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In vivo evidence suggesting a role for purine-catabolizing enzymes in the pathogenesis of cisplatin-induced nephrotoxicity in rats and effect of erdosteine against this toxicity

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The aim of this experimental study was to investigate the possible role of adenosine deaminase (AD) and xanthine oxidase (XO) in the pathogenesis of cisplatin-induced nephrotoxicity and the effect of erdosteine in decreasing the toxicity. The intraperitoneal injection of cisplatin ($7\,mg\,kg^{-1}$ body weight) induced a significant increase in plasma creatinine level and blood urea nitrogen (BUN), and plasma and damaged renal tissue activities of AD and XO in rats. Co-treatment with erdosteine ($10\,mg\,kg^{-1}day^{-1}$) attenuated the increase in the plasma creatinine and BUN levels, and significantly prevented the increase in tissue and plasma AD and XO activities ($P < 0.05$). The results of this study revealed that XO and AD may play an important role in the pathogenesis of cisplatin-induced nephrotoxicity. The potent free radical scavenger erdosteine may have protective potential in this process and it will become a promising drug in the prevention of this undesired side-effect of cisplatin, but further studies are needed to illuminate the exact protection mechanism of erdosteine against cisplatin-induced nephrotoxicity. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS — xanthine oxidase; adenosine deaminase; cisplatin; nephrotoxicity; erdosteine

INTRODUCTION

Cisplatin is one of the most effective antineoplastic agents and plays a major role in the treatment of a variety of human solid tumours including lung cancer. Nephrotoxicity is a major dose-limiting side-effect of this drug. Although the underlying mechanism of this side-effect is still unknown, in recent years evidence is accumulating has accumulated which indicates that the reactive oxygen metabolite may play a role in the pathogenesis of cisplatin-induced renal damage.[1] Kruidering *et al*. showed that freshly isolated porcine proximal tubular cells (PPTC) in suspension exposed to cisplatin at different concentrations suffered a loss of mitochondrial membrane potential, and inhibition of the mitochondrial respiratory chain which can generate excessive free oxygen radicals. This study also demonstrated that exposure of PPTC to 50 μM cisplatin caused a significant decrease in ATP content.[2]

XO produces large amounts of reactive oxygen species (ROS), especially superoxide ($O_2^{\cdot-}$), during the above-mentioned reaction.[3] Cisplatin induces excessive ATP catabolism in renal tubules with subsequent local release of its metabolites adenosine, xanthine and hypoxanthine. Subsequently the increased xanthine and adenosine which are substrates for XO and AD respectively, may amplify the activity and/or expression of XO and AD in the damaged tissue.[4] Hence, the increased XO activity may cause further

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tissue damage because of its free radical-generating effect. Because the reaction between superoxide radical originating from XO activity and nitric oxide gives the potent oxidant agent peroxynitirite ($ONOO^-$), this agent may cause further injury in the renal tissue. Therefore, we hypothesized that XO and AD enzymes may participate in the pathogenesis of cisplatin-induced acute renal damage. The role of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase in cisplatin-induced nephrotoxicity has been well documented[5–9] but AD and XO activities have not been investigated to date.

The free radical scavenging activity of erdosteine metabolites has attracted much attention by many research groups in recent years.[10–14] Our previous study demonstrated that oral erdosteine administration attenuated cisplatin-induced renal tubular damage in rats by its free radical-scavenging activity.[15] The structural feature responsible for the antioxidant and free radical-scavenging activity of erdosteine is the –SH group in the chemical structure.[10] Therefore, erdosteine seems to be a promising drug for the prevention of free radical-induced damage in many diseases.

The aims of the current study were (1) to investigate the possible role of AD and XO in the pathogenesis of cisplatin-induced nephrotoxicity in rats and (2) to explain whether there is a preventive effect of erdosteine on this undesired side-effect of cisplatin and on these enzymes activities.

MATERIALS AND METHODS

Animals

Female Wistar albino rats weighing 200–250 g were purchased from the Experimental Research Centre, University of Erciyes (Kayseri, Turkey) and housed in the animal laboratory at our university. The animals were fed with a standard diet and were maintained in a 12-h light–dark cycle.

Cisplatin-induced acute renal failure

A cisplatin model of acute renal failure was induced in rats as described by Zang *et al.*[16] The animals ($n = 9$) received an intraperitoneal injection of cisplatin (Cisplatinum Ebewe, 0.5 mg ml^{-1}) at a dose of 7 mg kg^{-1} body wt and were sacrificed 5 days after cisplatin injection. Blood and kidneys were obtained for the various measurements. Renal impairment was assessed by blood urea nitrogen (BUN) and plasma creatinine levels. BUN and creatinine were determined by the use of Sigma diagnostic kits.

Effect of erdosteine on the cisplatin model of acute renal failure

The erdosteine was obtained from a drug company (Ilsan, Turkey), dissolved in distilled water, and administered orally once a day at a dose of 10 mg kg^{-1} body weight via plastic disposable syringes ($n = 8$). The first dose of erdosteine was given 24 h prior to cisplatin injection and continued until sacrifice.

Control rats

Isotonic saline solution (in equal volume to cisplatin) was administered by intraperitoneal injection ($n = 6$). $NaHCO_3$, dissolved in distilled water, was given orally (in equal volume to erdosteine) 24 h prior to the saline solution injection and then once a day at the same dose until the animals were killed.

Tissue homogenization

All tissues were washed twice with cold saline solution, placed into glass bottles, labelled, and stored at −30°C until processing (maximum storage time 10 h). Tissues were homogenized in 4 volumes of ice-cold Tris-HCl buffer (50 mmol, pH 7.4) using an homogenizer (Tempest Virtishear, Model 278069; The Virtis Company, Gardiner, NY) for 2 min at 10 000 r.p.m. after the kidney had been cut into small pieces with scissors. The homogenate was then centrifuged at 5000 *g* for 60 min to remove debris. Measurements of XO and AD activities and protein concentration were carried out on the clear supernatant fluid. All preparative procedures were performed at +4°C.

Protein determinations

Protein was assayed by the method of Lowry *et al.*[17]

Xanthine oxidase determination

Plasma and tissue XO activities were measured by the method of Prajda and Weber,[18] where the activity is measured by determination of uric acid from xanthine. Supernatant derived from the tissue homogenate (50 µl) was incubated for 30 min at 37°C in 3 ml of phosphate buffer (pH 7.5, 50 mM) containing xanthine (4 mM). The reaction was stopped by addition of 0.1 ml 100% (w/v) TCA, the mixture was then centrifuged at 4000 *g* for 20 min. Urate was determined in the supernatant by measuring absorbance at 292 nm against a blank and expressed as units per g protein

in the tissue and units per milliliter ($U\,ml^{-1}$) in plasma. Results are reported as mean ± standard deviation. All samples were assayed in duplicate. All chemicals used were obtained from Sigma.

Adenosine deaminase determination

Plasma and tissue AD activities were estimated spectrophotometrically by the method of Giusti, which is based on the direct measurement of the ammonia produced when AD acts in excess of adenosine.[19] Results were expressed as units per g protein in the tissue and units per liter in plasma ($U\,l^{-1}$), and calculated as mean ± standard deviation. All samples were assayed in duplicate.

Data analysis

Data are expressed as means ± standard deviation. The one-way ANOVA analysis of variance and *post-hoc* multiple comparison tests (LSD) were performed on the data of biochemical variables to examine differences among groups. Results are expressed as mean ± standard deviation. All analyses were carried out using the SPSS statistical software package. A p-value <0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

In the kidney tissue, the activity of XO and AD in rats treated with cisplatin alone were significantly higher than those of the control animals ($P = 0.001$ for XO and $P = 0.014$ for AD). Erdosteine administration significantly prevented the elevation of XO ($P = 0.028$) and AD ($P = 0.016$) activities in the renal tissue of rats (Figure 1).

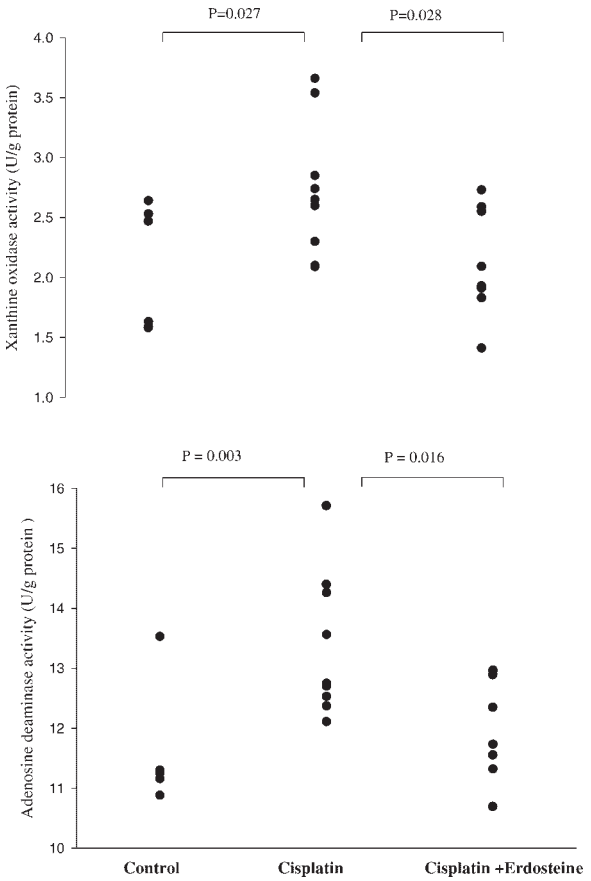

Figure 1. The scatter plot graph of xanthine oxidase and adenosine deaminase activity in the renal tissues of control, cisplatin and cisplatin plus prophylactic erdosteine-treated groups. Both enzymes increased significantly with cisplatin treatment. Erdosteine significantly prevented the increase in the enzyme activities

Plasma XO and AD activities were significantly increased from control values of 1.55 ± 0.44 (mean ± SD) and 45.5 ± 11.29 to 2.36 ± 0.68 and $61.33 \pm 11.15\,U\,l^{-1}$ ($P = 0.007$ and 0.01, respectively) in rats treated with cisplatin. The results obtained with rats treated with cisplatin and concurrently treated with erdosteine revealed that the drug had a preventive effect on the plasma activities of these enzymes (XO, 1.53 ± 0.43; AD, 51.12 ± 9.8, $P = 0.003$ and 0.032 respectively) as compared to rats receiving cisplatin alone (Figure 2). These results were similar to the tissue AD and XO activities.

In the cisplatin-treated animals, plasma creatinine and BUN levels increased from control values of 0.4 ± 0 and 19.50 ± 2.9 to 1.33 ± 1.3 and 78.77 ± 42.7, respectively, ($P = 0.002$ for BUN, $P = 0.049$ for creatinine). Erdosteine co-treatment partly prevented the increase of creatinine (1.15 ± 0.8) and BUN (69.75 ± 25.5) levels but this prevention did not reach a statistically significant level ($P > 0.05$).

This study indicates that XO and AD activities were increased in the renal tissue and plasma of rats in an experimental model of acute renal failure. Our observations also revealed an inhibitory effect of erdosteine on the increase in these enzyme activities in both plasma and damaged kidney tissue, although the erdosteine treatment at a dose of $10\,mg\,kg^{-1}$ body weight had a limited preventive effect on the rise in BUN and plasma creatinine levels. The biochemical mechanism of the inhibitory effect of erdosteine on the increase in the enzymes activities is not obvious. Erdosteine may directly interact with the superoxide

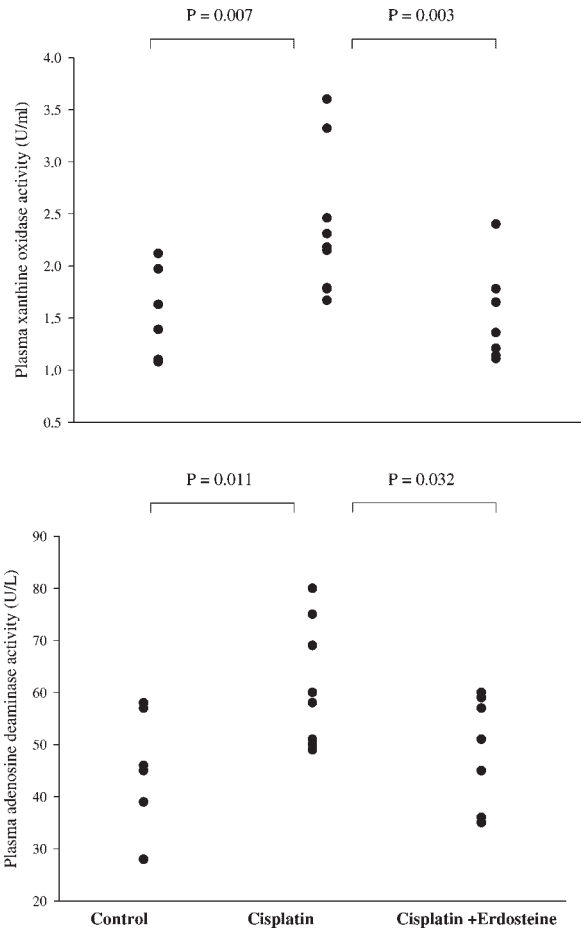

Figure 2. The scatter plot graph of xanthine oxidase and adenosine deaminase activity in the plasma of control, cisplatin and cisplatin plus prophylactic erdosteine-treated groups. Both enzymes increased significantly with cisplatin treatment. Erdosteine significantly prevented the increase in the enzyme activities

generator XO or act as a general radical scavenger for reactive oxygen species during cisplatin nephrotoxicity.

Xanthine oxidase exists in oxidase (XO) and dehydrogenase (XDH) isoforms.[20] In most tissues the NAD^+-dependent dehydrogenase predominates, but when oxidation of its thiol groups occurs, or when it undergoes limited proteolysis, it is converted to its oxidase form.[21] Xanthine dehydrogenase–oxidase conversion, and hypoxanthine formation have been implicated in the oxidative damage of reperfusion injury following reoxygenation of most major organs.[22] Elevated levels of XO have also been reported in the plasma of patients with Acute Respiratory Distress Syndrome[23] and ischaemia and reperfusion injury in many organs.[3,24,25] In accord with our previous studies, the increased levels of plasma XO and AD probably originated from the injured kidney.[26,27] Xia and Zweier demonstrated that substrate availability is the primary cofactor in triggering and controlling XO-mediating free radical generation in the post-ischaemic heart.[28]

Recently, it has been proposed that mitochondrial dysfunction including loss of the mitochondrial membrane potential caused by cisplatin, plays an important role in the pathogenesis of cisplatin-induced nephrotoxicity.[2,29,30] The mitochondrial membrane dysfunction leads to impaired ATP metabolism with increased production of purine degradation products such as adenosine, inosine, hypoxanthine, and xanthine which are substrates for AD and XO. In our study, the presence of significantly increased activities of XO and AD in the kidney tissue and plasma obtained from rats treated with cispatin may be due to the excessive substrate production for these enzymes during the above-mentioned process of mitochondrial membrane.

McCord proposed that the process of increased substrate formation could be an important factor in triggering XO-mediating free radical generation.[21] Abd-Elfattah *et al*. have noted with *in vivo* surgical canine models of global ischaemia and reflow that XO substrate formation is an important factor in the process of post-ischaemic injury and have shown that pharmacological intervention aimed at decreasing substrate concentration can decrease the severity of this injury.[31]

Many free radical-scavenging compounds have been tested in the prevention of cisplatin-induced renal failure with some elimination of this nephrotoxicity.[5–8,32,33] Erdosteine administration in the present study significantly attenuated the increase of renal tissue and plasma AD and XO activities but had a limiting effect on the improvement of renal function. These data indicate that the presence of XO and AD may be important requisite factors in the process of free radical generation in cispatin-induced acute renal failure. Although erdosteine significantly prevents the increase of XO activity, it cannot improve the impaired renal function. These data suggest that XO induced-free radical generation is not the only process involved in the pathogenesis of cisplatin nephrotoxicity. There may be other mechanisms that disturb renal function in this experimental model of acute renal failure.

Since substrate availability plays a limiting role in the process of free radical generation by XO,

pharmacological approaches that decrease substrate formation may provide an alternative approach to that of radical scavenging compounds or direct oxidase blockers in preventing free radical generation in cisplatin nephrotoxicity and subsequent oxidant-mediated renal tissue injury. Conversely, increased XO substrate formation that would occur from increased ATP degradation would be expected to result in increased XO-mediating free radical formation and postoxidant-induced tissue injury, further exacerbating the process of renal damage which occurs secondary to ATP depletion. Using inhibitors of the enzymes, such as allopurinol in such an experimental cisplatin-induced nephrotoxicity model may help to provide a better understanding of the underlying mechanism. The potent free radical scavenger erdosteine may have a protective effect against this process and be a promising drug in the prevention of this undesired side-effect of cisplatin.

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