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Cisplatin-induced Acute Renal Failure is Ameliorated by Erdosteine in a Dose-dependent Manner

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Key words: cisplatin; renal failure; erdosteine.

The aim of this study was to investigate the optimum dosage of erdosteine to ameliorate cisplatin-induced nephrotoxicity. Three different doses of erdosteine at 25, 50 and 75 mg kg^{-1} were studied in rats. Intraperitoneal administration of 7 mg kg^{-1} cisplatin led to acute renal failure, as indicated by kidney histology and increases in plasma creatinine and blood urea nitrogen (BUN) levels. At 5 days after cisplatin injection the BUN level was increased significantly from 15.1 ± 4.3 to 126.7 ± 152.6 mg dl^{-1} and plasma creatinine levels increased from 0.37 ± 0.005 to 1.68 ± 1.9 mg dl^{-1}. When the rats were administered 50 and 75 mg kg^{-1} erdosteine 24 h before cisplatin injection that was continued until sacrifice (total of 6 days), the BUN and creatinine levels remained similar to control levels and the grade of histology was similar. Erdosteine at doses of 50 and 75 mg kg^{-1} ameliorates cisplatin-induced renal failure. The optimum dose of erdosteine may be 50 mg kg^{-1} in this study.

INTRODUCTION

Cisplatin is effective in the treatment of a variety of human solid tumours, including lung, testicular, bladder and ovarian cancer. The most common side-effect limiting the use of cisplatin is nephrotoxicity (Walker and Gale, 1981). Although vigorous hydration with or without mannitol diuresis may substantially reduce the nephrotoxicity, a reduction in the glomerular filtration rate occurs in 20–30% of patients despite prophylactic intensive hydration and forced diuresis (Hartmann *et al.*, 1999).

Although the underlying mechanism of this side-effect is still unknown, in recent years there has been accumulating evidence for a role of reactive oxygen metabolites in the pathogenesis of cisplatin-induced renal damage (Baliga *et al.*, 1999). Thus many antioxidant agents have been studied in experimental and clinical studies to reduce or prevent cisplatin-induced nephrotoxicity (Xia and Zweier, 1995; Greggi *et al.*, 2000; Davis *et al.*, 2001). However, most of them are not clinically available. Moreover, the effective doses of these compounds are so high as to produce other undesirable side-effects.

Erdosteine is a mucolytic agent that was approved for the therapy of chronic obstructive pulmonary diseases in Turkey and many European countries. The free-radical scavenging activity of erdosteine metabolites has been studied by many research groups in recent years (Biagi *et al.*, 1989; Vagliasindi and Fregman 1989; Dechant and Noble, 1996; Braga *et al.*, 2000; Fadillioglu *et al.*, 2003). The structural feature responsible for the antioxidant and free-radical scavenging activity of erdosteine is the –SH group in the chemical structure (Dechant and Noble, 1996) after its hepatic metabolism. Therefore, it seems to be a promising drug in the prevention or treatment of diseases in which free radicals are involved in the pathogenesis.

Our previous study demonstrated that oral administration of erdosteine at a dose of 10 mg kg^{-1} significantly inhibited the depletion of antioxidant enzyme activity such as catalase and glutathione peroxidase and the increase in malondialdehyde and nitric oxide levels in cisplatin-induced kidney-damaged rats (Yildirim *et al.*, 2003). However, in the above-mentioned study erdosteine showed a limited preventive effect on renal function measured by blood urea nitrogen (BUN) and creatinine levels and histological assessment. We suggest that this weak protective effect against cisplatin-induced nephrotoxicity may be due to the inappropriate dose of this drug given to the rats. The appropriate dose of erdosteine to prevent cisplatin-induced acute renal failure in rats is unknown, therefore the present study was designed to define the optimum dosage of erdosteine to prevent cisplatin-induced nephrotoxicity. We tested three different doses of erdosteine at 25, 50 and 75 mg kg^{-1} in this model of cisplatin-induced nephrotoxicity.

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MATERIALS AND METHODS

Animals

Female Wistar albino rats weighing 200–250 g were purchased from the experimental research centre of the University of Erciyes (Kayseri, Turkey). They were housed in cages and kept in a room maintained at a constant temperature of 22 °C, 50–60% humidity and a 12-h light/dark cycle. The animals were fed with a standard diet obtained from Inonu University Animal Laboratory. Rats were divided into five equal groups (n = 10): rats given cisplatin as a cisplatin-induced acute renal failure model; rats given cisplatin plus erdosteine at doses of 25, 50 and 75 mg kg^{-1} body wt.; and rats given isotonic saline solution alone as a control group.

Cisplatin-induced acute renal failure

The cisplatin model of acute renal failure was induced in rats as described by Zhang *et al.* (1999) and in our previous study (Yildirim *et al.*, 2003). The animals received a single intraperitoneal injection of 7 mg kg^{-1} body wt. of cisplatin (Cisplatinum Ebewe, 0.5 mg ml^{-1}, Liba Drug Company, Turkey) and were sacrificed 5 days after cisplatin injection. Blood and kidneys were obtained for the various measurements. Renal impairment was assessed by BUN and plasma creatinine levels, as well as by the kidney histology. The BUN and creatinine levels were determined with an autoanalyser (Olympus AU600, Japan) by using commercial Sigma diagnostic kits. Kidney histology was performed as described in the following section.

Effect of erdosteine on cisplatin model of acute renal failure

The erdosteine as a suspension of 200 mg per 5 ml was obtained from the drug company ILSAN-ILTAS (Turkey) and administered orally once a day via plastic disposable syringes. The first dose of erdosteine was given 24 h prior to cisplatin injection and was continued until sacrifice (i.e. a total of 6 days). Erdosteine-treated rats (n = 30) were divided into three equal groups that were treated with 25, 50 or 75 mg kg^{-1} body wt. of erdosteine per day. Control rats were administered an isotonic saline solution (0.2 ml) by intraperitoneal injection. In addition, 0.2 ml of $NaHCO_3$ dissolved in distilled water was given orally instead of erdosteine 24 h prior to the saline injection and then once a day at the same dose until sacrifice.

Kidney histology

Five kidneys from each group were sectioned and fixed in 10% formalin, dehydrated and embedded in paraffin. Tissues then were sectioned at 3 μm and stained with haematoxylin and eosin (H&E), followed by semiquantitative analysis of the kidney sections by a pathologist (S. Ozen) using a double-blind protocol as described previously (Baliga *et al.*, 2001). The entire kidney sections were reviewed at a magnification of ×100. Each successive field was assessed individually for severity of tubulointerstitial damage and graded as follows: (0) normal; (I) areas of tubular epithelial cell swelling, vacuolar degeneration, necrosis and desquamation involving <25% of cortical tubules; (II) similar changes involving >25% but <50% of cortical tubules; (III) similar changes involving >50% but <75% of cortical tubules; (IV) similar changes involving >75% of cortical tubules. The mean score of all fields was taken as the renal injury score of that kidney section.

Biochemical measurements

All tissues were washed twice with cold saline solution, placed into glass bottles, labelled and stored in a deep freeze (–30 °C) until processing (maximum of 10 h). Tissues were homogenized in four volumes of ice-cold TRIS·HCl buffer (50 mmol, pH 7.4) using a glass Teflon homogenizer (Tempest Virtishear, Model 278069; The Virtis Company, Gardiner, NY) after cutting the kidney into small pieces with scissors (for 2 min at 5000 rpm). Malondialdehyde (MDA) measurements were made at this stage. The homogenate then was centrifuged at 5000 *g* for 60 min to remove debris. The clear upper supernatant fluid was taken to determine the catalase (CAT) and glutathione peroxidase (GSH-Px) activities and protein concentration at this stage. The supernatant solution was extracted with an equal volume of ethanol–chloroform (5 : 3 v/v). After centrifugation at 5000 *g* for 30 min, the clear upper layer (the ethanol phase) was used for the superoxide dismutase (SOD) activity and protein assays. All preparation procedures were performed at 4 °C.

Kidney tissue MDA determination. The kidney MDA level was determined by a method based on reaction with thiobarbituric acid (TBA) at 90–100 °C (Wasowicz *et al.*, 1993). In the TBA test reaction, MDA or MDA-like substances and TBA react together to produce a pink pigment with an absorption maximum at 532 nm. The reaction was performed at pH 2–3 and 90 °C for 15 min. The sample was mixed with two volumes of cold 10% (w/v) trichloroacetic acid to precipitate the protein. The precipitate was pelleted by centrifugation and an aliquot of the supernatant was reacted with an equal volume of 0.67% (w/v) TBA in a boiling water-bath for 10 min. After cooling, the absorbance was read at 532 nm (Ultraspec Plus, Pharmacia LKB Biochrom, UK). The results are expressed as nanomoles per gram of kidney tissue protein (nmol g^{-1} protein) according to a standard graphic prepared with serial dilutions of standard 1,1,3,3-tetramethoxypropane.

Kidney tissue myeloperoxidase (MPO) analysis. The MPO activity was determined using a 4-aminoantipyrine–phenol solution as the substrate for MPO-mediated oxidation by hydrogen peroxide (H_2O_2), and changes in absorbance at 510 nm (A_{510}) were recorded (Wei and Frenkel, 1993). One unit of MPO activity is defined as that which degrades 1 μmol H_2O_2 min^{-1} at 25 °C. Data are presented as U g^{-1} protein. All samples were assayed in duplicate.

Kidney tissue antioxidant enzyme analysis. Total (Cu–Zn and Mn) SOD (EC 1.15.1.1) activity was determined according to the method of Sun *et al.* (1988). The principle of the method is based on inhibition of nitroblue tetrazolium (NBT) reduction by the xanthine–xanthine oxidase system as a superoxide generator. Activity was assessed in the ethanol phase of the supernatant after 1.0 ml of ethanol–chloroform mixture (5 : 3, v/v) was added to the same volume of sample and centrifuged. One unit of SOD was defined as the amount causing 50% inhibition in the

NBT reduction rate. The SOD activity is expressed as U mg^{-1} protein.

Glutathione peroxidase (GSH-Px) activity was measured by the method of Paglia and Valentine (1967). The enzymatic reaction in the tube — containing NADPH, reduced glutathione (GSH), sodium azide and glutathione reductase — was initiated by addition of H_2O_2 and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity is expressed as U g^{-1} protein. Catalase (CAT) activity was determined according to Aebi's method (Aebi, 1974). The principle of the method was based on determination of the rate constant k (s^{-1}) of the H_2O_2 decomposition at 240 nm. Results are expressed as k g^{-1} protein. All samples were assayed in duplicate.

Protein determinations. Protein assays were made by the method of Lowry *et al.* (1951).

Data analysis

Data are expressed as means ± SD. A one-way ANOVA and *post-hoc* multiple comparison tests (LSD) were performed on the data of biochemical variables to examine the differences among the groups. All analyses were made using the SPSS statistical software package. A P value of <0.05 was considered as statistically significant.

RESULTS

Administration of cisplatin at a single dose of 7 mg kg^{-1} body wt. resulted in acute renal failure similar to that in our previous study (Yildirim *et al.*, 2003). At 5 days after cisplatin injection the BUN level was increased significantly from 15.1 ± 4.3 to 126.7 ± 152.6 mg dl^{-1} ($P < 0.0001$) and plasma creatinine levels increased from 0.37 ± 0.005 to 1.68 ± 1.9 mg dl^{-1} ($P < 0.0001$) (Fig. 1). As shown in Table 1, SOD, GSH-Px and catalase activities in renal tissue of rats treated with cisplatin alone were decreased significantly compared with control rats ($P < 0.005$). Kidney tissue MDA levels and MPO activities were increased significantly by cisplatin treatment ($P = 0.003$) (Figs 2 and 3).

Prophylactic erdosteine treatment at a dose of 25 mg kg^{-1} leads to some attenuation of the cisplatin-induced elevation of plasma levels of BUN and creatinine (Fig. 1). Erdosteine at this dose also produced attenuation of the loss of antioxidant enzymes, but this attenuation reached a statistically significant level only in SOD. When the effect of erdosteine at a dose of 50 or 75 mg kg^{-1} on the kidney tissue antioxidant enzymes activities was examined, it was observed that erdosteine significantly inhibited the depletion of CAT, SOD and GSH-Px activities (Table 1).

On the other hand, prophylactic erdosteine administration at the three doses provided marked protection from an increase in MDA level (a marker of lipid peroxidation) and MPO activity (an indicator of tissue neutrophil accumulation) in the kidney of cisplatin-treated rats (Figs 2 and 3).

Erdosteine, when treated at 50 and 75 mg kg^{-1} once a day for 6 days, ameliorated cisplatin-induced acute renal failure, as measured by BUN and creatinine levels (Fig. 1) and histological study (Fig. 4). For histology study, we obtained five rats from each group. The histological

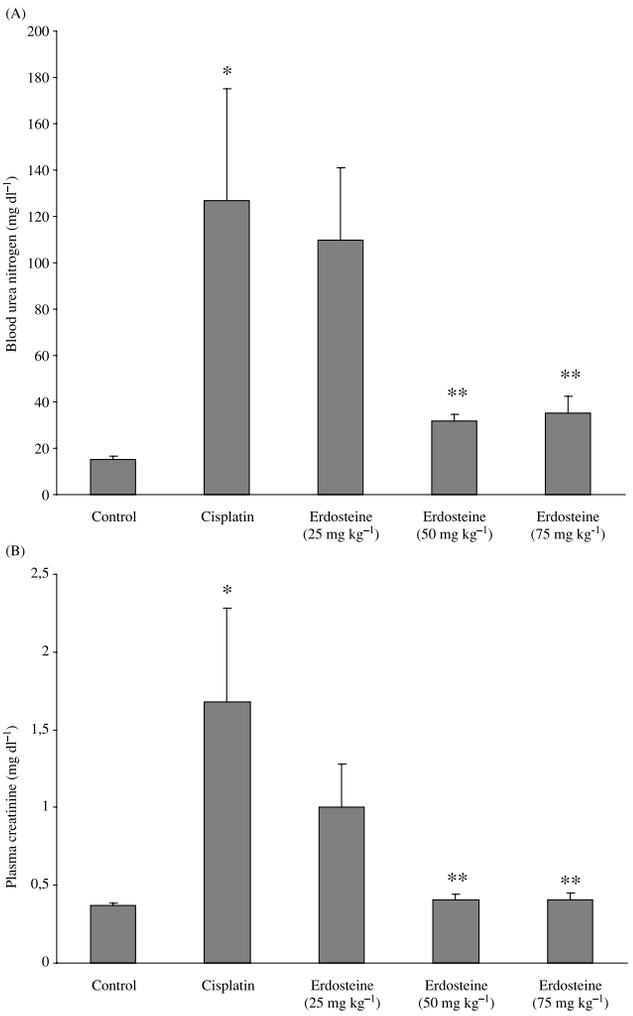

Figure 1. Effect of erdosteine on cisplatin-induced acute renal failure as measured by blood urea nitrogen (A) and plasma creatinine (B) levels. Values are means ± SEM; * $P < 0.0001$ compared with other groups and ** $P < 0.0001$ compared with cisplatin treatment alone and erdosteine treatments at a dose of 25 mg kg^{-1}.

changes, graded as described in Materials and Methods, are summarized in Table 2. Compared with control rats (Fig. 4A), cisplatin-treated rats had extensive epithelial cell vacuolization, swelling and desquamation (grade 4) occurring predominantly in the proximal tubules (Figure 4B). There was a clear reduction in the extent of tubular damage (grade I) in those animals treated with erdosteine at a dose of 50 or 75 mg kg^{-1} (Figs 4D and 4E). As shown in Fig. 4C, although 25 mg kg^{-1} erdosteine treatment results in some protection from the tubular damage in rats treated with cisplatin, erdosteine at a dose of 50 or 75 mg/kg^{-1} provides more protection.

There were no significant differences between the effects of erdosteine at doses of 50 and 75 mg kg^{-1} on any of the parameters assessed in this study.

DISCUSSION

The present study shows for the first time that the clinically available radical scavenger erdosteine at doses of

Table 1—Renal tissue antioxidant enzyme activity changes in the experimental groups: cisplatin administration caused a significant depletion of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activity, and prophylactic erdosteine treatment ameliorated the depletion of these enzymes activities in the damaged renal tissue by cisplatin

	SOD[a] (U mg^{-1} protein)	CAT[b] (kg^{-1} protein)	GSH-Px[b] (U g^{-1} protein)
Controls ($n = 10$)	0.17 ± 0.03	0.44 ± 0.08	1.30 ± 0.37
Cisplatin ($n = 10$)	0.13 ± 0.03	0.17 ± 0.04	0.80 ± 0.24
Erdosteine, 25 mg kg^{-1} ($n = 10$)	0.16 ± 0.04	0.19 ± 0.04	1.00 ± 0.14
Erdosteine, 50 mg kg^{-1} ($n = 10$)	0.16 ± 0.04	0.19 ± 0.04	1.06 ± 0.28
Erdosteine, 75 mg kg^{-1} ($n = 10$)	0.15 ± 0.03	0.22 ± 0.07	1.01 ± 0.30

[a] $P < 0.005$ compared with control and cisplatin groups and $P < 0.05$ compared with erdosteine (50 and 75 mg kg^{-1}) and cisplatin groups.
[b] $P < 0.001$ compared with control and cisplatin groups and $P < 0.05$ compared with erdosteine (50 and 75 mg kg^{-1}) and cisplatin groups.

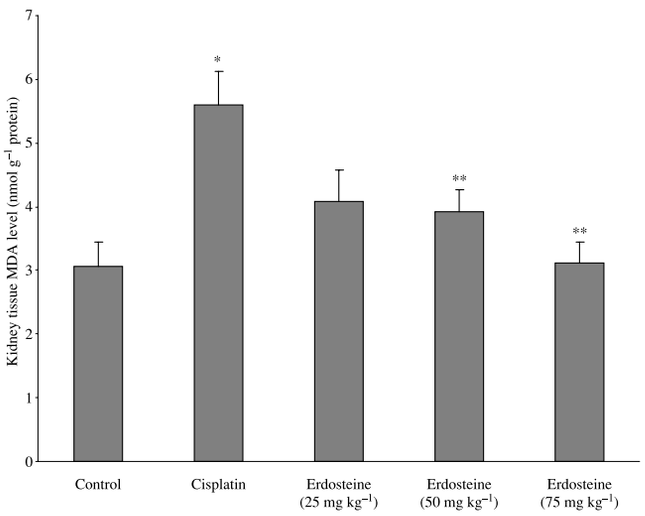

Figure 2. Effect of cisplatin and protective effect of erdosteine on malondialdehyde (MDA) formation in kidney tissue of rats treated with cisplatin. Values are means ± SEM; (*) significantly higher than all other groups ($P < 0.0001$); (**) significantly lower than other groups except for control group ($P < 0.0001$).

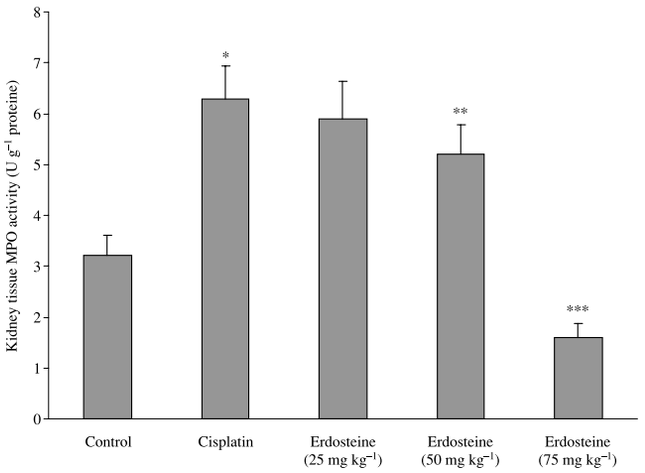

Figure 3. Effect of cisplatin and protective effect of erdosteine on myeloperoxidase (MPO) activity in kidney tissue of rats treated with cisplatin. Values are means ± SEM; (*) significantly higher than all other groups ($P < 0.0001$) and (**) significantly lower than other groups except for control group ($P < 0.0001$) compared with cisplatin treatment alone; (***) significantly lower than all other groups ($P < 0.0001$).

Table 2—The distribution of grade of nephrotoxicity in experimental groups

	Grade 0	Grade I	Grade II	Grade III	Grade IV
Control	1	4			
Cisplatin			1	2	2
Erdosteine, 25 mg kg^{-1}		1	2	2	
Erdosteine, 50 mg kg^{-1}		3	2		
Erdosteine, 75 mg kg^{-1}		4	1		

50 and 75 mg kg^{-1} dramatically inhibits cisplatin-induced *in vivo* nephrotoxicity in rats. The mechanisms underlying cisplatin-induced nephrotoxicity have not been fully understood but one possibility is unopposed intracellular generation of free-radical species induced by cisplatin administration. Cisplatin has been shown to induce oxidative injury, including tissue lipid peroxidation, enzyme inactivation, changes in the cellular non-enzymatic and enzymatic antioxidant defence system and glutathione status (Baliga *et al.*, 1999; Davis *et al.*, 2001; Nisar and Feinfeld, 2002). Several radical scavengers and antioxidants, such as edaravone (Sueishi *et al.*, 2002), WR-2721 (Amifostine) (Johnsson and Wennerberg, 1999), superoxide dismutase (Davis *et al.*, 2001), Vitamins E and C (Appenroth *et al.*, 1997) and caffeic acid phenethyl ester (Ozen *et al.*, 2004), are reported to attenuate or inhibit cisplatin-induced renal toxicity. However, most of them are either clinically non-available or only effective at intolerable doses.

Lipid peroxidation is frequently used as an index of tissue oxidative stress resulting from free-radical damage to membrane components of cells. Using tissue MDA as a criterion of lipid peroxidation, we show, both in the present and the previous study (Yildirim *et al.*, 2003), that lipid peroxidation in kidney cells significantly increased in rats treated with cisplatin alone. Prophylactic erdosteine treatment significantly ameliorated the increase in kidney tissue MDA levels. On the other hand, the activities of

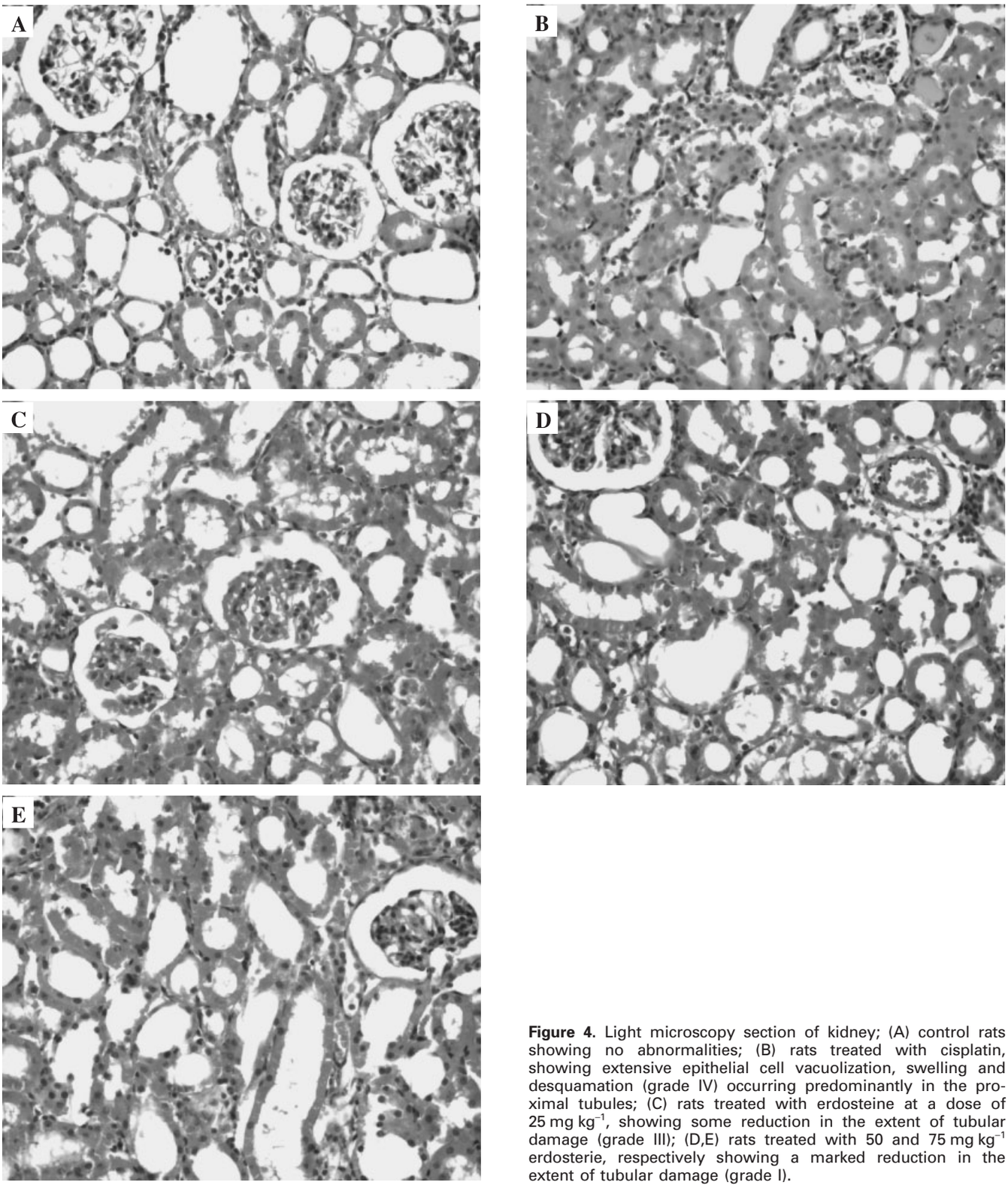

Figure 4. Light microscopy section of kidney; (A) control rats showing no abnormalities; (B) rats treated with cisplatin, showing extensive epithelial cell vacuolization, swelling and desquamation (grade IV) occurring predominantly in the proximal tubules; (C) rats treated with erdosteine at a dose of 25 mg kg^{-1}, showing some reduction in the extent of tubular damage (grade III); (D,E) rats treated with 50 and 75 mg kg^{-1} erdosterie, respectively showing a marked reduction in the extent of tubular damage (grade I).

antioxidant enzymes such as SOD, CAT and GSH-Px (on indirect indicator of free-radical production; Ozyurt *et al.*, 2001) in the kidney tissue produced by cisplatin were significantly depleted, and the depletion of these enzymes activities was significantly reduced by the erdosteine treatment, especially at doses of 50 and 75 mg kg^{-1}. These findings indicate the potent free-radical scavenger properties of erdosteine reported in many previous studies (Hayashi *et al.*, 2000; Fadillioglu and Erdogan, 2003; Fadillioglu *et al.*, 2003; Yildirim *et al.*, 2003; Fadillioglu *et al.*, 2003). Thus, it is suggested that the protective effect of erdosteine observed in the presented study is due primarily to the inactivation of free radicals. However, it may be assumed that the doses of erdosteine used in this study were higher than the clinically tolerable dose. Previous studies indicate that the tolerable dose of erdosteine without side-effects was 900 mg day^{-1} in humans and 500–1000 mg kg^{-1} in rats (Ricevuti *et al.*, 1988; Giovanni

et al., 1991). The most common side-effect of this group of drugs (such as *N*-acetylcysteine) is gastric mucosa irritation due to the mucolytic affect, and this side-effect is rarely seen in erdosteine because the mucolytic effect of erdosteine emerges after hepatic metabolism (Giovanni *et al.*, 1991). Our previous study in which we used 10 mg kg^{-1} erdosteine demonstrated that erdosteine had a similar effect on the protection of antioxidant enzyme activities and products of lipid peroxidation in renal tissue but limited protective activity for BUN and creatinine levels and histopathological changes. However, the present study clearly demonstrates that erdosteine at doses of 50 and 75 mg kg^{-1} reduced the increase in BUN and creatinine levels in plasma and the histopathological changes. These findings also demonstrate that the 50 mg kg^{-1} dose of erdosteine was better than the 25 mg kg^{-1} dose for the protection of cisplatin-induced renal failure model in rats.

The tissue MPO activity has been used as a biochemical marker for the tissue content of polymorphonuclear leukocytes (Mollar *et al.*, 2002). Previous studies demonstrated that antioxidant agents ameliorated the accumulation of leukocytes in the damaged lung tissue produced by bleomycin in rats (Hagiwara *et al.*, 2000; Mollar *et al.*, 2002). Although one study suggests that leukocyte accumulation plays a role in the pathogenesis of cisplatin-induced renal failure (Kelly *et al.*, 1999), the role of accumulation of leukocytes in the kidney of rats treated with cisplatin is clearly unknown. Acute renal injury from cisplatin and acute lung injury from bleomycin are known to share many common features, such as free-radical production and benefit from antioxidant treatment. Thus, to determine the role of inhibition of cellular infiltration, we examined the effect of erdosteine on leukocyte accumulation in the kidneys caused by cisplatin. In this study, cisplatin treatment produced a significant increase in the kidney tissue MPO activity, which indicated leukocyte accumulation in the kidney tissue, compared with control animals. Histological examination supported this finding. We observed that prophylactic erdosteine administration produced a marked inhibition in this increase of MPO activity produced by cisplatin. The inhibitor effect of erdosteine may reflect a reduction in the migration of neutrophils and other inflammatory cells, thereby mitigating tissue damage in the kidney.

In conclusion, cisplatin-induced renal failure was ameliorated by the clinically available free-radical scavenger erdosteine at doses of 50 and 75 mg kg^{-1}. The optimum dose of erdosteine is 25–50 mg kg^{-1} in this study.

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