Renoprotective effects of megadose vitamin C on cisplatin-induced kidney injury

Supa Sithanukul1  Chollada Buranakarl*  Chutamas Benjanirut1  Anudep Rungsipipat2

Abstract

Cisplatin (CDDP)-induced nephrotoxicity is mediated via oxidative stress and may be alleviated using antioxidant activity. Although megadose of vitamin C may cause oxalate nephropathy and apoptosis, the antioxidant effect of vitamin C may be beneficial when given along with CDDP. The objective of this study was to investigate the renoprotective effects of megadose vitamin C on rats receiving CDDP. Rats were divided into 4 groups: group 1, control rats (CONT); group 2, vitamin C-treated rats (VIT C); group 3, CDDP-treated rats (CDDP) and group 4, CDDP + vitamin C-treated rats (CDDP + VIT C). Vitamin C (1000 mg/kg) was given intravenously to the VIT C and CDDP + VIT C groups while CDDP (6 mg/kg) was injected intraperitoneally into the CDDP and CDDP + VIT C groups. Renal function, oxidative stress, apoptosis and histopathology were investigated. At 5 days after injection, CDDP caused renal impairment as shown by significant increases in plasma concentrations of creatinine (PCr), urea nitrogen (PUN), urinary excretion of electrolytes (Na+, K+ and Cl-) and protein (P<0.05). Plasma malondialdehyde (P-MDA) and urinary MDA and creatinine ratio (U-MDA/Cr) were also increased (P<0.05). Kidney PCR products of antiapoptosis/proapoptosis (Bcl-2/Bax) were reduced by CDDP while histopathologic results showed severe massive tubular necrosis. Giving megadose vitamin C along with CDDP showed improvement on all renal function parameters and oxidative stress parameters except proteinuria. The vitamin C caused improvement on tubular cell lesions, but did not alter the Bcl-2/Bax level. It is concluded that megadose vitamin C can provide protection against CDDP-induced kidney injury by antioxidant activity.

Keywords: apoptosis, cisplatin, kidney injury, megadose vitamin C, oxidative stress

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Introduction

Cisplatin (cis-diaminedichloroplatinum II, CDDP) has been widely used as a part of cancer treatment regimen in both humans and dogs (Barabas et al., 2008; Miller et al., 2010). However, this aquated platinum complex can cause DNA lesion, resulting in replication arrest, transcription inhibition, cell-cycle arrest, DNA repair, apoptosis and cell death (Rabik and Dolan, 2007). Moreover, CDDP can cause nephrotoxicity (Miller, 2010). A report on the apoptosis of kidney cells by CDDP was found (Sheikh-Hamad et al., 2004), which involves multiple pathways including extrinsic pathway, intrinsic mitochondrial pathway and endoplasmic reticulum stress pathway (Miller, 2010).

It has been demonstrated that oxidative stress may play a central role in renal toxicity of platinum-based anticancer drugs (Chirino and Pedraza-Chaverri, 2009; Santos et al., 2008; Santos et al., 2007). Using antioxidants in combination with chemotherapy could diminish the risk of CDDP-induced nephropathy by lowering oxidative damage (Ajith et al., 2009; Santos et al., 2008; Tarladacalisir et al., 2008). Giving vitamin C (500 mg/kg) and E in combination orally showed a protective effect against CDDP-induced nephrotoxicity in mice (Ajith et al., 2009). However, high dose or megadose vitamin C (1000 mg/kg) may exacerbate renal impairment since vitamin C enhances oxalate excretion (Pena de la Vega et al., 2004), which could be associated with oxalate nephropathy (Lamarche et al., 2011). Moreover, ascorbate radical and hydrogen peroxide may be involved in the oxidative damage demonstrated in renal epithelial cell (Thamilseivan et al., 2003). Thus, the effects of megadose vitamin C when co-administered with CDDP on renal function are still questionable although the protocol was applied for alternative therapies in cancer treatment (Ohno et al., 2009). Therefore, this study was carried out to investigate the beneficial effects of CDDP and megadose vitamin C in combination on renal functions, renal oxidative stress and renal apoptosis compared with CDDP alone.

Materials and Methods

Animals and experimental design: The experiment was performed in accordance with the institutional guidelines and conformed to CU-IACUC, Faculty of Veterinary Science, Chulalongkorn University. Male Sprague Dawley rats obtained from the National Laboratory Animal Center, Mahidol University (NLAC-MU), weighing between 250-300 g and aged between 7 to 8 weeks old, were housed under standard condition of light and dark cycle (LD = 12:12 hr) with free access to water and food (Perfect Companion Co., Ltd, Thailand). Body weight, food intake and water intake were recorded daily. The rats were randomly divided into four groups: CONT group (n=9), VIT C group (n=9), CDDP group (n=11) and CDDP + VIT C group (n=16). The CONT group served as control, to which isotonic sodium chloride solution was administered intravenously (iv) via tail vein followed by intraperitoneally (ip) to substitute for vitamin C and cisplatin (CDDP), respectively. Megadose vitamin C (1000 mg/kg body weight) was administered iv to the VIT C group and the CDDP + VIT C group while cisplatin (6 mg/kg body weight) was administered ip to the CDDP group and the CDDP + VIT C group. The intravenous injection was started 15 minutes before the intraperitoneal injection in all groups.

Sample collection: During pre-treatment period, blood pressure was measured using the tail-cuff method. Blood volume of 0.5 ml was collected in a heparinized tube by clipping the tip of the tail for measurement of packed cell volume (PCV), plasma urea nitrogen (PUN) and creatinine (PCr) concentrations to serve as pretreatment values. Afterward, the rats were housed in a metabolic cage for 24 hr urine collection to determine urine volume (V), concentrations of protein, creatinine, malondialdehyde (MDA), protein carbonyl (PCO), total antioxidant status (TAS) and osmolarity (pre-treatment period). After urine collection, all rats were anesthetized with pentobarbital sodium at a dose of 60 mg/kg intraperitoneally. Each rat was subjected to a single iv injection followed by an ip injection of megadose vitamin C and/or CDDP, respectively. Four days after drug administration, blood pressure and urine collection was reperformed (post-treatment period). At the end of the experiment, all animals were euthanized under pentobarbital anesthesia while 5 ml of blood was collected via cardiac puncture. The heparinized blood samples were used to determine chemistries, electrolyte concentrations, osmolarity and MDA while the EDTA blood samples were used for TAS and PCO measurement. Both kidneys were immediately excised and kept at -70°C for analyses of oxidative stress and apoptotic markers while some tissues were kept in 10% neutral buffered formalin for histopathologic study.

Determination of renal function and oxidative stress markers: Concentrations of Na+ and K+ were measured using a flame photometer (Flame photometer 410C, Ciba Corning Inc., USA) while Cl- concentration was determined by a chloridometer (Chloride analyzer 925, Ciba Corning Inc., USA). Osmolarity was determined using an osmometer (Osmometer 3D3; Advance Instruments Inc., Norwood, MA, USA). Systolic blood pressure was measured by the tail-cuff method (The ML125/R NIBP System for rats, ADinstruments CO., USA). PUN and creatinine concentrations were measured using an automate analyzer (The IL ILab 650 Chemistry Analyzer, Diamond diagnostic, MA, USA). Urinary protein concentration was measured by precipitating with sulfosalicylic acid. TAS was determined by a modified method as described previously (Janaszewska and Bartosz, 2002) while PCO was measured by the spectrophotometric dinitrophenylhydrazine (DNPH) assay following a modified method of Levine et al. (1990). MDA was assayed in the form of thiobarbituric acid reacting substances (TBARS) using a modified method of Ohkawa et al. (1979). Kidney MDA value was expressed as nmol of TBARS per milligram protein which was determined by the method of Lowly et al. (1951). Kidney (K) GSH content was determined following a modified method of Beutler et al. (1963) while CAT activity was measured by a modified
method of Aebi (1983) and was expressed as sec⁻¹ per milligram protein which was determined by Bradford protein assay (Bradford, 1976). Kidney Bcl-2 and Bax mRNA expression was measured by real-time PCR.

**Measurement of Bcl-2 and Bax:** Total RNA was extracted from 20 mg frozen kidneys using Total RNA Mini Kit (Tissue) (Geneaid Biotech Ltd.). A spectrophotometer was used for quantitating the amount of RNA. Readings were taken at wavelengths of 260 nm and 280 nm. Total kidney RNA was reverse transcribed using Random primers and iScript Select cDNA synthesis Kit (Bio-rad). The first-strand synthesis cDNA obtained was synthesized from 1 µg of total RNA according to the manufacturer’s instructions (Bio-rad laboratories). Two microliter of the first-strand synthesis cDNA was used as templates and amplified by real-time PCR using SYBR Green PCR Master Mix (Applied Biosystems, Forter City, CA, USA) with primers for rat Bcl-2, Bax and GAPDH genes. These reactions were performed on a 0.2 ml 96-well plate in a thermal cycler sample block of ABI 7500 Real-time PCR machine (Applied Biosystems, Forter City, CA, USA). Real-time-PCR Master Mix E4 (GeneOn) reactions were initially activated at 95°C for 10 min. Then, the typical two-temperature cycle for a PCR was run, which consisted of a denaturation step at 95°C for 15 sec, followed by an annealing-extension step at 60°C for 1 min. Each reaction was amplified in triplicate and a control without cDNA was performed in parallel with each assay. Relative changes in the interested gene expression and the ratio were calculated based on the comparative Ct method (2⁻ΔΔCt method). The housekeeping gene (GAPDH) was used as an internal control. Sequences of specific primers for Bcl-2, Bax and GAPDH were used as shown in Table 1.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence 5’-3’</th>
<th>Accession number</th>
<th>Size of product (bp)</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Rat Bcl-2 | F - GGGATGCCCTTGTGGAACATATG  
R - CAGCCAGGAGAAATCAAACAGA | NM016993 | 62 | Cheng et al. 2009 |
| Rat Bax | F - ATGGAGCTGCAGAAGGATATT  
R - TGAAGTGTCAATGACGAAAAC | NM017059 | 97 | Ding et al. 2011 |
| Rat GAPDH | F - TCCCTACAAGATTTGCGACAA  
R - AGATCCACAACCGGATAATT | NM017008 | 309 | Gong et al. 2008 |

Two and four percentage agarose gel electrophoreses were performed to confirm product size of each primer.

**Morphological studies:** Renal tissues were randomly collected from 3 rats of each group and were fixed in 10% neutral buffered formalin, routinely histologically processed, embedded in paraffin wax and cut at 4-6 µm. The sections were stained with Hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS). The histopathological lesions were scored under a light microscope by a veterinary pathologist. Each parameter was assessed using the average of ten high power fields (40X) for each sample, where the tubular damage was prominent. Parameters including tubular cast, tubular dilatation and interstitial cell infiltration were scored on the scale of 0 to 3, ranging from none, <25% (0); mild, 25-50% (1); moderate, 51-75% (2); to severe, >75% (3).

**Statistical analysis:** All numerical data were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (One-way ANOVA) was used to compare data between groups followed by post-hoc analysis with Tukey test. The paired t-test or Wilcoxon signed-rank test was used to compare data before and after treatment within the same group. Pearson correlations were used to determine relationships. P value of less than 0.05 was considered to be statistically significant.

**Results**

**Effects of megadose vitamin C on body weight, food intake and water intake:** The body weights during pre-treatment period were not different among the rat groups (CONT, 358.3 ± 5.3; VIT C, 353.6 ± 8.2; CDDP, 356.9 ± 6.5; CDDP + VIT C, 367.3 ± 4.9 g). At the end of the study, the body weights of the CDDP group were significantly lower than those of the other groups (304.3 ± 10.1 vs CONT, 335.5 ± 5.7; VIT C, 332.6 ± 7.9; CDDP + VIT C, 326.7 ± 5.4 g) (P<0.05). The body weights of both CDDP and CDDP + VIT C groups were also significantly decreased compared to those at the day of drug injection (356.9 ± 6.5 and 367.3 ± 4.9 g, respectively) (P<0.001). The food intake of the CDDP group was significantly lower than those of the CONT and VIT C groups starting the next day after the CDDP injection. At the end of the experiment, the CDDP group had significantly lower food intake compared with the other groups (P<0.05) and the pre-treatment value (P<0.001). The rats receiving vitamin C alone or in combination with CDDP had higher food intake compared with the rats receiving CDDP alone (P<0.05) and were not different from the CONT group. The water intake was similar among the groups throughout the experiment.

**Effects of megadose vitamin C on renal function:** When comparing among the groups, the systolic blood pressures (SAP) were not different both before (CONT, 93 ± 1; VIT C, 96 ± 4; CDDP, 105 ± 2; CDDP + VIT C, 106 ± 3 mmHg) and after the CDDP treatment (CONT, 107 ± 2; VIT C, 104 ± 2; CDDP, 99 ± 5; CDDP + VIT C, 103 ± 3 mmHg). A significant increase in SAP was found only in the CONT group after the CDDP treatment (P<0.001). The packed cell volume was slightly decreased in all groups, although it was still in normal limit. The plasma concentrations of Na⁺, K⁺ and
Cl− post-treatment were in normal range and not different among the groups.

In the CDDP group, the values of PCr and PUN after the CDDP treatment were significantly higher than those in the CONT and VIT C groups (P<0.05) (Figs. 1a and 1b). In the CDDP + VIT C group, they were significantly lower than those in the group receiving CDDP alone (P<0.05). The urine volume, urinary protein creatinine ratio and urinary protein excretion in the CDDP and CDDP + VIT C groups were increased significantly after the CDDP treatment (P<0.001). The urinary protein excretion was higher in both CDDP and CDDP + VIT C groups compared with the groups not receiving CDDP (P<0.05) (Table 2).

At post-treatment, the values of urinary fraction excretions (FE) of Na+, K+ and Cl− of the CDDP group were significantly higher than those in the CONT and VIT C groups (P<0.05) while lower values were found after receiving both CDDP and VIT C (P<0.05) (Figs. 1c and 1d). The Uosm/Posm was lower in the rats receiving CDDP alone and CDDP + VIT C (P<0.05) compared with the CONT group (Table 2).

![Bar charts](image)

**Table 2** Urine flow, protein excretion and urinary plasma osmolarity ratio during pre- and post-treatment periods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONT (n=9)</td>
</tr>
<tr>
<td>V (µl/g/day)</td>
<td>Pre: 23.78 ± 3.46</td>
</tr>
<tr>
<td></td>
<td>Post: 30.56 ± 4.01</td>
</tr>
<tr>
<td>UPC ratio (mg/mgCr)</td>
<td>Pre: 2.23 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Post: 1.742 ± 0.212</td>
</tr>
<tr>
<td>Uprot.V (mg/g/day)</td>
<td>Pre: 37.06 ± 1.62</td>
</tr>
<tr>
<td></td>
<td>Post: 33.41 ± 4.11</td>
</tr>
<tr>
<td>Uosm/Posm</td>
<td>Post: 3.93 ± 0.97</td>
</tr>
</tbody>
</table>

Abbreviations: V, urine volume; Uprot.V, urinary protein excretion; UPC ratio, urinary protein creatinine ratio; Uosm, urinary osmolarity; Posm, plasma osmolarity.

Data are shown as mean ± SEM.

Different superscripts (a, b) indicate statistically significance among groups using one-way ANOVA.

* = P<0.05, ** = P<0.01 and *** = P<0.001 significant differences from the values before treatment using paired t-test.

† = P<0.05 and ††† = P<0.001 significant differences from the values before treatment using Wilcoxon signed-rank test.
Effects of megadose vitamin C on oxidative stress markers: The plasma MDA (PMDA) concentration was highest in the CDDP group while the P-TAS level was lowest in the CDDP + VIT C group (P<0.05) (Fig. 2a, Table 3). The P-PCO concentrations were similar among the groups. After CDDP treatment, U-MDA/Cr increased significantly in only the CDDP group (P<0.05) with a slightly lower value in the CDDP + VIT C group (Fig. 2b). Lower U-TAS was found in the CDDP group. U-PCO/Cr was significantly higher in the CDDP (P<0.01) and CDDP + VIT C groups (P<0.05) when compared to the pre-treatment values. The K-GSH levels in the groups receiving CDDP were significantly higher than those in the groups without CDDP (P<0.05) (Table 3).

**Table 3** Oxidative stress markers in plasma, urine and kidney during pre- and post-treatment periods

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>CONT (n=9)</th>
<th>VIT C (n=9)</th>
<th>CDDP (n=11)</th>
<th>CDDP + VIT C (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-MDA (nmol/ml)</td>
<td>Post</td>
<td>3.341 ± 0.219 †</td>
<td>3.446 ± 0.310 †</td>
<td>6.511 ± 0.704 †</td>
<td>3.763 ± 0.289 †</td>
</tr>
<tr>
<td>P-PCO (nmol/mg prot)</td>
<td>Post</td>
<td>1.462 ± 0.088</td>
<td>1.512 ± 0.088</td>
<td>1.559 ± 0.076</td>
<td>1.796 ± 0.125</td>
</tr>
<tr>
<td>P-TAS (AA%)</td>
<td>Post</td>
<td>23.39 ± 1.24b</td>
<td>23.53 ± 1.69b</td>
<td>20.47 ± 2.25b</td>
<td>16.64 ± 1.66b</td>
</tr>
<tr>
<td>U-MDA/Cr (nmol/mgCr)</td>
<td>Post</td>
<td>18.57 ± 1.57</td>
<td>19.56 ± 1.59</td>
<td>19.19 ± 2.03</td>
<td>16.87 ± 1.47</td>
</tr>
<tr>
<td>U-PCO/Cr (nmol/mg prot)</td>
<td>Pre</td>
<td>12.29 ± 2.01</td>
<td>14.30 ± 3.44</td>
<td>18.01 ± 4.38</td>
<td>11.98 ± 1.62</td>
</tr>
<tr>
<td>U-TAS (AA%)</td>
<td>Post</td>
<td>74.30 ± 1.40</td>
<td>73.19 ± 2.98</td>
<td>70.29 ± 3.33</td>
<td>69.57 ± 2.57</td>
</tr>
<tr>
<td>K-MDA (nmol/mg prot)</td>
<td>Post</td>
<td>0.672 ± 0.042</td>
<td>0.644 ± 0.025</td>
<td>0.683 ± 0.047</td>
<td>0.610 ± 0.047</td>
</tr>
<tr>
<td>K-GSH (µmol/mg prot)</td>
<td>Post</td>
<td>0.452 ± 0.03b</td>
<td>0.481 ± 0.022b</td>
<td>0.607 ± 0.028b</td>
<td>0.612 ± 0.022b</td>
</tr>
<tr>
<td>K-CAT (Unit/mg prot)</td>
<td>Post</td>
<td>203.6 ± 20.7</td>
<td>201.2 ± 23.3</td>
<td>184.8 ± 13.2</td>
<td>177.3 ± 6.9</td>
</tr>
</tbody>
</table>

**Figure 2** Effects of CDDP treatment on plasma malondialdehyde (a) and urinary MDA creatinine ratio (b). Different superscripts mean differ significantly (P<0.05) among groups using one-way ANOVA and one way ANOVA on rank, respectively (P<0.05).

**Effects of megadose vitamin C on apoptotic gene expression:** The relative mRNA levels of Bcl-2 and Bax post-treatment in both control and experimental groups are shown in Fig. 3a. The relative mRNA levels of Bax tended to be higher in the CDDP and CDDP + VIT C groups, resulting in lower Bcl-2/Bax ratio compared with the CONT and VIT C groups (P<0.05) (Fig. 3b).

The agarose gel electrophoresis patterns confirmed the correct size of real-time PCR products of Bcl-2, Bax and internal standard, GAPDH. The GAPDH products of PCR in the kidney of the control rats were shown with molecular size of 309 (Fig. 4a) while the Bcl-2 and Bax products of PCR from the samples of the CDDP and CDDP + VIT C groups were shown with molecular sizes near 62 and 97, respectively (Fig. 4b).

**Effects of megadose vitamin C on histopathology of kidney tissues:** The semiquantitative changes in renal structures are shown in Table 4.

**Glomerular alterations:** Glomeruli in the CONT and CDDP groups revealed no remarkable lesions either cellular components; podocytes, mesangial and capillary endothelial cells; or basement membrane matrix (mucopolysaccharide PAS-positive substance in mesangium of glomerular tuft) (Figs. 5a and 5c). However, the glomerulus in the groups receiving VIT C with and without CDDP (VIT C and CDDP + VIT C
groups) showed swollen glomerular corpuscle with marked dilatation of glomerular capillary tuft (Figs. 5b and 5d). However, no hyaline droplet was found in all four groups.

Figure 3  Relative mRNA expression levels of Bcl-2 and Bax (a) and ratio of Bcl-2 to Bax (b). Different superscripts mean differ significantly (p<0.05) among groups using one-way ANOVA.

Figure 4  Agarose gel electrophoretic analysis of real-time PCR (RT-PCR) products. a; 100 bp ladder molecular sized marker and GAPDH products of RT-PCR were loaded in lanes 1 and 2, respectively. b; 10-300 bp ladder molecular sized marker was loaded in lane 1. Two samples in CDDP and CDDP + VIT C groups were run for Bcl-2 products of RT-PCR in lanes 2 and 3 and for Bax products of RT-PCR in lanes 4 and 5.

Table 4  Semi-quantitative assessment of renal tubular structural changes

<table>
<thead>
<tr>
<th></th>
<th>CONT</th>
<th>VIT C</th>
<th>CDDP</th>
<th>CDDP + VIT C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tubular cast</td>
<td>0±</td>
<td>0±</td>
<td>2.97 ± 0.03b</td>
<td>2.00 ± 0.20b</td>
</tr>
<tr>
<td>Tubular dilation</td>
<td>0±</td>
<td>0±</td>
<td>2.97 ± 0.03b</td>
<td>1.63 ± 0.18c</td>
</tr>
<tr>
<td>Interstitial cell infiltration</td>
<td>0±</td>
<td>0±</td>
<td>1.80 ± 0.12b</td>
<td>1.07 ± 0.13c</td>
</tr>
</tbody>
</table>

Data are shown as mean ± SEM. Different superscripts (a, b) indicate statistical significance using one-way ANOVA (P<0.05).
Tubular alterations: The proximal and distal convoluted tubules in the control and VIT C groups were normal (Figs. 6a and 6b). In the CDDP group, the kidneys revealed severe diffuse massive tubular necrosis especially in the cortico-medullary junction and the glomerulus showed moderate congestion (Fig. 6c). Moderate diffuse hydropic degeneration, characterized by cloudy swelling of cytoplasm of renal cortical tubular epithelium particularly proximal convoluted tubules, was found (Fig. 6d). Necrotic proximal and distal convoluted tubules were characterized by disruption of epithelial cells from tubular basement membrane, breakdown of pale eosinophilic cytoplasm and swelling, nuclear condensation and fragmentation with some protein debris accumulation in tubular lumen (PAS positive) (Fig. 6d). The tubular dilation was remarkable with significant changes in the interstitial tissue characterized by infiltration of acute inflammatory cells (neutrophils and macrophages) compared with the CONT and VIT C groups (Table 4). However, in the CDDP group, the lesions of proximal and distal convoluted tubules were significantly milder than those of the CDDP group (Figs. 6e and 6f, Table 4). There were some hyaline casts accumulating in the necrotic tubular lumen of the CDDP group particularly descending tubules and collecting ducts (Fig. 6g). Some tubules of the CDDP + VIT C group showed accumulation of necrotic tubular cells, protein debris and hyaline cast in their lumens (Fig. 6h, Table 4).

Correlation between PUN and other parameters: Since PUN is a good marker for renal function, correlations were made between PUN and the other parameters of renal function and oxidative stress. By using simple Pearson correlation on post-treatment, negative correlations were found between PUN and both body weight (r = 0.369, P = <0.05) and SAP (r = 0.335, P<0.05). PUN positively correlated with PCr (r = 0.623, P<0.001), FE (Na+, r = 0.317, P<0.05; K+, r = 0.312, P<0.05 and Cl−, r = 0.879, P<0.001) and UPC ratio (r = 0.393, P<0.01), and negatively correlated with Uosm/Posm (r = 0.313, P<0.05).

PUN positively correlated with P-MDA (r = 0.584, P<0.001), U-MDA/Cr (r = 0.778, P<0.001), U-PCO/Cr (r = 0.606, P<0.001) and K-GSH (r = 0.385, P<0.01), and negatively correlated with U-TAS (r = 0.581, P<0.001) and Bcl-2/Bax (r = 0.396, P<0.01).

Discussion

Acute nephrotoxicity caused by CDDP in relation to oxidative stress has been demonstrated earlier (Chirino and Pedraza-Chaverri, 2009; Santos et al., 2007). Using antioxidant could ameliorate renal damage and apoptosis (Santos et al., 2008). Giving vitamin C alone or in combination with vitamin E was proved to prevent CDDP-induced kidney injury in mice and rats (Ajith et al., 2009; Antunes et al., 2000; Fatima et al., 2007; Tarladacalisir et al., 2008). However, the dose of vitamin C used in all studies was considered much lower than the dose used in treatment of cancer. Thus, this is the first study to
demonstrate the effect of megadose of vitamin C in CDDP-induced kidney injury.

Both CDDP and vitamin C when given alone or in combination had no effects on blood pressure. These results are similar to those of a previous report recorded 9 days after CDDP treatment (Bagnis et al., 2001), but different from those of another study in which a reduction was found on day 5 (Ali et al., 2011). The prevention of blood pressure elevation when giving CDDP alone or in combination with vitamin C in the present study may be due to diuresis with lower Uosm/Posm.

Figure 6  Tubular alterations of rats at light microscopic level. (a) CONT group: renal tubular structure showing normal appearance (periodic acid-Schiff (PAS) stain; original magnification, x 400). (b) VIT C group: renal tubules showing no remarkable lesions (PAS stain; original magnification, x 400). (c) CDDP group: massive necrosis of renal tubules in cortico-medullary area (arrow) (hematoxylin-eosin (HE) stain; original magnification, x 100). (d) CDDP group: massive acute tubular necrosis with protein debris accumulated in renal tubular lumen (arrow) (PAS stain; original magnification, x 400). (e) CDDP + VIT C group: moderate necrosis of renal tubules in cortico-medullary area (arrow) (HE stain; original magnification, x 100). (f) CDDP + VIT C group: diffuse hydropic degeneration of renal tubules and protein debris accumulated in their lumens (arrow) (PAS stain; original magnification, x 400). (g) CDDP group: accumulation of hyaline casts in degenerated renal tubular lumen (arrow) (PAS stain; original magnification, x 400). (h) CDDP + VIT C group: accumulation of necrotic tubular cell casts and protein debris in renal tubular lumen (arrow) (PAS stain; original magnification, x 400).
The pathophysiology of CDDP nephrotoxicity is a complex process including vascular, tubular, glomerular and interstitial injuries which may lead to chronic renal disease. These effects of CDDP-induced nephrotoxicity increase with dose and frequency of administration (Miller et al., 2010). In the present study, the single dose of CDDP administration resulted in the significant increases in PCr and PUN in the CDDP group. A previous study using the same dose of CDDP showed that the peak level of renal functional loss was on day 5 and then it decreased to the basal level on day 14 (Zhou et al., 2006). Proteinuria caused by CDDP, despite the fact that lesions could not be demonstrated from histopathology, may be due to glomerular damage and/or decreases in receptor-mediated endocytosis of protein as previously described (Takano et al., 2002). The body weight was decreased significantly after receiving CDDP and was less in the group receiving CDDP alone. The decrease in body weight correlated with degree of azotemia.

CDDP was also involved in the tubular cell damage. In the present study, the CDDP administration increased FENa, FEK* and FECI. Increased urinary excretion of electrolyte has been well documented to correlate with severity of kidney impairment in dog (Buranakarl et al., 2007). The results of our study are in agreement with those of previous reports after 5 day of CDDP administration (van Angelen et al., 2013). Therefore, tubular reabsorptive capacity of both ion and water was diminished. Change in renal function may not be mediated via angiotensin II since the nonpeptide angiotensin II (All) receptor blocker, losartan, could not alter the onset or severity of cisplatin nephrotoxicity in rats (Rastghalam et al., 2014).

The histopathology of kidney tissue showed lesions caused by CDDP including acute tubular necrosis of both proximal and distal convoluted tubules. By determining mRNA expression for the apoptosis markers, the ratio of anti-apoptotic level (Bcl-2) to pro-apoptotic level (Bax) appears to function as a rheostat that determines survival or death. In the present study, a significantly lower Bcl-2/Bax was found in the CDDP group. Similar results were reported by Sheikh-Hamad et al. (2004) that CDDP (5 mg/kg) significantly increased the levels of Bax expression but did not alter the expression of Bcl-2 in rat kidney at 5 days after drug injection, resulting in the decline in Bcl-2/Bax.

Giving megadose vitamin C alone had no adverse effect on the renal function although oxalate nephropathy was reported when receiving vitamin C chronically (de la Vega et al., 2004; Gurum et al., 2012; Lamarche et al., 2011) or in patient with systemic lupus erythematosus (Cossey et al., 2013). In the present study, no evidence of nephropathy was found when vitamin C was given. In previous studies, improvement in renal function by oral (50-500 mg/kg) (Ajith et al., 2009; Antunes et al., 2000) and parenteral administration (Fatima et al., 2007; Tarladacalisir et al., 2008) of vitamin C was demonstrated. However, the dosage of vitamin C was lower than that of the present study and was not considered as megadose. This was supported by a previous study in rats showing peak plasma concentration of 10 nM when giving 0.5 mg of ascorbate per gram of body weight. However, only 2 nM of ascorbate was found in the plasma when giving the same amount ip and even undetectable when giving by oral route (Chen et al., 2007). Thus, a pharmacological dose of vitamin C is considered at the concentration of 500-5000 mg/kg given intravenously. The present study is the first study to demonstrate the renoprotective effect of megadose vitamin C. Giving megadose vitamin C to the CDDP rats could improve their renal function by reducing PUN, PCr and urinary excretion of electrolytes compared with the group receiving CDDP alone. The improved glomerular filtration may be a result of the antioxidant activity of vitamin C improving endothelium-dependent vasodilatation via NO pathway activity as seen in hypertensive patients (Taddei et al., 1998) and in patients with chronic renal failure (Cross et al., 2003).

However, megadose vitamin C could not prevent proteinuria although the anti-proteinuric effect of vitamin C was demonstrated in diabetic rats (Craven et al., 1997; Lee et al., 2007). Vitamin C could lower oxidative stress and may be responsible for reduced slit pore caused by damage and stretching of podocyte with denuded glomerular basement membrane seen in diabetic rats. Nevertheless, the drinking water was supplemented with 10g/L vitamin C for a period of 8 and 32 weeks, respectively. The antiproteinuric effect could not be seen in the present study at 6 days after the CDDP and vitamin C treatment.

Less kidney histopathologic lesions were seen in the CDDP + VIT C group. The glomerulus of rats receiving vitamin C both with and without CDDP showed dilated glomerular capillaries, which suggested that vitamin C might cause vasodilation and increase blood flow. Whether higher blood flow is related to antioxidant activity needs further elucidation. The rats receiving both CDDP and vitamin C showed marked tubular improvement with regeneration although the hyaline cast and cellular debris persisted as corresponding to proteinuria. Improved glomerular and tubulointerstitial lesions along with decreased number of apoptotic cells by chronic oral administration of vitamin C for 8 months were demonstrated in diabetic rats (Lee et al., 2007). The rescue of tubular necrosis but not apoptosis by vitamin C in our experiment suggests that independent pathways may exist and occur depending on the time course of CDDP exposure.

CDDP nephrotoxicity can be induced by inhibition of DNA synthesis (Pabla and Dong, 2008) and involve oxidative stress and apoptosis (Miller et al., 2010; Santos et al., 2007). The role of oxidative stress in CDDP-induced kidney injury and its improvement by low dose of vitamin C were supported previously (Antunes et al., 2000). In the present study, the CDDP group had twice higher PCO/Cr with lower U-TAS, which were improved after the megadose of vitamin C was given. However, the reduction in P-TAS in the CDDP + VIT C group may be due to the megadose vitamin C promoting ROS generation via superoxide-driven Fenton reaction, which was demonstrated in vitro (Michels and Frei, 2013). The elevated K-GSH levels in
both CDDP and CDDP + VIT C groups could be explained by positive regulation in the glutathione biosynthesis under oxidative stress conditions resulting in increased glutathione levels (Tian et al., 1997). However, reduction in the renal glutathione level has been reported in rats in response to CDDP-induced oxidative stress (Fujieda et al., 2011). In the present study, PUN correlated with P-MDA, U-MDA/Cr, U-PCO/Cr, U-TAS and K-GSH. Therefore, renal functional loss by CDDP may be related to oxidative damage, which could be prevented by vitamin C.

A question is raised whether vitamin C alleviates the tumor killing effect mediated by generation of free radical. However, the tumor killing effect of cisplatin is due to not only free radical but also direct action on DNA damage. Vitamin C can improve antineoplastic activity of doxorubicin, cisplatin and paclixel demonstrated in human breast carcinoma cell lines (Kurbacher et al., 1996).

The renoprotective effects of megadose vitamin C on CDDP-induced kidney injury with reduced oxidative damage were demonstrated. Improved glomerular filtration and tubular reabsorptive capacity of electrolytes and water were seen without antiproteinuric effect. The renal functional improvement was supported by renal histopathology although renal apoptosis could not be prevented by vitamin C.

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**References**


บทคัดย่อ

ผลของการใช้วิตามินซีในขนาดสูงต่อการป้องกันการเสียหายของไตจากซิสพลาติน

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การเป็นพิษของไตเนื่องจากซิสพลาตินเป็นผลมาจากความเครียดออกซิเดชั่น ซึ่งสามารถทำให้ลดลงได้เมื่อใช้วิตามินซีในขนาดสูง แต่การเป็นพิษได้ตามความเครียดออกซิเดชั่น จะมีการเกิดการเมดิคินฮอร์โมนและเกิดโรคที่สูงขึ้น แต่การใช้วิตามินซีในขนาดสูงจะทำให้เกิดการก่อโรคที่สูงขึ้น เนื่องจากสารภัยและเกิดโรคที่สูงขึ้น แต่การใช้วิตามินซีในขนาดสูงจะทำให้เกิดการก่อโรคที่สูงขึ้น เนื่องจากสารภัยและเกิดโรคที่สูงขึ้น

วิตามินซีในขนาดสูงทำให้ไตเสียหายโดยมีการเพิ่มขึ้นของความเข้มข้นของครีอะทินีนและยูเรียไนโตรเจนในพลาสมา มีการเพิ่มของสารออกซิเดชั่นและสารออกซิเดชั่นในปัสสาวะ (P<0.05) ผลผลิตของ PCR จากไตซึ่งเป็นสัดส่วนของการต่อต้านการเกิดอะพอพโทซิสต่อการเกิดอะพอพโทซิส หรือ Bcl-2/Bax มีค่าลดลงเมื่อได้รับซิสพลาติน ในขณะที่ผลการจุลพยาธิวิทยาพบการตายของเซลล์ท่อไตอย่างกว้างขวาง เมื่อให้วิตามินซีขนาดสูงร่วมกับซิสพลาตินพบว่าการทำงานของไตและการเกิดความเครียดออกซิเดชั่นดีขึ้น แต่ไม่มีการรักษาให้กลับสู่ระดับปกติเหมือนการไม่ให้วิตามินซี แต่ยังมีการขับทิ้งโปรตีนทางปัสสาวะสูง การให้วิตามินซีขนาดสูงช่วยลดการเสียหายของไตเป็นผลจากซิสพลาตินผ่านการต่อต้านความเครียดออกซิเดชั่น

คำสำคัญ: อะพอพโทซิส ซิสพลาติน การเสียหายของไต วิตามินซี ขนาดสูง ความเครียดออกซิเดชั่น

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