have been tested for their ability to protect against cisplatin-induced nephrotoxicity in experimental animals. None of these compounds has proved to be clinically efficacious as complete protection in patients.

Carvedilol (CRV), beta blocker, is a unique multiple-action drug, a non-selective beta-blocker with vasodilatory properties due to alpha blockade^[16,17], and a scavenger of oxygen free radicals^[18]. Clinical studies on human subjects have shown that carvedilol improves both idiopathic and ischemic congestive heart failure^[19] furthermore, carvedilol is beneficial in renal dysfunction^[20,21]. Therefore the present study investigated the beneficial effects of carvedilol, at non-hypotensive doses, on the renal damage-induced by cisplatin in rats.

MATERIALS AND METHODS

Drugs and Chemicals: Cisplatin (David Bull Laboratories), Carvedilol (Roche), Thiobarbituric acid (TBA) Fluka (Buchs, Switzerland). N-(1-naphthyl) Ethylenediamine dihydrochloride (NEDD), Sulfanilamide (SULF), were all purchased from Sigma-Aldrich and were used without further purification. All other chemicals were of the highest analytical grade and obtained from commercial suppliers.

Animals: Male Wistar albino rats, weighing 150-200 g each, were used in all experiments. They were obtained from the Experimental Animal Care Center, King Saud University, Riyadh, Saudi Arabia. They were housed under conventional laboratory conditions in a room

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temperature maintained at $25 \pm 1^\circ\text{C}$ and a relative humidity range of 40% to 75% with a regular 12h light/dark cycle. Rats were fed a standard animal pellet diet and allowed free access to water unless otherwise indicated. Experimental procedures were reviewed and approved by the Institutional Ethical Committee of College of Pharmacy, King Saud University, Saudi Arabia.

Experimental Protocol: In this experiment, rats were randomly allocated into 4 groups, each consisting of eight animals. The first group received methylcellulose 0.5% in tap drinking water. The 2nd group, CRV group, received Carvedilol ($5 \text{ mg kg}^{-1}\text{day}^{-1}$), in drinking water with 0.5% methylcellulose to facilitate dissolution and absorption. A 3rd group, CIS group, received Cisplatin (7 mg kg^{-1} , i.p) on day 5. The 4th group, (CRV+CIS) group, was given CRV+CIS. Rats will be treated with CRV 5 days prior to CIS administration and thereafter throughout the study. Five days after CIS treatment, animals were anesthetized with ether and blood samples were collected by heart puncture. Serum was separated by centrifugation for 5 min at 1000 g and used for measurement of indices of nephrotoxicity and calcium concentration. Rats were sacrificed by cervical dislocation and the kidneys were quickly isolated, washed with ice-cold saline, blotted dry on a filter paper and weighed, decapsulated and homogenized in ice-cold KCl (1.15%, pH 7.4) to yield a 10% (w/v) tissue homogenates using Glas-Col homogenizer (USA).

Serum levels of creatinine, blood urea nitrogen (BUN) and albumin were measured according to the methods of Bartles *et al*^[22], Patton and Crouch^[23], Wrenn and Feichtmeir^[24] respectively. Serum calcium levels were determined using Randox kits (Randox Laboratories, Antrim, UK).

Lipid peroxides (LP) level in kidney homogenate was determined as thiobarbituric acid-reactive substances spectrophotometrically, the absorbance was measured at 532 nm by the method of Ohkawa^[25] and the concentrations were expressed as nanomole malondialdehyde (MDA) per gram tissue (nmol MDA g^{-1} kidney tissue). kidney homogenate contents of acid soluble thiols mainly reduced glutathione were measured according to the method of Ellman^[26]. Tissue glutathione peroxidase (GPx) activity was determined according to the method of Paglia and Valentine^[27].

The tissue level of total nitrate/nitrite (NOx) was determined by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride. Prior to the Griess reaction all nitrate was converted to nitrite using vanadium trichloride as described^[28]. Total protein content will be determined according to Lowry method^[29].

Statistical analysis: Data were expressed as means \pm SD. Statistical comparison between different groups were

done using one-way analysis of variance followed by Tukey-Kramer multiple comparisons test. Significance was accepted at $P < 0.05$.

RESULTS AND DISCUSSIONS

Administration of a single dose of CIS (7 mg kg^{-1} i.p.) to normal rats developed nephrotoxicity manifested by significant increase in serum creatinine and BUN levels and decrease in serum albumin and calcium levels (Table 1). In addition, CIS administration induced significant increases in kidney weight as a percentage of body weight and renal MDA production (136% & 320%) as compared with the normal untreated group respectively. On the other hand, CIS treatment depleted renal reduced glutathione content (78%) as compared to control group (Table 2). CIS administration also reduced renal catalase and GPx activities (51% and 65%) as compared with the normal untreated group respectively (Table 3).

However, pretreatment with CRV ($5 \text{ mg kg}^{-1}\text{day}^{-1}$), in drinking water to CIS-treated rats significantly improved the CIS-induced nephrotoxicity. It protected rats from CIS-induced nephrotoxicity. The rise in serum creatinine and BUN levels was significantly prevented by CRV. In addition, CRV treatment reduced the CIS-induced increases in kidney weight as a percentage of body weight and renal MDA production (40% & 78%) as compared with the CIS-treated rats respectively. Also, it increased the renal reduced GSH and restored the depleted antioxidants enzymes, renal catalase and GPx activities (125% & 147%, 193%) as compared with the CIS-treated rats respectively. Although CIS-treatment decreased total NOx content (76%) as compared with the normal untreated group, pretreatment with CRV did not significantly alter renal total NOx content as compared with the CIS-treated rats.

The aim of this work is to investigate the beneficial effects of carvedilol, at non-hypotensive doses, on the renal damage-induced by cisplatin in rats. In the present study, kidney damage induced by cisplatin treatment was characterized by significant increases in kidney weight as a percentage of total bodyweight, serum creatinine and serum BUN levels. On the other hand, there was decrease in serum albumin and calcium levels compared with untreated control animals. Cisplatin administration resulted in a significant reduction in renal total NOx. In addition, cisplatin induced a significant renal oxidative stress demonstrated by elevated renal MDA, reduction of GSH and reduced activities of renal catalase and GPx compared with untreated control animals.

Our results of cisplatin-induced renal damage in accordance with those of previous reports^[30]. The possible mechanisms of cisplatin-induced renal damage including elevation of caspase-3 activity with progression of renal

Table 1: The effect of carvedilol (5 mg kg⁻¹day⁻¹) on serum indices of cisplatin(7 mg kg⁻¹, i.p)-induced nephrotoxicity in rats.

Group	Creatinine (mg/dl)	BUN (mg/dl)	Albumin (g/dl)	Calcium (nm/L)
Control	0.41±0.042	20.92±1.71	4.28±0.077	2.59±0.132
Carvedilol	0.43±0.030	21.13±2.11	4.11±0.08	2.71±0.143
Cisplatin1	96±0.034 ^a	92±15.31 ^a	3.52±0.09 ^a	2.14±0.180 ^a
Carvedilol+Cisplatin	0.39±0.081 ^b	26.34±1.94 ^b	4.19±0.12 ^b	2.43±0.090 ^b

Data are mean ± SD (n=8). Statistical significance between means was analyzed using one way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. ^a, Significantly different from control group. ^b, Significantly different from cisplatin group (P < 0.05).

Table 2: The effect of carvedilol (5 mg kg⁻¹day⁻¹) on kidney weight as a percentage of body weight, renal GSH, MDA and NOx of cisplatin (7 mg kg⁻¹, i.p)-induced nephrotoxicity in rats.

Group	Kidney weight as % of body weight	GSH (μmol g ⁻¹ kidney tissue)	MDA (nmol g ⁻¹ kidney tissue)	Nox (μmol g ⁻¹ kidney tissue)
Control	0.42±0.025	6.63±0.41	403.2±19.3	349.6±24.1
Carvedilol	0.45±0.070	6.39±0.64	394.6±26.4	322.2±26.2
Cisplatin	0.57±0.028 ^a	5.19±0.32 ^a	1292±93.41 ^a	268.2±19.3 ^a
Carvedilol+Cisplatin	0.44±0.040 ^b	6.51±0.21 ^b	521±42.2 ^b	290.4±23.1

Data are mean ± SD (n=8). Statistical significance between means was analyzed using one way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. ^a, Significantly different from control group. ^b, Significantly different from cisplatin group (P < 0.05).

Table 3: The effect of carvedilol (5 mg kg⁻¹day⁻¹) on antioxidant enzymes, glutathione peroxidase and catalase of cisplatin (7 mg kg⁻¹, i.p)-induced nephrotoxicity in rats.

Group	Glutathione peroxidase (U g ⁻¹ kidney tissue)	Catalase (U mg ⁻¹ protein)
Control	33.21±2.13	5.12±0.32
Carvedilol	35.14±1.72	5.33±0.48
Cisplatin	21.71±1.41 ^a	2.60±0.34 ^a
Carvedilol+Cisplatin	31.89±1.18 ^b	5.04±0.51 ^b

Data are mean ± SD (n=8). Statistical significance between means was analyzed using one way ANOVA followed by Tukey-Kramer multiple comparisons post hoc test. ^a, Significantly different from control group. ^b, Significantly different from cisplatin group (P < 0.05).

cell apoptosis or protein kinase C activation^[31,32] in addition to oxidative stress and preoxidative alterations. A role for cisplatin-induced oxidative stress may provide an attractive hypothesis^[33]. Several studies have now documented the importance of reactive oxygen metabolites (ROM) in cisplatin-induced renal apoptosis. Mitochondria continuously scavenge ROM via the antioxidant enzymes such as glutathione peroxidase and catalase^[34]. Cisplatin is known to accumulate in mitochondria of renal epithelial cells. Several investigators have demonstrated that cisplatin induces ROS in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes and depletion of GSH^[33].

In our study, administration of carvedilol (5 mg kg⁻¹day⁻¹) 5 days prior to and 5 days post cisplatin treatment significantly attenuates cisplatin-induced nephrotoxicity. This was clearly manifested by the improvement in the most biochemical parameters changed in cisplatin-induced nephrotoxicity. The renoprotective effect of carvedilol against cisplatin-induced nephrotoxicity could be attributed to its antioxidant activity.

Carvedilol and certain of its metabolites are potent antioxidants. Its antioxidant activity has been attributed to the carbazole moiety of the drug^[35]. The antioxidant effects of carvedilol has been demonstrated and characterized in a variety of in vitro test systems, and have also been confirmed in vivo in animals and in humans^[36]. Carvedilol has been known to scavenge free radicals and inhibits lipid peroxidation rat brain homogenates and human low density lipoproteins^[37,38]. Carvedilol is approximately 10 fold more potent as an antioxidant than vit. E. Several metabolites of carvedilol are extremely potent antioxidants being 30 to 80 fold more potent as carvedilol and up to 1000 fold more potent than vit. E^[35].

Carvedilol prevented the cisplatin-induced rise in both serum urea and creatinine and the decrease in serum albumin and calcium which is in accordance with Singh *et al*^[20]. Also, carvedilol administration reduced the increased renal MDA level and restored the depleted renal GSH and antioxidant enzymes (catalase and GPx) which is in a good correlation with Singh *et al* and Ronsein *et al*^[18,39]. The antioxidant action of carvedilol may be speculated to be due to inhibition of direct cytotoxic action of free radicals, prevention of oxygen free radical from activating transcription factors and protection and replenishing the endogenous antioxidant defense mechanisms^[40].

In conclusion the findings of the present study strongly suggest the role of oxidative stress in the pathophysiology of cisplatin-induced nephrotoxicity and that carvedilol could be used for the protection of cisplatin-induced nephrotoxicity.

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