Protective Effects of Increasing Vitamin E and A Doses on Cisplatin-Induced Oxidative Damage to Kidney Tissue in Rats

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Abstract
Objective: Cisplatin (DDP, cis-diamminedichloroplatinum II) is one of the most potent chemotherapeutic anti-tumor drugs, but is able to generate reactive oxygen species (ROS) and it also inhibits the activity of antioxidant enzymes in renal tissue. In the present study, we investigated the preventive effect of 100, 200 and 400 mg/kg b.w. doses of vitamin E (VE), and 25, 50, and 100 mg/kg b.w. doses of vitamin A (VA) combination on malondialdehyde (MDA), nitric oxide (NO), and glutathione (GSH) levels and superoxide dismutase (SOD) activity in cisplatin-induced toxicity in rat kidneys. Our literature survey indicated a lack of any experimental study showing the beneficial effect of VA on cisplatin-induced MDA, NO, GSH and SOD changes. For this reason, we hoped that this study would provide a unique contribution in that respect. Materials and Methods: 59 Wistar rats (11 to replace prematurely lost animals) were used. 48 evaluable rats were divided into 8 groups (n = 6 in each group): control group, DDP alone (5 mg/kg b.w.) group, 3 VE combination treatment groups of VE100+DDP, VE200+DDP, and VE400+DDP, and 3 VA combination treatment groups of VA25+DDP, VA50+DDP, and VA100+DDP. Kidney MDA, GSH, NO levels and SOD activities were determined for the assessment of oxidant-antioxidant balance. Results: While in the DDP group the tissue levels of MDA and NO were found to be significantly higher than in the control group, GSH levels and SOD activities were significantly lower. MDA and NO levels were found to be significantly lower and GSH levels and SOD activities significantly higher in the VE200+DDP and VE400+DDP groups when compared with the DDP alone group. MDA and NO levels were found to be significantly lower in the VA50+DDP and VA100+DDP groups when compared with the DDP alone group. However, identical comparisons with the DDP alone group showed significantly higher GSH levels and SOD activities in the VA25+DDP, VA50+DDP, and VA100+DDP groups. Among the VE100+DDP, VE200+DDP, and VE400+DDP groups, and VA25+DDP, VA50+DDP, and VA100+DDP groups, MDA and NO levels decreased and GSH levels and SOD activities increased steadily and significantly as the doses of VE and VA increased. Conclusion: These vitamins would be effective in protecting against cisplatin-induced tissue damage in rat kidneys. It is possible that the toxic effect of cisplatin is somehow minimized by a compensatory mechanism involving VE and VA via induction of antioxidant enzyme activities following intraperitoneal injection of DDP.

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Introduction

Cisplatin (DDP, cis-diaminedichloroplatinum II) is an effective antitumor agent with a wide spectrum of activity against various solid tumors [1]. Activity has been demonstrated against a variety of tumors (testicular, ovarian, bladder and small cell lung cancers) [2, 3]. However, high doses produce nephrotoxic side effects, and the dose of cisplatin must often be limited [4]. The alterations in the kidney functions induced by cisplatin are characterized by signs of injury, such as glutathione (GSH) status, and cisplatin-induced nephropathy is closely associated with an increase in lipid peroxidation [2, 5]. Cisplatin side effects on non-tumor cells includes free radical generation [6] and is also closely related to reactive oxygen species (ROS), such as superoxide anions and hydroxyl radicals [6–8]. Potentially toxic ROS are produced in normal cellular metabolism and in abundance of pro-oxidant states [9]; administration of cisplatin causes an increase in lipid peroxide (malondialdehyde: MDA) levels and a decrease in the activity of enzymes such as superoxide dismutase (SOD) and GSH. Therefore, cisplatin chemotherapy induces a fall in plasma antioxidant levels, which may result in a failure of the antioxidant defense mechanism against oxidative damage induced by commonly used antitumor drugs [10]. There is some evidence to suggest that the free radical gas nitric oxide (NO) acts as a novel transcellular messenger molecule in many key physiological processes [11]. Endogenous NO is of a dual role in specialized tissues and cells; it is an essential physiological signalling molecule not only mediating various cell functions but also inducing cytotoxic and mutagenic effects when present in excess. NO reacts rapidly with superoxide anion to form peroxynitrite, which may be cytotoxic by itself or easily decompose to the highly reactive and toxic hydroxyl radical and nitrogen dioxide [12].

Most carotenoids and vitamin E (VE) have antioxidant activity [13]. The basic function of VE is to protect all membrane lipids and unsaturated fatty acids against oxidative degradation [14]. β-Carotene can be metabolized to vitamin A (VA, retinol) [15]. Antioxidant potency of VA and β-carotene may scavenge oxygen radicals and protect against cancer occurrence [16]. Our literature survey failed to disclose any experimental study showing the beneficial effect of VA on cisplatin-induced MDA, NO, GSH and SOD changes.

In the present study, we investigated the preventive effect of 100, 200 and 400 mg/kg b.w. doses of VE, and 25, 50, and 100 mg/kg b.w. doses of VA combination on MDA, NO, and GSH levels and SOD activity in cisplatin-induced toxicity in rat kidneys.

Material and Methods

Chemicals

All chemicals and VE (DL-α-tocopheryl acetate) and VA (retinol palmitate) were obtained from Sigma Chemical Inc. (Deisenhofen, Germany). The injectable form of cisplatin (Faulding Pharmaceuticals Plc, Royal Leamington Spa,Warwks.,UK) was purchased from local pharmacies. All reagents were analytical grade and were prepared each day.

Animals

Albino Wistar rats, aged 6 weeks and weighing 110–190 g, were used. Ethical approval was given by the Ethics Committee of Kocaeli University Faculty of Medicine. Experimental groups were organized as 8 groups which included 6 male animals each. Animals lost prematurely were replaced for completion of the groups' total number; a total of 11 additional animals were used (overall 59 animals). All animals were fed with commercial rat diet.

Study Groups and Sampling

Control (n = 6): received olive oil (0.5 ml/100 g b.w.) by gavage.

DDP group (n = 6): cisplatin (5 mg/kg b.w.) was injected intraperitoneally 24 h before killing the rats.

VE100+DDP group (n = 6): received VE (100 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

VE200+DDP group (n = 6): received VE (200 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

VE400+DDP group (n = 6): received VE (400 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

VA25+DDP group (n = 6): received VA (25 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

VA50+DDP group (n = 6): received VA (50 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

VA100+DDP group (n = 6): received VA (100 mg/kg b.w.) once a day for 2 days by gavage before cisplatin (5 mg/kg b.w.) injection.

All animals were killed by cervical dislocation 24 h after cisplatin injection.

Homogenate Preparation

The kidney tissues were removed and washed three times in cold isotonic saline (0.9%). Tissues were homogenized with cold Tris-HCl buffer (pH 7.4) to make a 10% homogenate (w/v). Tissue lipid peroxide levels, expressed in terms of MDA, were determined according to the method of Ebell and Aust [17]. The results were expressed as nmol/100 mg protein. Tissue GSH was measured according to the method of Ellman [18] with 5,5′-dithiobis-(2-nitrobenzoate). One milliliter of 10% TCA was added to 1 ml of homogenate. After centrifugation, 0.5 ml supernatant was taken and 4.5 ml of Ellman reagent in phosphate buffer (0.1 M, pH 8.0) was added. Absorbance was measured at 412 nm and the results were
expressed as nmol/mg protein. Cu-ZnSOD activity was measured kinetically by the method of Sun et al. [19]. The activity was expressed as U/mg protein. Since NO measurement is very difficult in biological specimens, tissue nitrite and nitrate were estimated as an index of NO production. The method for nitrite and nitrate levels was based on the Griess reagent which consists of sulfanilamide and N-(1-naphthyl)ethylenediamine [20]. Samples were initially deproteinized with Somogyi reagent. Total nitrite (nitrite + nitrate) was measured after conversion of nitrate to nitrite by copperized cadmium granules by a spectrophotometer at 545 nm. A standard curve was established with a set of serial dilutions (10^{-8}–10^{-3}) of sodium nitrite. The resulting equation was then used to calculate the unknown sample concentrations. The results were expressed as U/mg protein. Since NO measurement is very difficult kinetically by the method of Sun et al. [19]. The activity was expressed as nmol/mg protein. Statistical analyses were performed by the one-way analysis of variance (ANOVA) for the comparison of continuous variables in more than two groups. Continuous variables between two groups were analyzed using Student’s t test. Differences between groups were considered to be statistically significant at the level of p < 0.05.

**Results**

Table 1 displays the effects of DDP treatment with and without VE and VA on MDA, NO, and GSH levels, and SOD activities in the VE200+DDP (GSH: p < 0.05; SOD: p < 0.01) and VE400+DDP (GSH: p < 0.01; SOD: p < 0.0001) groups.

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 6)</th>
<th>DDP (n = 6)</th>
<th>VE100 + DDP (n = 6)</th>
<th>VE200 + DDP (n = 6)</th>
<th>VE400 + DDP (n = 6)</th>
<th>VA25 + DDP (n = 6)</th>
<th>VA50 + DDP (n = 6)</th>
<th>VA100 + DDP (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney MDA, nmol/100 mg protein</td>
<td>39.43 ± 8.20</td>
<td>62.71 ± 4.42</td>
<td>57.61 ± 5.10</td>
<td>50.67 ± 9.07</td>
<td>45.67 ± 2.89d</td>
<td>61.34 ± 6.09d</td>
<td>55.73 ± 5.04d f</td>
<td>48.59 ± 6.11e</td>
</tr>
<tr>
<td>Kidney NO, nmol/100 mg protein</td>
<td>19.27 ± 1.57</td>
<td>48.13 ± 9.22</td>
<td>43.09 ± 6.63</td>
<td>35.30 ± 9.45</td>
<td>27.03 ± 7.55c</td>
<td>41.25 ± 4.89</td>
<td>38.44 ± 2.82c</td>
<td>33.88 ± 5.18e</td>
</tr>
<tr>
<td>Kidney GSH, nmol/mg protein</td>
<td>60.04 ± 10.95</td>
<td>30.06 ± 7.45</td>
<td>35.57 ± 6.55</td>
<td>41.28 ± 6.76</td>
<td>49.06 ± 5.42</td>
<td>48.67 ± 7.53</td>
<td>56.41 ± 6.73</td>
<td>63.61 ± 4.32</td>
</tr>
<tr>
<td>Kidney SOD, U/mg protein</td>
<td>4.91 ± 1.25</td>
<td>1.53 ± 0.22</td>
<td>2.39 ± 1.10</td>
<td>3.27 ± 0.95</td>
<td>4.13 ± 0.38d</td>
<td>1.93 ± 0.33d</td>
<td>2.44 ± 0.57c</td>
<td>3.26 ± 0.94</td>
</tr>
</tbody>
</table>

Significantly different from control group, a p < 0.0001, b p < 0.01, c p < 0.05. Significantly different from DDP group, d p < 0.0001, e p < 0.01, f p < 0.05.

**Discussion**

Cisplatin is one of the more common antitumor agents for several types of cancer and has been widely used for chemotherapy [22]. It acts mostly on the proximal renal tubule of the kidney. Proximal tubular epithelial cells take up cisplatin actively and the concentration of the drug in these cells exceeds plasma concentration by a factor of 5 [23]. Cisplatin toxicity in proximal tubular cells is morphologically characterized by tubular necrosis [24].

Data from this study revealed that cisplatin significantly increased MDA and NO levels in kidney tissues. MDA is released from free radical damage to membrane...
components of the cells [23]. This speculation agreed with previous studies which have demonstrated the involvement of oxidative stress, lipid peroxidation, and mitochondrial dysfunction in cisplatin-induced nephrotoxicity [25–27]. Cisplatin leads also to generation of free radicals both in vitro and in vivo. The in vivo production of $\text{H}_2\text{O}_2$ and $\text{O}_2^-$ by polymorphonuclear leukocytes of patients treated with a wide range of cytotoxic agents for both hematological and solid malignancies increased significantly compared with the pretreatment values [2, 28]. The decreased kidney GSH levels and SOD activities found in rats treated with cisplatin compared to the control group indicate that lipid-phase antioxidant defense is impaired during cisplatin treatment. GSH and SOD in the cells are important components of several naturally occurring antioxidant defense mechanisms to prevent oxidative injury [2]. There is also a hypothesis that decreased levels of natural antioxidants and diminished scavenging enzyme capacity may be responsible for the excess of ROS observed in cisplatin-induced toxicity [2, 29].

NO has an important role in modulating oxidant stress and tissue damage [30]. It has been demonstrated that oxidant stress to epithelial cells caused an increase in immunodetectable inducible NO synthase (iNOS), which results in an elevation in NO release, nitrite production, and decreased cell viability [31]. The mechanism mediating induction of iNOS due to the free radical exposure remains unknown. It has been recently hypothesized that the cytotoxic effect of NO production depends on the redox state of the cell and its ability to generate peroxynitrite ($\text{ONOO}^-$) anion. Peroxynitrite, a highly reactive oxidant formed during the interaction between NO and $\text{O}_2^-$, can attack a wide variety of biological targets. The present study indicated the significant elevation in NO level in damaged kidney tissue of the cisplatin-treated rats and VA and VE significantly attenuated this increment. This increment of NO generation in the renal tissue of cisplatin-administrated rats support the above-mentioned mechanism relating generation of NO caused by free radicals under oxidative stress [32].

In our study, the positive effect of VE and VA supplementation, even at maximum doses, could not exceed significantly in terms of any of the studied parameters (SOD, GSH, MDA and NO) when compared with controls; GSH exceeded the control level, but this did not reach significance (table 1). On the other hand, when compared with the DDP alone group, although minimum doses of VA (25 mg/kg b.w.) and VE (100 mg/kg b.w.) achieved a positive effect by increasing SOD and GSH and decreasing MDA and NO, this effect reached significance at intermediate doses, and the significance was further increased at maximum doses (100 and 400 mg/kg b.w.) of these vitamins. VE is the major lipophilic chain-breaking antioxidant present within cell membranes [33]. It suppresses the oxidative stress of membranes. Given the potential role of the ROS in mediating tissue damage, cells containing the enzyme SOD are important components of several naturally occurring antioxidant defense mechanisms preventing oxidative injury [2]. Antioxidant potency of VA and β-carotene may scavenge oxygen radicals and protect against cancer occurrence [16]. These antioxidants may be protecting against cisplatin-induced toxicity by inhibition of the inactivation of GSH and antioxidant system by cisplatin-induced nephropathy formation of cancer patient.

In conclusion, these vitamins would be effective in protecting against cisplatin-induced tissue damage in rat kidneys. It is possible that the toxic effect of cisplatin is somehow minimized by a compensatory mechanism involving VE and VA via induction of antioxidant enzyme activities following intraperitoneal injection of DDP. On the other hand, since our literature survey failed to disclose any experimental study showing the beneficial effect of VA on cisplatin-induced MDA, NO, GSH and SOD changes, this study would provide a significant contribution in that respect.

Acknowledgement

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References