Cisplatin induced nephrotoxicity and the modulating effect of glutathione ester

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Abstract

The objective of this study was to assess the therapeutic advantage of glutathione ester along with cisplatin. Comparisons were made with renal reduced glutathione, enzymatic antioxidants, and lipid peroxidation levels. Cisplatin caused differential toxic effects on renal antioxidants and lipid peroxidation. However administration of glutathione ester modulates the toxic effects of cisplatin observed in renal antioxidants and lipid peroxidation. The finding that glutathione ester co-administration along with cisplatin is more effective and advantageous in protecting against the nephrotoxicity of cisplatin when it was given alone. (Mol Cell Biochem 144: 7-11, 1995)

Key words: cisplatin, glutathione ester, reduced glutathione, antioxidants, lipid peroxidation, nephrotoxicity

Introduction

Cisplatin (Cis-diaminedichloroplatinum II) is an important chemotherapeutic agent. It is a highly effective antitumor compound for several types of cancer including testicular, bladder, head and neck, ovarian, osteogenic, and uterine cervix carcinomas [1, 2]. These types of tumors are treated most effectively by using relatively high dose regimens of chemotherapeutic agents. Unfortunately, the importance of adverse effects are dose-limiting. Toxicities associated with Cisplatin include cumulative nephrotoxicity [3, 4].

The reactivity of cisplatin in its aquated/hydroxylated form with nuclear DNA has been well documented [5] and may mediate the inhibition of DNA synthesis in tumor tissues [6, 7]. On this basis it seems reasonable to speculate that such an electrophilic complex may bind to essential macromolecules of the kidney, resulting in nephrotoxicity.

The search for methods to suppress these toxic reactions has been a continuing one in which many substances have been reported to produce some benefit. The use of nucleophiles to reduce the nephrotoxicity of platinum complexes with antitumor activity has considerable history. There is evidence that cisplatin binds to thiols such as cysteine and glutathione [8]. It has been reported that administration of glutathione before treatment with cisplatin led to a decrease in nephrotoxicity [9]. Monoesters of glutathione in which the glycine carboxyl group of this tripeptide is esterified are effective delivery agents for glutathione [10, 11]. The effective transport of glutathione monoesters has led to their use in the protection of cells against radiation [12] and various toxic compounds such as acetaminophen [11], heavy metal ions [13], and certain anticancer agents (eg: Melphelan, Cisplatinum and Cyclophosphamide). Administration of glutathione monoester also protects against cellular damage (eg: mitochondrial degeneration, cataracts [14, 15]) that is produced by administration of buthionine sulfoximine, a selective inhibitor of glutathione synthesis [16]. Hence it seems to be important to learn whether administration of glutathione monoesters may protect against nephrotoxicity and also to examine their potential value in ameliorating a variety of conditions thought to be produced by free radicals and reactive oxygen compounds [17, 18], that are likely to be generated by cisplatin.
Materials and methods

Healthy adult albino rats of the Wistar strain weighing 120-160 g were obtained from the Fredrick Institute of Plant Protection and Toxicology, Padappai, Madras, India. They were kept in clean cages, housed in a well-ventilated animal house with controlled illumination (14 h light/10 h darkness). Standard rat feed (Hindustan Lever Limited, Bombay, India) and clean drinking water were made available ad libitum.

Cisplatin was obtained as a raw material from Tamil Nadu Dadha Pharmaceuticals Limited, Madras, India. It was reconstituted in sterile saline at a concentration of 1 mg/ml and sterilised by filtering through a 0.2 μm microfilter under the laminar flow. It was sealed in glass vials and stored in a refrigerator.

The glutathione ester was freshly prepared by mixing glutathione plus isopropanol plus sodium sulphate (1:1:0.5; molar ratio) (adjusted to pH 6.8 with NaOH) by the method of Anderson and Meister [9, 19]. From this 10 mM solution was prepared and was administered subcutaneously to the experimental animals.

The rats were divided into three groups. Group I served as control animals which received the vehicle (0.9% saline, I.P.) and Group II animals received cisplatin (6 mg/kg body weight) intraaperitoneally once a week for two successive weeks. This dosage was selected for a statistically significant increase in lipid peroxidation in rat kidney after injection of cisplatin with dose-limiting side effect of nephrotoxicity [20] and Group III animals received glutathione ester 10 mM, (10 ml/kg body weight) subcutaneously along with cisplatin simultaneously.

After the experimental period of fifteen days, the animals were killed by cervical decapitation. The kidneys were immediately removed and washed in ice-cold saline and a portion of the kidneys were homogenised in 0.1 M, Tris-HCl buffer pH 7.4 and were used for the assay of the biochemical parameters.

Histopathological study

Tissues for pathological evaluation were placed in 10% neutral-buffered formalin immediately after dissection and were subsequently embedded in paraffin, sectioned, and stained with haematoxylin and eosin for examination with the light microscope.

In the tissue homogenate, reduced glutathione concentration was assayed by the method of Moran [21]. The enzymatic antioxidants, superoxide dismutase [22], catalase [23] and glutathione peroxidase [24] were also assayed. The activity of lipid peroxidation was determined by following the procedure of Hogberg et al. [25] and protein content of the tissue homogenates was measured by the method of Lowry et al. [26]. Students 't' test was used to determine statistically significant differences between control and treated groups.

Results

The levels of reduced glutathione and the activities of the antioxidant enzymes in kidneys of control and experimental animals were given in Table 1.

The reduced glutathione level of kidney was found to be significantly decreased (p<0.001) in cisplatin treated animals when compared to control animals. The levels got increased (p<0.001) and restored to near normal during the administration of glutathione ester.

Superoxide dismutase activity got significantly decreased (p<0.001) in cisplatin treated animals, when compared to controls and increased (p<0.01) in glutathione ester administered animals.

The activity of catalase in kidney of cisplatin treated animals got significantly reduced (p<0.001), when compared to controls. However a moderate increase (p<0.001) was observed in glutathione ester administered animals and was found to be restored to near normal.

Table 1: Effect of glutathione ester on renal reduced glutathione and antioxidant enzymes in cisplatin treated rats

<table>
<thead>
<tr>
<th>Renal glutathione and antioxidant enzymes</th>
<th>Group I control</th>
<th>Group II cisplatin treated</th>
<th>Group III cisplatin + glutathione ester treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reduced glutathione</td>
<td>6.51 ± 0.23</td>
<td>3.24 ± 0.43***</td>
<td>5.69 ± 0.21***</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>4.59 ± 0.32</td>
<td>3.31 ± 0.11***</td>
<td>3.86 ± 0.38**</td>
</tr>
<tr>
<td>Catalase</td>
<td>37.59 ± 0.28</td>
<td>29.52 ± 0.42***</td>
<td>38.50 ± 0.30***</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>3.35 ± 0.26</td>
<td>2.42 ± 0.25**</td>
<td>3.56 ± 0.38**</td>
</tr>
</tbody>
</table>

Values are expressed as μg/mg protein for reduced glutathione, units/mg protein for superoxide dismutase, μmoles of H₂O₂ utilized/min/mg protein for catalase and μg glutathione utilized/min/mg protein for glutathione peroxidase. Values are given as mean ± S.D for six animals in each group.

For statistical significance Group II was compared with Group I and Group III was compared with Group II *p<0.05, **p<0.01, ***p<0.001.
In the case of glutathione peroxidase, the activity was found to be significantly decreased (p<0.001) in cisplatin treated animals, when compared to controls. However, a significant increase (p<0.001) was observed in glutathione ester administered animals and also got restored to near normal level. The changes in the level of lipid peroxidation in control and experimental animals were given in Table 2.

The lipid peroxidation was found to be significantly increased (p<0.001) in the kidney of experimental animals, when compared to controls. However, a significant decrease (p<0.001) was observed in glutathione ester treated animals. In cisplatin treated animals, the lipid peroxidation was found to be significantly elevated in the presence of promoters like ascorbate and ferrous sulphate.

**Table 2. Effect of glutathione ester on the activity of lipid peroxidation in cisplatin treated rats**

<table>
<thead>
<tr>
<th>Lipid peroxidation</th>
<th>Group I (control)</th>
<th>Group II (cisplatin treated)</th>
<th>Group III (cisplatin + glutathione ester treated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>8.64 ± 0.21</td>
<td>11.55 ± 0.29***</td>
<td>8.51 ± 0.16***</td>
</tr>
<tr>
<td>Ascorbate</td>
<td>9.38 ± 0.24</td>
<td>13.59 ± 0.20***</td>
<td>9.54 ± 0.16***</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>10.42 ± 0.18</td>
<td>16.72 ± 0.12***</td>
<td>11.36 ± 0.15***</td>
</tr>
</tbody>
</table>

Values are expressed as n moles of malondialdehyde released/mg/mm protein. Values are given as mean ± S.D for six animals in each group.

For statistical significance, Group II was compared with Group I and Group III was compared with Group II. \*p<0.05, \**p<0.01, \***p<0.001.

**Discussion**

The decrease in glutathione levels and depression of macromolecule synthesis in the kidney may play a role in cisplatin induced nephrotoxicity. Cellular glutathione mechanism against metal toxicity is an important factor in the elevation of the acute and chronic toxicity of metals. Cellular glutathione plays an important role in the detoxification of metals. Depletion of glutathione may increase susceptibility of animals to the toxicity or lethality of mercury [27] or cadmium [13]. The finding that glutathione ester is more effective than glutathione in protecting against the toxicity of cisplatin suggests that use of glutathione ester may be therapeutically advantageous [9].

Superoxide dismutase has been reported to contain arginine and histidine in its active site [28]. Metal ions like copper and zinc play an important role in the activity of superoxide dismutase and interaction of hydroxyl radicals was reported to induce loss of activity [29]. In cisplatin treated rats, there was a significant decrease in superoxide dismutase activity. Sharma [30] has also demonstrated that renal excretion of magnesium, zinc and copper were markedly increased during cisplatin treatment. So a decrease in superoxide dismutase activity in cisplatin treated rats may be attributed to the increased excretion of copper and zinc, which were not available for superoxide dismutase activity.

The activity of the antioxidant enzyme catalase was found to be significantly decreased in cisplatin treated rats. Catalase has been shown to be responsible for the detoxification of significant amounts of hydrogen peroxide [31] and the decrease in catalase activity could be due to free radicals, which are expected to be generated by the administration of cisplatin in experimental rats. Catalase has also been shown to be inactivated by hydrogen peroxide and superoxide anions [32].

The selenium dependent glutathione peroxidase activity was significantly decreased in cisplatin treated rats. This shows the defect in the enzymatic detoxification of free radicals in kidney. Intestinal abnormalities have been reported to decrease the activity of selenium dependent glutathione peroxidase. Gastrointestinal toxicity is known to occur as a result of cisplatin treatment, which also affects the gastric smooth muscle contractility [33]. Hence, the decrease in renal glutathione peroxidase activity may be correlated closely with the defective enzymatic detoxification of free radicals generated by cisplatin [34, 35].

A significant increase in lipid peroxidation may be due to abnormal levels of reactive oxygen radicals which react with membrane lipids, causing an increase in lipid peroxidation [34]. Due to the decreased levels of antioxidant enzymes, the lipid peroxidation reaction is not neutralised and hence there is enhanced susceptibility to lipid peroxidation. The lipid peroxidation reaction was found to be increased in rats treated with cisplatin. This suggests that cisplatin treated rats may be less resistant and more susceptible in lipid peroxidation in the presence of promoters like ascorbate and ferrous sulphate [36]. These results provide evidence that cisplatin may generate free radicals, which can interact with membrane lipids and consequently cause the production of lipid hydroperoxides.

Decreased antioxidants and antioxidant enzymes with increased lipid peroxidation reactions have also been reported in several conditions, such as Vit-E deficiency, atherosclerosis and diabetes mellitus [36].

Glutathione plays an important role in maintaining membrane structure and participates in a variety of active transport process. It is known to protect the cellular system against the toxic effects of lipid peroxidation [37].

In summary, we conclude that the reduction in activities of renal reduced glutathione, antioxidant enzymes and increased lipid peroxidation along with the observed histopathological changes in the kidneys (Figs 1–3) supports the evidence that part of the mechanism of nephrotoxicity in cisplatin treated rats is related to free radical formation and lipid peroxidation. Administration of glutathione ester was found to be effective.
in protecting against the toxicity of cisplatin suggests that use of glutathione ester along with cisplatin may be therapeutically advantageous in minimizing the cytotoxic effect of cisplatin.

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References

Protective Role of Vitamin E and Acetazolamide in Cisplatin-Induced Changes in Lipid Peroxidation and Antioxidant Enzyme Levels in Albino Rats

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Summary  Cisplatin, an antitumor chemotherapeutic agent with dose-limiting nephrotoxic side effects, was administered i.p. to male albino rats at a concentration of 6 mg/kg body wt. The antioxidant vitamin E (400 mg/kg body wt., orally) and the diuretic acetazolamide (ACZ) (20 mg/kg body wt., i.p.) were co-administered as modulators. The effect of cisplatin on lipid peroxidation and antioxidant enzyme levels was then examined. The agent increased the level of lipid peroxidation and significantly decreased the activities of antioxidant enzymes. These effects may have been due to the formation of free radicals and were considered to be one of the reasons for cisplatin-induced nephrotoxicity. Interestingly, these changes were found to be very much counteracted by vitamin E and acetazolamide when they were co-administered along with the cisplatin. Hence, we suggest that co-administration of vitamin E and acetazolamide along with cisplatin will be clinically advantageous in attenuating cisplatin-induced nephrotoxicity during chemotherapy.

Key Words: cisplatin, vitamin E, acetazolamide, lipid peroxidation, antioxidant enzymes

Cisplatin (cis-diammine dichloro platinum-II), a platinum containing coordination complex, is an antitumor agent with proven efficacy as a single agent against a wide spectrum of malignancies [1]. Toxicological studies in humans and animals indicate that higher doses produce enzyme inhibition and histological changes in liver, kidney, gastrointestinal tract, and bone marrow [2]. However, the chief dose-limiting side effect of cisplatin is its pronounced nephrotoxicity, which occurs at doses lower than those that damage other organs [3]. The underlying molecular mechanism of the nephrotoxicity induced by cisplatin remains unclear.

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Cisplatin has been shown to cause depletion of sulphydryl (SH) groups, which action has been implicated as a cause of the nephrotoxicity. The histopathological profile of cisplatin nephrotoxicity appears similar to that of other heavy metals. It is often thought that cisplatin nephrotoxicity is related to the platinum moiety [4]. However, McGuinness et al. [5] and Sugihara et al. [6] suggested that the cisplatin-induced nephrotoxicity is related to an increase in lipid peroxide levels in the kidney. Sadzuka [7] also reported that the lipid peroxide levels increased after cisplatin treatment, especially that in the kidney. Abnormal levels of free radicals generated by cisplatin may react with polyunsaturated fatty acids in plasma and subcellular membranes, resulting in an increase in lipid peroxidation and cellular disturbances. These findings might indicate that the generation of free radicals and subsequent lipid peroxidation at least play a considerable role in cisplatin-induced nephrotoxicity.

Various procedures have been examined clinically to reduce this toxicity. Among these vitamin E, a lipid-soluble biological antioxidant, is known to protect cells from a variety of oxidizing agents including drugs, environmental pollutants, and molecular oxygen [8]. Vitamin E is thought to be incorporated into the lipid bilayer of biological membranes to an extent proportional to the amount of polyunsaturated fatty acids present in the membrane [9]. Inside the membrane, vitamin E scavenges free radicals and thus makes the membrane more resistant to peroxidative damage [10].

Another manoeuvre used to decrease the nephrotoxic effects of cisplatin include hydration by the administration of diuretics [11]. In animals, furosemide- or mannitol-induced diuresis concurrent with cisplatin administration has been reported to decrease cisplatin renal toxicity in some studies, but to aggravate renal toxicity in others [12]. The exact mechanism of the diuretic-induced reduction of nephrotoxicity is not yet known, although the reduction of platinum concentration in the tubular fluid caused by the diuretics is assumed to account for the protection. Acetazolamide, a sulphur-containing diuretic, has recently been studied and shown to possess several advantages that make it recommendable for use with cisplatin [13].

Therefore, in this present study, we assessed the combined attenuating effect of vitamin E and acetazolamide on malondialdehyde formation and on the activities of free radical-scavenging enzymes in cisplatin-induced nephrotoxicity.

**MATERIALS AND METHODS**

*Modulators.* Vitamin E was obtained from S.D., Fine Chemicals, Ltd., Bombay, India, and was administered daily for 15 days as an antioxidant [14]. Acetazolamide (Dimox) was purchased from Lederle Laboratories Division, Pearl River, New York, and was administered as a diuretic 30 min prior to cisplatin injection [13].
Cisplatin. Cisplatin was obtained as a gift from Tamil Nadu Dadha Pharmaceutical, Ltd., Madras, India. It was reconstituted in sterile saline at a concentration of 1 mg/ml and sterilized by filtering through a 0.2 μm microliter under a laminar flow hood. It was sealed in glass vials and stored in a refrigerator prior to use.

Animal studies. Male albino rats of the Wistar strain, weighing between 180 and 200 g, were obtained from King Institute of Preventive Medicine, Guindy, Madras, India. They were housed in hygienic cages in a well-ventilated animal house with controlled illumination (14 h light/10 h darkness), and fed standard rat chow (Hindustan Lever, Ltd., Bombay, India).

The experimental animals were divided into five groups. The first group (control) was treated with physiological saline (0.9%, i.p.) alone in a volume equal to that used for the drug-treated groups. The second group was treated with cisplatin (6 mg/kg body wt., i.p.) once in a week for two successive weeks. The third group was given the cisplatin treatment and vitamin E (400 mg/kg body wt., orally for 15 days). The fourth group was treated with cisplatin and acetazolamide (20 mg/kg body wt., i.p.) which was given 30 min prior to the cisplatin administration. The fifth group was treated with cisplatin, vitamin E, and acetazolamide by the above protocols. Food and pure drinking water were made available ad libitum to all the experimental groups.

After the experimental period of 15 days the animals were killed by cervical decapitation. Immediately, the kidneys were dissected out and placed in ice-cold saline. A known amount of fresh kidney tissue was homogenized with Tris-HCl buffer, pH 7.5, and used for the estimations of lipid peroxidation [15], superoxide dismutase [16], catalase [17], and glutathione peroxidase [18]. The total protein content was also estimated [19].

Statistical analysis. Statistical analysis was done by Student’s t-test.

RESULTS

Table 1 depicts the activities of renal superoxide dismutase, catalase, and glutathione peroxidase in all the experimental groups.

Superoxide dismutase (SOD)

The activity of SOD was markedly reduced (p < 0.001) after cisplatin administration in Group-II animals; but when one or both attenuators were present, the levels were notably greater (p < 0.01) in Group-III and -IV animals, and much greater (p < 0.001, to near normal) in Group-V ones compared with the level in Group-II animals.

Catalase (CAT)

This enzyme level was significantly reduced after cisplatin treatment in Group-II (p < 0.001) animals. As in the case of SOD, the level was greater in...
Table 1  Effect of vitamin E and acetazolamide (ACZ) on the renal antioxidant enzymes in cisplatin-treated rats

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Group I (Control)</th>
<th>Group II (Cisplatin treated)</th>
<th>Group III (Cisplatin + Vit E) treated</th>
<th>Group IV (Cisplatin + ACZ) treated</th>
<th>Group V (Cisplatin + Vit E + ACZ) treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>17.65 ± 2.31</td>
<td>8.32 ± 1.70**</td>
<td>13.57 ± 2.04*</td>
<td>12.36 ± 1.85*</td>
<td>16.84 ± 2.17*</td>
</tr>
<tr>
<td>Catalase</td>
<td>2.94 ± 0.35</td>
<td>1.32 ± 0.17**</td>
<td>2.23 ± 0.28**</td>
<td>1.67 ± 0.24*</td>
<td>2.65 ± 0.28**</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>78.46 ± 7.38</td>
<td>32.75 ± 5.64**</td>
<td>59.94 ± 6.82**</td>
<td>45.92 ± 4.82**</td>
<td>69.53 ± 6.48**</td>
</tr>
</tbody>
</table>

Values are expressed as units/mg protein for superoxide dismutase, μmol of H₂O₂ utilized/min/mg protein for catalase, and μg of glutathione utilized/min/mg protein for glutathione peroxidase. Values are given as the mean ± SD for six animals in each group. For statistical significance, Group II was compared with Group I, and Groups III, IV, and V were compared with Group II. *p < 0.01; **p < 0.001

Table 2  Effect of vitamin E and acetazolamide (ACZ) on the level of lipid peroxidation in cisplatin-treated rats

<table>
<thead>
<tr>
<th>Lipid peroxidation</th>
<th>Group I (Control)</th>
<th>Group II (Cisplatin treated)</th>
<th>Group III (Cisplatin + Vit E) treated</th>
<th>Group IV (Cisplatin + ACZ) treated</th>
<th>Group V (Cisplatin + Vit E + ACZ) treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0.57 ± 0.03</td>
<td>1.13 ± 0.07*</td>
<td>0.59 ± 0.04*</td>
<td>0.80 ± 0.06*</td>
<td>0.45 ± 0.04*</td>
</tr>
<tr>
<td>Ascorbate induced</td>
<td>0.68 ± 0.04</td>
<td>1.55 ± 0.09*</td>
<td>0.75 ± 0.07*</td>
<td>0.93 ± 0.03*</td>
<td>0.71 ± 0.05*</td>
</tr>
<tr>
<td>Ferrous sulphate induced</td>
<td>0.74 ± 0.02</td>
<td>1.92 ± 0.10*</td>
<td>0.97 ± 0.08*</td>
<td>1.05 ± 0.07*</td>
<td>0.85 ± 0.05*</td>
</tr>
</tbody>
</table>

Values are expressed as nmol of malondialdehyde/mg protein. Values are given as the mean ± SD for six animals in each group. For statistical significance, Group II was compared with Group I, and Groups III, IV, and V were compared with Group II. *p < 0.001

Group-III (p < 0.001), Group-IV (p < 0.01), and Group-V (p < 0.001, to normal level) animals than in Group-II ones when the animals were treated with the modulators.

Glutathione peroxidase (GPx)

The activity of GPx was similarly affected by cisplatin, and the vitamin E and acetazolamide were similarly effective in protecting against the loss of GPx activity.

Table 2 depicts the level of renal lipid peroxidation in all the experimental groups with promoters FeSO₄ and ascorbate.

Lipid peroxidation (LPO)

The basal lipid peroxidation was found to be significantly increased (p <
0.001) in Group-II animals when compared with that in Group-I animals; and the levels were markedly lower \((p<0.001)\) in Group-III, -IV, and -V animals than in Group-II ones. Lipid peroxidation promoted by FeSO\(_4\) or ascorbate was similarly affected by cisplatin; and, again, the attenuation by vitamin E and/or acetazolamide was evident.

**DISCUSSION**

Cisplatin-induced nephrotoxicity represents a life-threatening complication and is a major limiting factor in its wide spread use as an antitumor agent [20]. The nephrotoxicity of cisplatin may be attributed to free radical-mediated lipid peroxidation similar to that caused by the heavy metal cadmium [21]. This toxicity was alleviated by orgotein, which contains superoxide dismutase [5], and also by the antioxidant N-N'-diphenyl-p-phenylene diamine(DPPD) [14], which findings suggest that pathologic levels of oxygen radicals initiate lipid peroxidation.

The primary defense against cytotoxic oxygen radical is provided by antioxidant enzymes that scavenge intermediates of oxygen reduction [22]. It is well known that superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase play an important role as protective enzymes against lipid peroxidation in tissues. Regarding changes in the activity of these enzymes in rat kidney tissue after cisplatin administration, it is important in connection with its nephrotoxicity to clarify the mechanism by which cisplatin induces increased lipid peroxidation in the kidney.

Superoxide dismutase is responsible for the catalytic dismutation of the potentially toxic superoxide anion radical, \(\cdot O_2^-\) to \(H_2O_2\) [23]. Metal ions such as copper and zinc play an important role in the activity of SOD, and the interaction of hydroxy radicals with these ions was reported to induce a loss of SOD activity [24]. In this study, it is worthy to note that the SOD activity after cisplatin administration was significantly decreased in the kidney. Actually, Mn-SOD is distributed in the mitochondrial fraction, whereas Cu Zn-SOD is found mainly in the cytosolic fraction [23]. Histological studies on the subcellular distribution of platinum have shown that mitochondria and cytosol contained the highest concentration of platinum and that marked vacuolation occurred in the mitochondria of proximal tubule cells [4]; i.e., cisplatin-induced toxicity involves disruption of the mitochondria. So, this might be one of the reasons for the decreased SOD activity in kidney. Recently, Babu et al. [25] suggested that the decreased SOD activity may be due to increased excretion of Mn, Zn, and Cu in the urine of cisplatin-administered rats [26], which would lower the availability of these ions for use in enzyme activity.

The other antioxidant enzymes examined, GPx and catalase, are very important in the hydroperoxide scavenging system [18, 27]. The toxicity of superoxide anions is considered to be low, whereas the hydroxy radicals are extremely toxic.
The latter are produced by the reaction between superoxide anions and hydroperoxide [28]. In our study, the decreased activity of catalase and GPx in kidney after cisplatin administration could be due to enhanced production of hydroperoxide. Therefore, the production of hydroxy radicals may be increased by the enhanced production of hydroperoxides and superoxide anions, and this is considered to damage the cells [29]. However, Sakthisekaran et al. [30] also reported that the decreased GPx activity may closely correlate with defective detoxification of free radicals generated by cisplatin.

Based on the above hypothesis it is evident that the increased lipid peroxidation might be due to the decreased activities of free radical-scavenging enzymes and increased generation of hydroperoxide after cisplatin administration. Our results also suggest that cisplatin-treated rats may be less resistant and more susceptible to lipid peroxidation in the presence of promoters like ascorbate and ferrous sulphate [31]. So, it is possible that cisplatin could produce free radicals that cause subsequent lipid peroxidation to an acceptable extent.

The attenuant vitamin E is a well-accepted first line defense mechanism against lipid peroxidation. It protects poly unsaturated fatty acids in the cell membrane through its free radical-scavenging activity at an early stage of free radical attack [32]. Because of the phenolic hydroxyl group of the chromanol moiety, a structural feature of vitamin E-active compounds, a hydrogen can be transferred from phenolic group to free radicals, which are formed randomly or by the metabolic process during lipid peroxidation [33].

In disease conditions, membrane damage often occurs in some organs or tissues; and such damage provokes lipid peroxidation in the membranes and accelerates the disorder in structure and functions of the membrane [34]. There is ample evidence that vitamin E protects biological membranes against lipid peroxidation in vivo [35]. In our study also, the increased level of lipid peroxidation and decreased activities of antioxidant enzymes observed in cisplatin-treated animals were not nearly so pronounced in those also administered vitamin E.

The another attenuant, diuretic acetazolamide showed a considerable correcting effect in this system. Although several suggestions have been made, the mechanism of action of acetazolamide in protecting against cisplatin nephrotoxicity is still unclear. Acetazolamide is an organic acid which may competitively decrease tubular reabsorption of cisplatin [13]. Also, acetazolamide is a sulphur-containing drug and several sulphur-containing compounds have been shown to be effective in blocking or reducing cisplatin toxicity [36] besides which, methionine-P complex has been identified in the urine following cisplatin administration [37]. Acetazolamide thus may reduce cisplatin nephrotoxicity by a chemical interaction with reactive sites on the cisplatin molecule. So, the presence of sulphur (-s-) group in the structure of acetazolamide and its effect on producing alkaline urine [38], which facilitates increased excretion of platinum under alkaline pH may be closely correlated with the correcting effect of acetazolamide. However, the protection in this system was more pronounced when vitamin E was administered in addition.
VITAMIN E/ACETAZOLAMIDE AND CISPLATIN-INDUCED CHANGES

E and acetazolamide were administered in combination.

From all the above observations, we consider the reduced level of enzymic antioxidants and increased lipid peroxidation to be two reasons for cisplatin-induced nephrotoxicity. Further, we propose that administration of vitamin E and acetazolamide may be clinically advantageous to reduce cisplatin-induced nephrotoxicity, especially when they are given together with the cisplatin.

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