

PROTECTIVE EFFECTS OF VITAMIN C AGAINST CISPLATIN-INDUCED NEPHROTOXICITY AND LIPID PEROXIDATION IN ADULT RATS: A DOSE-DEPENDENT STUDY

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Cisplatin is one of the most active cytotoxic agents in the treatment of cancer, but its clinical use is associated with nephrotoxicity. In the present study we report the effects of different amounts of vitamin C (50, 100 or 200 mg kg⁻¹ body wt.) in rat kidneys treated with cisplatin (5 mg kg⁻¹ body wt.), using single doses of both compounds. Cisplatin administration induced lipid peroxidation which was accompanied by a decrease in renal glutathione level in animals killed 7 days after treatments. Furthermore, an increase in serum creatinine has been observed. Treatment of animals with vitamin C 10 min prior to the cisplatin inhibited cisplatin-mediated damage. Seven days after vitamin C plus cisplatin treatments, the depleted level of glutathione and changes in the creatinine clearance recovered to significant levels ($P < 0.05$). Similarly, the enhanced serum creatinine levels which are indicative of renal injury showed a significant reduction ($P < 0.05$) with the three doses of vitamin C tested. The protective effect of vitamin C was dose-dependent. The results suggest that vitamin C is an effective chemoprotective agent against nephrotoxicity induced by the antitumoral cisplatin in Wistar adult rats. © 2000 Academic Press

KEY WORDS: nephrotoxicity, cisplatin, vitamin C, rats, antioxidant.

INTRODUCTION

Cisplatin (*cis*-diamminedichloroplatinum II) is one of the most potent chemotherapeutic antitumor drugs. Activity has been demonstrated against a variety of neoplasms, particularly for head and neck, testicular, ovarian, bladder and small-cell lung cancers [1]. Cisplatin also has been shown to be effective against virally-induced, chemically-induced and transplantable tumours in animals [2]. High doses of cisplatin produce hepatotoxicity, but the impairment of kidney function by cisplatin is recognized as the main side-effect and the most important dose-limiting factor [3, 4]. The alterations induced by cisplatin in the kidney functions were characterized by signs of injury, such as changes in urine volume, in glutathione status, increase of products of lipid peroxidation, and changes in creatinine clearance [5].

Cisplatin-induced nephrotoxicity is closely associ-

ated with an increase in lipid peroxidation in the kidney tissues [6, 7]. This antitumoral was able to generate active oxygen species, such as superoxide anion and hydroxyl radical [8–10], and to inhibit the activity of antioxidant enzymes in renal tissue [11]. Furthermore, cisplatin-induced glutathione depletion is a determinant step in oxidative stress in the kidney tissue that leads to nephrotoxicity [12].

Much attention has been given to the possible role that dietary antioxidants play in protecting against cisplatin-induced nephrotoxicity [11, 13, 14]. Selenium, vitamins C and E, and cysteine were able to protect against the nephrotoxicity induced by cisplatin [11]. Vitamin C, an essential nutrient and active reducing agent, is involved in many biological processes [15, 16]. However, few papers have reported on the effects of this vitamin in cisplatin-treated rats, and usually only one dose has been used [11].

As vitamin C has been shown to protect against various kinds of injuries and neoplasms involving oxidative stress, it is possible that treatment of

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animals with vitamin C may attenuate cisplatin-induced lipid peroxidation, glutathione depletion, and a decrease in the glomerular filtration rate. The main objective of the present study was to obtain information about the possible protective effects dose-dependent of the different doses of vitamin C in Wistar rats treated with cisplatin.

MATERIALS AND METHODS

Chemicals

Vitamin C (Redoxon®, Roche Chemicals and Pharmaceuticals) was purchased on the local market. Cisplatin (Platinil®, Quiral Química do Brasil) was a gift. All other chemicals and reagents used were of analytical grade.

Animals and treatments

Adult male Wistar rats, provided by the Animal House of the Prefeitura Administrativa do Campus de Ribeirão Preto, Universidade de São Paulo, were utilized in this study. Rats weighting 200 ± 10 g, had free access to standard rat chow and water, housed individually in metal cages, and kept in a room maintained at $23 \pm 2^\circ\text{C}$ with a 12-h light/dark cycle.

The animals were divided into eight groups of six rats each. Group I received distilled water by gavage and 10 min after was injected with saline intraperitoneally. This group served as negative control. Groups II, III and IV received a single dose of vitamin C by gavage (50, 100 or 200 mg kg⁻¹ body wt., respectively) and 10 min later saline i.p. Animals of group V received distilled water by gavage and were injected with cisplatin (5 mg kg⁻¹ body wt.) i.p. Groups VI, VII and VIII received the respective doses of vitamin C by gavage and were injected 10 min later with cisplatin i.p. At the end of experiments, animals of each group were killed by decapitation.

Rats were killed 24 h or 7 days after cisplatin or saline administration to study the effect of vitamin C on cisplatin-mediated alterations in renal glutathione levels. Blood samples were collected in heparinized tubes from the rats killed 7 days after cisplatin or saline administration and their kidneys were removed and kept in an ice bath until homogenization. Serum was used for the estimation of creatinine and kidney tissue for the determination of contents of renal glutathione and lipid peroxidation. Changes in urinary volume and development of body weight were also determined during the experiments.

Biochemical assays

Reduced glutathione (GSH) was determined as non-protein and total sulphhydryl contents in rat kidneys using the method of Sedlak and Lindsay [17].

Briefly, tissues were homogenized in 5.0 ml of cold KCl (1.15%) solution using motor-driven tissue homogenizers. The samples were precipitated with trichloroacetic acid and then subjected to centrifugation at 1200 g for 10 min. The reaction mixture contained 0.5 ml of supernatant, 2.0 ml of Tris-EDTA buffer (pH 8.9), and 0.1 ml 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB). The solution was kept at room temperature for 15 min, and then was read at 412 nm on a spectrophotometer (Genesys, Spectronic Instruments). The results were expressed in $\mu\text{mol GSH g}^{-1}$ of protein.

The content of thiobarbituric acid-reactive substances (TBARS) was measured by the method of Uchiyama and Mihara [18] to assess lipid peroxidation. To the tubes 0.5 ml of the same homogenized tissue, 3.0 ml of H₃PO₄ and 1.0 ml of thiobarbituric acid (0.6%) were added. The tubes were heated for 45 min in a boiling waterbath and the reaction mixture was then cooled in an ice bath followed by the addition of 4.0 ml of *n*-butanol. The contents were mixed for 40 s with a vortex mixer, centrifuged at 1200 g for 10 min, and the absorbance of the organic layer was measured at 520 and 535 nm. The results were expressed by nmol MDA g⁻¹ of tissue. Serum and urinary creatinine were estimated by alkaline picrate method with a test kit (Labtest Diagnóstica S.A.) and served as kidney glomerular function parameters. The absorbance was recorded at 520 nm. Creatinine clearance was calculated from the values of urinary and serum creatinine, time (last 24 h) and body weight. All assays were done using samples in triplicate of each animal.

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). Differences between treatments were determined by the Mann-Whitney's test. The results were expressed as the mean \pm SD of six values in each group, and a statistical probability of $P < 0.05$ was considered to be significant.

RESULTS

The effects of different doses of vitamin C on cisplatin (5 mg kg⁻¹ body wt.) nephrotoxicity was evaluated by urinary volume. Control rats and those that received only vitamin C (50, 100 or 200 mg kg⁻¹ body wt.) excreted relatively constant amounts of urine ($P > 0.05$). The groups of cisplatin-treated rats excreted significantly higher urinary volumes (213.7%) 7 days post-treatment than control rats (Fig. 1). Interestingly, increases in urinary volumes were also observed in all groups treated with cisplatin 1, 3 and 5 days after treatment (data not shown). No differences in body weight were detected

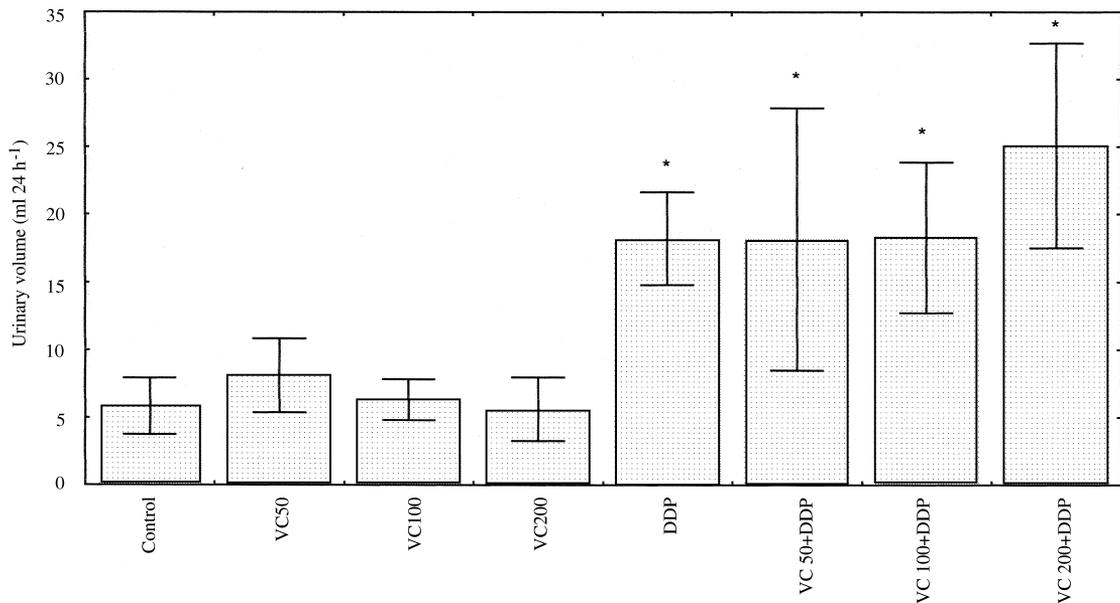


Fig. 1. Effect of vitamin C (VC; 50; 100 or 200 mg kg⁻¹ body wt.) on urinary volume 7 days after the administration of cisplatin (DDP; 5 mg kg⁻¹ body wt.). Each value represents mean \pm SD of six animals. *Statistically significant when compared to control ($P < 0.05$).

among the groups that received only vitamin C compared to the control ($P > 0.05$). At the end of the experimental period (seventh day), the development of body weight was depressed in all animals treated with cisplatin. This reduction was statistically significant when compared to the control ($P < 0.05$). Decrease in body weight in cisplatin-treated rats was not changed with the administration of different doses of vitamin C (Fig. 2).

TBARS content in the kidney tissue had been used as a measure of lipid peroxidation. The TBARS content was higher in groups treated with vitamin C than in the control, but this effect was not statistically significant ($P > 0.05$). Figure 3 shows the results in all groups. A single dose of cisplatin enhanced the formation of lipid peroxides in 57.7% compared to the control. This increase was prevented by concurrent administration of vitamin C at

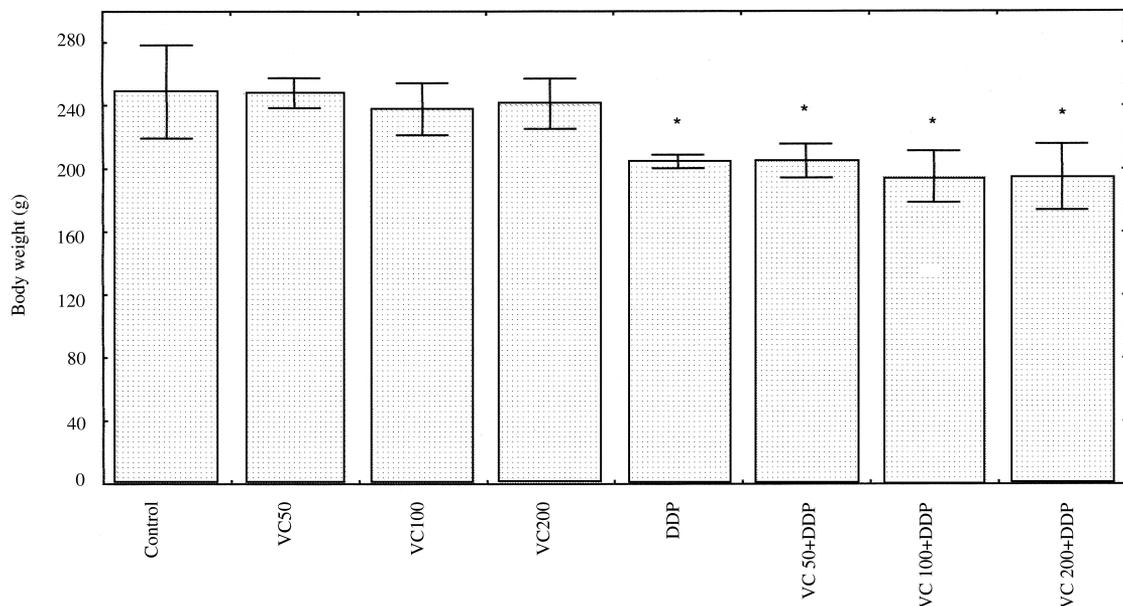


Fig. 2. Effect of vitamin C (VC; 50; 100 or 200 mg kg⁻¹ body wt.) in development of body weight 7 days after the administration of cisplatin (DDP; 5 mg kg⁻¹ body wt.). Each value represents mean \pm SD of six animals. *Statistically significant when compared to control ($P < 0.05$).

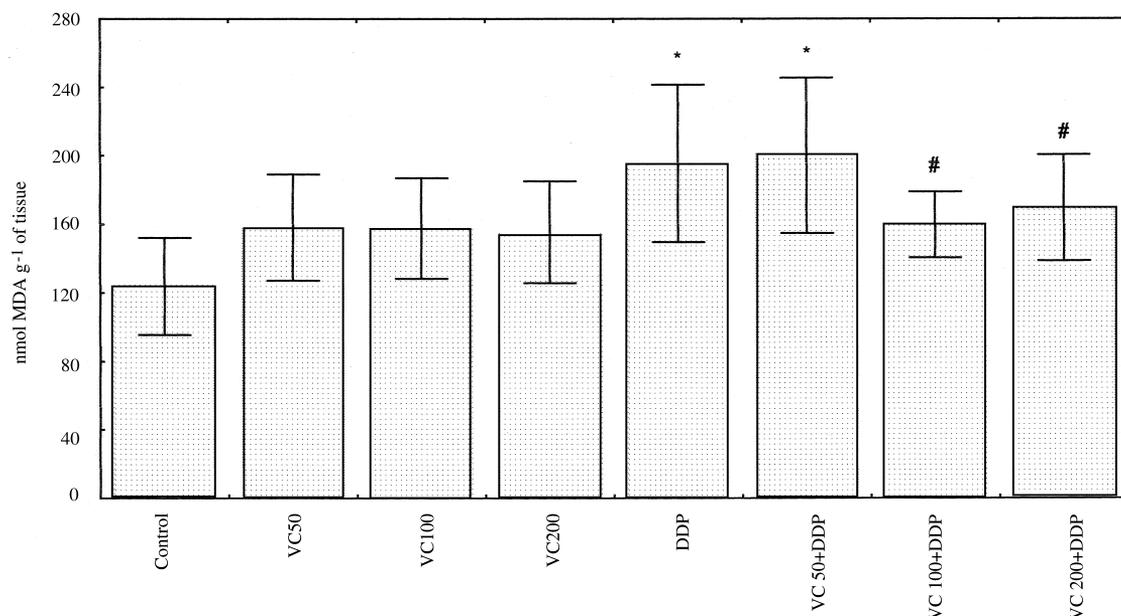


Fig. 3. Effect of treatment of adult rats with vitamin C (VC; 50; 100 or 200 mg kg⁻¹ body wt.) on cisplatin (DDP; 5 mg kg⁻¹ body wt.)-mediated enhancement of lipid peroxidation in renal tissue. Each value represents mean \pm SD of six animals. *Statistically significant when compared to control ($P < 0.05$). #Statistically significant when compared to DDP alone ($P < 0.05$).

doses of 100 or 200 mg kg⁻¹ body wt. ($P < 0.05$). Although lipid peroxidation was statistically reduced in the animals treated with the middle and higher doses of vitamin C plus cisplatin, it was still 28.8 and 37.0%, respectively, higher than in the control (Fig. 3).

The effect of treatment of adult rats with vitamin C on cisplatin-mediated changes in the levels of renal glutathione are shown in Table I. The cisplatin treatment resulted in a 55% increase in renal glutathione 24 h after cisplatin administration compared to respective controls ($P < 0.05$). Vitamin C induced a slight decrease on cisplatin-mediated enhancement of renal glutathione ($P > 0.05$). On the

other hand, glutathione content was depleted, but not significantly ($P > 0.05$), in cisplatin-treated rats 7 days post-treatment. The levels of renal glutathione were restored to approximately normal levels in the rats treated with vitamin C plus cisplatin. This protection provided by vitamin C on glutathione depletion was dose-dependent.

The effects of vitamin C on cisplatin-mediated increases in serum creatinine and decreases in creatinine clearance 7 days post-treatment are shown in Table II. Cisplatin alone led to approximately 65% enhancement in the value of serum creatinine compared to the control ($P < 0.05$). Administration of vitamin C to rats that received cisplatin led to a

Table I
Effect of cisplatin (DDP; 5 mg kg⁻¹ body wt.) or its combination with three doses of vitamin C (VC; 50, 100 or 200 mg kg⁻¹ body wt.) on levels of renal glutathione (GSH) in adult rats killed 24 h or 7 days after cisplatin administration

Groups	24 h ($\mu\text{mol GSH g}^{-1}$ of protein)	% of control	7 days ($\mu\text{mol GSH g}^{-1}$ of protein)	% of control
Control	23.7 \pm 4.85	100	28.0 \pm 6.04	100
VC 50	21.5 \pm 5.82	91	24.1 \pm 6.61	86
VC 100	20.7 \pm 4.40	87	24.0 \pm 5.41	86
VC 200	20.3 \pm 3.29	86	29.9 \pm 9.16	106
DDP 5.0	36.7 \pm 5.44*	155	22.2 \pm 4.91	79
+ VC 50	34.8 \pm 6.73*	147	25.1 \pm 8.65	89
+ VC 100	31.1 \pm 5.40*	131	34.7 \pm 6.83†	123
+ VC 200	32.8 \pm 6.94*	138	36.4 \pm 7.10†	130

Each value represents mean \pm SD of six animals.

*Statistically significant when compared to control ($P < 0.05$).

†Statistically significant when compared to DDP alone ($P < 0.05$).

Table II
Effect of three doses of vitamin C (VC; 50, 100 or 200 mg kg⁻¹ body wt.) to adult rats on cisplatin (DDP; 5 mg kg⁻¹ body wt.)-mediated enhancement in the value of serum creatinine and decrease in creatinine clearance, 7 days after cisplatin administration

Groups	Serum creatinine (mg 100 ml ⁻¹)	% of control	Creatinine clearance ml min ⁻¹ 100 g ⁻¹	% of control
Control	0.40 ± 0.17	100	0.26 ± 0.13	100
VC 50	0.43 ± 0.19	107	0.22 ± 0.08	84
VC 100	0.37 ± 0.06	92	0.26 ± 0.06	100
VC 200	0.39 ± 0.09	97	0.22 ± 0.04	84
DDP 5.0	0.66 ± 0.16*	165	0.15 ± 0.03*	57
+ VC 50	0.46 ± 0.21†	115	0.18 ± 0.07*	69
+ VC 100	0.41 ± 0.06†	102	0.33 ± 0.05†	126
+ VC 200	0.47 ± 0.14†	117	0.30 ± 0.07†	115

Each value represents mean ± SD of six animals.

*Statistically significant when compared to control ($P < 0.05$).

†Statistically significant when compared to DDP alone ($P < 0.05$).

significant reduction in these values. In the experiments with adult rats treated with cisplatin alone a significant reduction ($P < 0.05$) was observed in creatinine clearance compared to the control. This indicates glomerular damage. Treatment with vitamin C resulted in significantly less reduction in creatine clearance induced by cisplatin.

DISCUSSION

Different strategies have been proposed to inhibit cisplatin-induced nephrotoxicity [19]. Development of therapies to prevent the action or generation of free radicals may influence the progression of oxidative renal damage, along with the appearance of acute renal damage cisplatin-induced. Diet-controlled natural antioxidants such as vitamin C can be easily and safely increased in the tissues by dietary supplementation since ideal doses are known [20]. In this study we investigated the effects of different amounts of vitamin C on cisplatin-treated adult rats.

Choi *et al.* [21] observed that cisplatin caused necrosis in the proximal and distal tubules in rat kidneys, with a maximum peak of lesions observed 7 days after cisplatin injection. In the present study, the animals were killed 7 days after the administration of vitamin C and/or cisplatin. During the experiment, the animals treated with this antitumoral did not show the same development in body weight when compared with the control. The administration of vitamin C did not influence on this parameter investigated. This weight loss, already reported by other authors, may be due to the gastrointestinal toxicity of cisplatin [11, 21]. All the groups which received cisplatin had a greater urinary volume than the control group, showing the renal lesion caused by this antitumoral. Matsushima *et al.* [10] also observed that cisplatin injection caused increase in urine volume and induced a significant decrease in

body weight compared with control rats. Contrary to the results obtained by Appenroth *et al.* [11] with administration of vitamin E in rats treated with cisplatin, the present study showed that the administration of vitamin C did not inhibit loss of body weight and increase in urine volume induced by this antitumoral drug.

One of the most important intracellular antioxidant systems is the glutathione redox cycle. Glutathione is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism [22]. The exact mechanisms of the cisplatin-induced changes in renal glutathione concentrations are not completely elucidated. Thus, glutathione may modulate metal reduction, and the thiol portion is very reactive with several chemical compounds, mainly with alkylating agents such as cisplatin [23].

The depletion of levels of renal glutathione has been observed in rats in response to oxidative stress caused by cisplatin 7 days after its administration [12, 24]. Although the interaction of cisplatin with the enzymes which contain sulphhydryl groups is fairly well known, some investigations have shown that the nephrotoxicity of cisplatin is not necessarily associated with depletion in renal glutathione content. Results of Bräunlich *et al.* [19] showed that the kidney damage caused by cisplatin is not associated with decreased renal glutathione.

Tian *et al.* [25] suggested that under oxidative stress conditions or oscillations in the glutathione level, there may occur positive regulation in the biosynthesis of glutathione, contributing to the increase in its intracellular contents. The glutathione uptake appears to be the primary mechanism in tubular cells to maintain intracellular thiol redox status [26]. The ability of cells to rapidly increase glutathione synthesis and use glutathione for detoxification suggest that *in vivo* it may be an important protective mechanism against chemical injury [27].

This could justify the increase in glutathione content at 24 h following treatment of rats with cisplatin compared to the control, regardless of the combination with different vitamin C doses. On the other hand, in the animals which were killed 7 days after cisplatin administration, it was observed that the treatment with vitamin C prevented the depletion of renal glutathione caused by cisplatin, resulting in values close to those observed in the control group. These results suggest the possible involvement of vitamin C-mediated protection against cisplatin-induced depletion of renal glutathione. The cooperation between vitamin C and glutathione in various protective effects has been reviewed by Meister [28, 29].

The effect of cisplatin administration on renal excretion, glutathione depletion, increase in substances reactive to thiobarbituric acid and other parameters analysed in the present investigation is in accordance with literature data [6, 11, 12, 19, 30]. Lipid peroxidation constitutes a complex chain reaction of free radicals, which leads to a degradation of polyunsaturated fatty acid in cell membranes [31]. Several studies have shown the effects of chemoprotective agents on cisplatin-induced lipid peroxidation [13, 19].

In the present investigation the increase in lipid peroxidation induced by cisplatin was inhibited by the prior administration of middle and higher doses of vitamin C. It was suggested that low concentrations of vitamin C would increase lipid peroxidation by reducing iron, whereas a high concentration of vitamin C may reduce lipid peroxidation [31]. The ability of vitamin C to significantly decrease renal lipid peroxidation caused by cisplatin *in vivo* supports the idea that supplementation with dietary antioxidants could maintain the integrity of lipids in kidney tissue under oxidative stress induced during chemotherapy.

The changes in renal function observed in the rat system correlate well with the nephrotoxic effects of cisplatin in man [32, 33]. Alterations in values of creatinine clearance and serum creatinine levels observed in the treatment with cisplatin are taken as indications of an abnormal glomerular function [34, 35].

Vitamin C pretreatment inhibited the enhancement of serum creatinine and the reduction of creatinine clearance induced by cisplatin. Vitamin C, as an antioxidant agent, may have inhibited the chain reactions of the cisplatin-generated free radicals or scavenged the free radicals before they reached the cell targets damaging the glomerular kidney functions. Appenroth *et al.* [11] showed that the beneficial effects of the C and E vitamin combination was the results of the antioxidant action for these vitamins.

Other studies show the protection of other vitamins over renal oxidative damage. Vitamin E pre-

treatment for 1 week or garlic oil administration ameliorated iron nitritoacetate-induced lipid peroxidation and inhibited depletion of glutathione levels in renal tissue of rats [36, 37]. Pretreatment with vitamin A reduced the lipid peroxidation process in the kidney of mice after nickel administration [38]. Nevertheless, results in the literature show that the administration of vitamins and other antioxidants may not totally protect the kidneys from damage caused by the increase in free radical generation [6, 14]. In an *in vivo* study, pretreatment with desferrioxamine in a single dose prior to cisplatin administration was not protective against cisplatin nephrotoxicity [39]. The antioxidant silibinin partly counteracts the nephrotoxic side-effects of cisplatin in Wistar rats [34]. More recent studies obtained by Durak *et al.* [40] show that the combination of vitamin E and C at 200 mg kg⁻¹ dose did not completely hinder the oxidative damage in rabbit kidneys treated with cyclosporine.

A beneficial effect on kidney function was found when adult rats treated with cisplatin received different amounts of vitamin C. Under the experimental condition of the present study, vitamin C showed protection in a dose-dependent manner on cisplatin-induced oxidative damage on adult Wistar rat kidneys.

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