Oxytocin Ameliorates Cisplatin-Induced Nephrotoxicity in Wistar Rats

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Abstract

Background: Cisplatin (CP) is one of the most effective chemotherapeutic agents and plays a major role in the treatment of a variety of human solid tumours. However, the clinical use of CP is frequently limited, because of its renal toxicity and production of reactive oxygen species (ROS) that intensify the cytotoxic effects. The use of antioxidants could effectively counteract such cytotoxic effects of CP. Oxytocin (OT) was previously shown to have antioxidant properties and anti-inflammatory effects in different inflammatory models. Accordingly, the main aim of the present study is to examine the possible protective influence of oxytocin administration in CP-induced nephrotoxicity.

Methods: Male Wistar albino rats were classified into 4 groups: Control group, OT only-treated group which received OT twice (500 µg/kg; i.p.) 30 minutes and just before saline administration, CP-induced nephrotoxicity group which received a single dose of CP (7.5 mg/kg; i.p.) and treated with saline, and CP + OT group in the same previous doses. After 72 hours of CP administration, rats were sacrificed and blood was withdrawn for determination of urea, creatinine, albumin and lactate dehydrogenase (LDH). Kidneys were dissected out for histopathological examination, and determination of the tissue levels of reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO) and thiobarbituric acid reactive substances (TBARS). Glutathione S-transferase (GST), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx) and myeloperoxidase (MPO) activities were also assessed.

Results: CP showed histopathological damage and significantly increased serum levels of urea, creatinine and LDH as well as levels of TBARS, MDA, NO and MPO in renal tissues. Meanwhile, serum albumin and tissue levels of GSH, GST, SOD, CAT and GPx decreased. Alterations in these biochemical and histopathological indices due to CP were attenuated by OT.

Conclusion: Oxytocin exerts a protective effect against cisplatin-induced nephrotoxicity and this protection is partially mediated through its antioxidant activity.

Key Words: Cisplatin – Oxytocin – Nephrotoxicity.

Introduction

CISPLATIN (CP) is an inorganic complex composed of an atom of platinum surrounded by chloride and amine groups in the cis position. Cisplatin therapy is highly effective against many tumors [1]. Despite its profound chemotherapeutic properties, the clinical use of CP is manifested by severe nephrotoxicity [2]. Irreversible renal damage occurs in about one third of CP-treated patients [3]. Thus, prevention of the side effects of CP is one of the major issues in treating cancer patients [4]. Oxidative stress process was reported to contribute to CP-induced nephrotoxicity [5,6]. CP generates reactive oxygen species (ROS), such as superoxide anion and hydroxyl radical and consequently, depletes glutathione (GSH) and inhibits the activity of antioxidant enzymes in renal tissues. Some antioxidants were tested for their ability to protect against CP-induced nephrotoxicity in experimental animals [7,8].

Oxytocin (OT) is a neurohypophysial peptide synthesized in the paraventricular and supraoptical nuclei of the hypothalamus. The well-known effect of OT is the stimulation of the uterine contractions at parturition and myoepithelial contractions in the mammary gland during suckling [9]. OT actions are thought to be mediated through its G-protein coupled receptors, which are widely distributed in the central nervous system [10], endothelial and smooth muscle cells of blood vessels [11]. OT has a vasoconstrictor effect on small cerebral and peripheral vessels, but a vasodilator effect on the larger cerebral arteries [12]. OT receptors have also been identified in other tissues, including the kidney, heart, thymus, pancreas and adipocytes [13]. It was found that OT may decrease the release of some interleukins and increase the survival of ischemic skin flaps in rats via the activation of the
Animals: –70ºC for further analysis. The abdomen of each animal was opened and kidneys were rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. The right kidneys were fixed in 10% formalin for histopathological examination. For subsequent analysis, 10% homogenate was prepared from left kidneys in phosphate buffer (0.05mol/l, pH 7.0) using a polytron homogenizer (PT 10/35, Brinkmann, Ilinos, USA) at 4ºC. The homogenate was centrifuged at 1000 xg for 10 minutes at 4ºC to remove the cell debris.

Based on this background, the current study was designed to investigate the possible protective effect of oxytocin against cisplatin-induced nephrotoxicity in Wistar rats.

Material and Methods

Chemicals and drugs:

CP was purchased from Bristol-Myers Squibb, NY, USA as a solution (2mg/ml). OT and all other chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Animals:

Forty eight male Wistar albino rats (250-300g) were used in this study in accordance with ethical norms approved by local ethics committee for the care and use of laboratory animals. Animals were housed in a well ventilated temperature controlled room at 22±3ºC with 12 hours light and dark cycles. Food consisted of standard laboratory rat chow with free access to water. All experimental procedures were performed between 8-10 a.m. and care was taken to avoid all stressful conditions.

Experimental design:

Rats were divided into 4 equal groups in separate clean cages, 12 rats in each group. Group I (Control group) received saline intraperitoneally (i.p.), 1ml/kg. Group II (OT group) received OT alone (500 μg/kg, i.p.) 30 minutes and immediately before saline administration (instead of CP). The dose of OT is based on a previous study by Düsünceli et al.,[17]. Group III (CP group) received saline (1ml/kg) 30 minutes and immediately before CP administration. CP was given in a dose of 7.5mg /kg once i.p., the dose which is documented to induce nephotoxicity in rats.[18]. Group IV (OT + CP group) received both OT and CP in the same previous doses.

Seventy two hours after CP administration, rats were sacrificed and blood was collected directly by a cardiac puncture from the heart of each animal. The blood was centrifuged at 3000 xg for 10 minutes to obtain clear sera which were stored at −70ºC for further analysis. The abdomen of each rat was opened and kidneys were rapidly dissected out, washed in ice-cold isotonic saline and blotted between two filter papers. The right kidneys were fixed in 10% formalin for histopathological examination. For subsequent analysis, 10% homogenate was prepared from left kidneys in phosphate buffer (0.05mol/l, pH 7.0) using a polytron homogenizer (PT 10/35, Brinkmann, Ilinos, USA) at 4ºC. The homogenate was centrifuged at 1000 xg for 10 minutes at 4ºC to remove the cell debris.

Serum biochemical assays:

In order to assess the renal function, serum albumin concentrations were determined by a spectrophotometric brom cresol green method as described by Doumas et al.,[19]. Serum lactate dehydrogenase (LDH), blood urea nitrogen (BUN) and serum creatinine levels were also determined spectrophotometrically by the methods described by Martinek[20], Talke and Schuber[21] and Slot[22] respectively.

Renal biochemical assays:

Reduced glutathione (GSH) was determined according to the method of Moron et al.,[23] based on the formation of a yellow-colored complex with Ellman’s reagent. Lipid peroxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) in tissue homogenates referring to the malondialdehyde (MDA) standard calibration curve according to the method of Uchiyama and Mihara[24]. Nitric oxide (NO) was determined by a colorimetric method based on the Greiss Reaction[25].

Determination of renal enzymatic activity:

The activity of glutathione S-transferase (GST) was measured according to the method of Habig et al.,[26]. P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 310nm using UV-Double Beam Spectrophotometer (Labomed, Inc., CA, USA). Catalase (CAT) activity was determined from the rate of decomposition of H2O2, monitored by a decrease of 240nm following the addition of tissue homogenate[27]. Superoxide dismutase (SOD) levels in the kidney tissue were determined according to the modified method of Kakkar et al.,[28]. Glutathione peroxidase (GPx) activity was determined by the method of Hafeman et al.,[29] based on the degradation of H2O2 in the presence of GSH. Myeloperoxidase activity (MPO) was determined using a 4-aminoanipyrine/phenol solution as a substrate for MPO-mediated oxidation by H2O2 and changes in absorbance at 510nm (A510) were recorded[30].
Histopathological study:

All tissue specimens were fixed in 10% formalin, embedded in paraffin, sectioned at 5 µm and stained with hematoxylin & eosin. Histopathological sections of kidney from all groups were evaluated using light microscopy (Meiji-Microscopes, 109-L, Meiji techno Co., Saitama, Japan).

Statistical analysis:

The GraphPad InStat 3 (GraphPad Software Inc, La Jolla, CA, USA) computer program was used to conduct regression analysis and to plot the collected data. All data were expressed as means ± SEM. Assessment of these results was performed using one-way ANOVA procedure followed by Tukey-Kramer multiple comparisons tests using Software GraphPad Prism, Version 4. Statistical significance was determined as a p-value <0.05.

Results

Effect on serum markers:

Administration of CP to rats induced a marked renal impairment, characterized by a significant increase in BUN and serum creatinine levels, as well as a significant reduction of serum albumin in the studied groups of Wistar rats. Serum LDH level (a marker of tissue damage) was significantly increased in CP group (3473±10.7). Pretreatment with OT significantly suppressed serum LDH level, reverting it back close to the control level (2250±7.2) (Fig. 1).

Effect on tissue enzymatic activity:

Activities of the major enzymes of the antioxidant defense system, namely GST, SOD, CAT and GPx, were significantly decreased (p<0.05) in CP group. Administration of OT was found to elevate these levels significantly (Table 3). MPO activity, which is an indicator of neutrophil infiltration, was significantly higher in the renal tissue of CP group (3.96±0.19) than that of the control group (1.28 ±0.11). On the other hand, OT treatment in the OT + CP group significantly decreased the renal tissue level of MPO (1.94±0.18), which was approximating the control group values (Fig. 2).

Histopathological results:

The light microscopic study of the control and OT-only treated groups revealed a regular morphology of renal parenchyma with well-designated glomeruli and tubules (Fig. 3A,B). In the CP group, CP caused severe and widespread tubular and interstitial damage evidenced by tubular lumen dilatation due to flattening of tubular cells with brush border loss, cast formation and inflammatory cell infiltration (Fig. 3C). In the OT-treated CP group, there was a remarkable improvement in the histological features of the kidney. The reduced tubular damage and interstitial inflammation were the features indicating regeneration and improvement (Fig. 3D).

Effect on tissue markers:

In CP-injected rats, a significant (p<0.05) depletion of GSH and a significant increase in TBARS, MDA, and NO levels were observed compared with the control group. OT administration significantly alleviated these changes in OT + CP group compared with CP group (Table 2).

Table (1): Levels of blood urea, serum creatinine and serum albumin in the studied groups of Wistar rats.

<table>
<thead>
<tr>
<th></th>
<th>Urea (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>24.3±2.4</td>
<td>0.4±0.05</td>
<td>5.3±0.4</td>
</tr>
<tr>
<td>OT</td>
<td>29.6±3.1</td>
<td>0.5±0.06</td>
<td>5.1±0.1</td>
</tr>
<tr>
<td>CP</td>
<td>130±5.2a</td>
<td>7.2±0.6a</td>
<td>3.8±0.2a</td>
</tr>
<tr>
<td>OT + CP</td>
<td>62±3.5b</td>
<td>0.6±0.1b</td>
<td>4.1±0.5</td>
</tr>
</tbody>
</table>

Table (2): Renal tissue levels of Reduced Glutathione (GSH), Thiobarbituric acid-reactive substances (TBARS), Malondialdehyde (MDA) and Nitric Oxide (NO) in the studied groups of Wistar rats.

<table>
<thead>
<tr>
<th></th>
<th>GSH (μmol/g protein)</th>
<th>TBARS (nmol/g protein)</th>
<th>MDA (nmol/g protein)</th>
<th>NO (μmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.80±0.21</td>
<td>20.6±2.2</td>
<td>24.2±0.3</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>OT</td>
<td>1.43±0.11</td>
<td>23.2±1.9</td>
<td>25.3±0.4</td>
<td>0.11±0.06</td>
</tr>
<tr>
<td>CP</td>
<td>0.20±0.04a</td>
<td>51.3±4.2a</td>
<td>52.4±8.8a</td>
<td>0.38±0.9a</td>
</tr>
<tr>
<td>OT + CP</td>
<td>1.20±0.16b</td>
<td>25.8±2.8b</td>
<td>32.3±0.7b</td>
<td>0.18±0.07b</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM of 12 rats in each group. a p<0.05 vs. control group. b p<0.05 vs. CP group.

Table (3): Renal tissue levels of Reduced Glutathione (GSH), Thiobarbituric acid-reactive substances (TBARS), Malondialdehyde (MDA) and Nitric Oxide (NO) in the studied groups of Wistar rats.

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<th>GSH (μmol/g protein)</th>
<th>TBARS (nmol/g protein)</th>
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<th>NO (μmol/mg protein)</th>
</tr>
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<tr>
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</tbody>
</table>

Data are the mean ± SEM of 12 rats in each group. a p<0.05 vs. control group. b p<0.05 vs. CP group.

Fig. (1): Mean levels of serum lactate dehydrogenase (LDH) in the studied groups of Wistar rats a p<0.05 vs. control group; b p<0.05 vs. CP group.
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Table (3): Renal tissue levels of Glutathione S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Glutathione peroxidase (GPx) in the studied groups of Wistar rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>GST (U/mg protein)</th>
<th>CAT (U/mg protein)</th>
<th>SOD (U/mg protein)</th>
<th>GPx (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.82±0.07</td>
<td>5.13±0.27</td>
<td>53.1±2.12</td>
<td>29.12±1.12</td>
</tr>
<tr>
<td>OT</td>
<td>0.88±0.03</td>
<td>5.05±0.23</td>
<td>58.6±1.53</td>
<td>30.03±2.17</td>
</tr>
<tr>
<td>CP</td>
<td>0.36±0.03a</td>
<td>2.46±0.17a</td>
<td>30.6±1.21a</td>
<td>19.82±1.10a</td>
</tr>
<tr>
<td>OT + CP</td>
<td>0.58±0.04b</td>
<td>3.72±0.16b</td>
<td>39.1±1.16b</td>
<td>23.26±1.18b</td>
</tr>
</tbody>
</table>

Data are the mean ± SEM of 12 rats in each group.

a p<0.05 vs. control group.
b p<0.05 vs. CP group.

Fig. (2): Mean values of Myeloperoxidase (MPO) activity in the studied groups of Wistar rats. a p<0.05 vs. control group; b p<0.05 vs. CP group.

Fig. (3): Histopathological findings (H & E, x200) from (A) control group and (B) Oxytocin (OT) only treated group showing normal histopathological distribution; (C) Cisplatin-treated (CP) group showing prominent tubular dilatation with flat epithelial lining, cast formation and interstitial inflammation (D) OT plus CP group displaying remarkable improvement in the histological appearance with marked reduction in CP-induced tubular damage, compared to samples treated with CP alone.

Discussion

Nephrotoxicity has limited the clinical use of CP as a chemotherapeutic agent in 25-30% of patients, even since the first dose [31]. Less toxic platinum compounds have been developed, yet, CP remains the drug of choice in platinum based therapy regimens [32]. Therefore, strategies of ameliorating the nephrotoxicity of CP are of clinical interest.

In the current study, a single dose of CP induced renal damage evidenced by serum biochemical and tissue parameter changes as well as histopathological damage. BUN and serum creatinine were elevated with significant decrease in serum albumin indicating renal impairment. Similarly, LDH was found to be significantly elevated after CP administration indicating nonspecific cellular injury. The exact mechanism of CP-induced nephrotoxicity is not completely understood. However, oxidative stress and free radical production in renal tubular cells have been suggested to be responsible for the oxidative renal damage [33]. Mansour et al., [34] demonstrated that CP induces ROS by decreasing
the activity of antioxidant enzymes and by depleting intracellular concentrations of GSH. Glutathione has a direct antioxidant function by reacting with superoxide radicals, peroxy radicals and singlet oxygen followed by the formation of oxidized glutathione (GS-SG) and other disulfides. Both GST and GPx are GSH-dependent antioxidant enzymes [35]. SOD catalyzes dismutation of the superoxide anion (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$), which is then detoxified to H$_2$O by catalase [36]. Moreover, CAT is a common enzyme found in nearly all living organisms catalyzing the decomposition of hydrogen peroxide to water and oxygen [37].

The present results support the hypothesis that the mechanism of CP toxicity is related to the depletion of the antioxidant defense system. A significant decline in antioxidant enzymes activities and increase in free radicals in experimental models as well as in human studies is typical during CP treatment [38]. A significant increase in renal MDA and decrease in the activities of antioxidant enzymes were reported by many researchers upon similar CP treatment of rats [8, 39, 40]. In the present study, CP caused an elevation in tissue MPO activity indicating the presence of enhanced polymorph nuclear leukocytes recruitment in the inflamed tissue, while the increased renal MDA level, an indicator of lipid peroxidation, verified the oxidative damage in the renal tissue. Oxytocin pretreatment decreased the MPO and MDA levels and inflammatory cell infiltration which was in accordance with Cetinel et al., [41] who found similar effects of OT on both MPO and MDA in stress induced colitis.

In the present study, OT administration before CP prevented both oxidative renal injury and tissue neutrophil accumulation. A possible mediator behind the decrease in MPO activity in response to OT could be NO [42]. Previous reports suggested that the protective effects of OT pretreatment on ischemia-reperfusion damage may be caused by the release of NO [17] and NO may inhibit the adhesion and accumulation of neutrophils [43]. Some studies confirmed the antioxidant property of OT in colitis [44], pyelonephritis [45], and sepsis-induced inflammatory models [46]. It was also shown that in brain membranes, OT displayed antioxidant properties in aqueous medium, scavenging free peroxy radicals, preventing LDL oxidation and inhibiting lipid peroxidation [16]. Another possibility to consider is that OT may release atrial natriuretic peptide (ANP), which is a vasodilator and has antioxidantive properties [47]. In previous studies, it was shown that ANP protects the kidney [48] and liver [49] from ischaemia-reperfusion injury. On the other hand, it is generally accepted that OT activates two types of receptors, OT and vasopressin receptors. Activation of the vasopressin receptors evokes coronary constriction and contributes to the anti-inflammatory effects of OT, whereas activation of the endothelial OT receptors leads to vasodilatation [50].

Conclusion:

In conclusion, it was found that oxytocin has effectively attenuated cisplatin-induced renal tissue injury including biochemical changes and histopathological damages, making it a possible clinical candidate to ameliorate CP-induced nephrotoxicity through its antioxidant effect.

References


