Research Paper: Cisplatin Induce Urinary Space Obstruction and Tubular Necrosis in Rat Kidney

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ABSTRACT

Introduction: Cisplatin is a platinum based antineoplastic drug, which is widely used for treatment of solid tumors. The present investigation was carried out to study the nephrotoxic effects of double dose injection of cisplatin in rats, as an experimental model.

Methods: In this experimental study, 45 adult male Sprague Dawley rats with average weight of 200±30 g were randomly divided into two experimental (n=30) and one control (n=15) groups. Rats of experimental groups received two repeated doses of cisplatin intraperitoneally (2.5 mg/kg, experimental group E1 & 5 mg/kg, experimental group E2) in the beginning of first and fifth week of the experiment. Eight weeks after injection, rats of all groups were given deep anesthesia and killed. Blood samples were collected directly from their hearts for biochemical evaluation. Tissue samples were removed and prepared sections were stained with H&E, PAS, Masson trichrome, and PNA methods. Prepared microscopic slides were utilized for both histopathological and morphometrical studies. Collected data were analyzed by ANOVA and Tukey post hoc test using SPSS.

Results: Cisplatin administration induced a significant decrease in urinary space diameter of renal corpuscles in the experimental groups compared to the control group. This ultimately led to the urinary space obstruction in up to 95% of nephrons in experimental groups (P<0.001). There were significant difference between control and experimental groups (P<0.001) with regard to epithelial thickness of collecting duct, proximal and distal convoluted tubules. Moreover, there was a significant difference between control and experimental groups (P<0.001) with regard to the diameter of vasa recta. Acute tubular necrosis and urinary space obstruction were the main histological features of the experimental groups. There were also a significant elevation in serum level of BUN and creatinine in experimental group compared to those of control group.

Conclusion: Cisplatin induces acute tubular necrosis and urinary space obstruction and some other morphological changes in rat kidney, in a dose dependent manner.

Key Words:
Cisplatin, Convoluted tubule, Glomerulus, Vasa recta, Rat
1. Introduction

Cisplatin (Cis-diamine dichloroplatinum [II]), a platinum derivative and anti-neoplastic drug, is an inorganic platinum based agent widely used for treatment of solid tumors, including head and neck, lung, testis, ovarian, and bladder cancers. The clinical use of cisplatin is highly restricted due to its significant side effects such as bone marrow suppression, nephrotoxicity, hepatotoxicity, and peripheral neuropathy. Acute tubular necrosis, apoptosis, inflammation, oxidative stress, and kidney vascular injury in association with a rapid decrease in glomerular filtration rate are the main nephrotoxic side effects of cisplatin cytotoxicity. Kidneys are the main target organ for the cytotoxic effects of chemical agents, toxins, and drugs. It seems that drug nephrotoxicity is responsible for approximately 19% of Acute Kidney Injury (AKI) in critically ill patients [1-2].

Kidneys are essential for the metabolism and elimination of the toxic agents and drugs such as cisplatin [3]. After entrance of cisplatin into cells, chloride atoms of cisplatin are replaced with water molecules which produce highly reactive electrophilic products. In cancer and other proliferating cells, cisplatin-DNA inter- and intra-stand crosslink complexes of cisplatin with purine bases form. This reaction alters secondary structure of DNA, finally lead to cell cytotoxicity and inhibition of DNA transcription and duplication [4-5].

Renal blood flow can reduce within 3 hours after cisplatin infusion, and Glomerular Filtration Rate (GFR) drops after the decrease in renal blood flow. GFR changes and renal blood flow probably reflect higher renal vascular resistance due to tubular-glomerular feedback because of higher sodium chloride delivery to the macula densa. Most patients have a reversible reduction in glomerular filtration, but some have an irreversible decrease [6]. Experimental models of cisplatin-induced nephrotoxicity show an increase in the expression of proinflammatory cytokines, including tumor necrosis factor, interleukin 6, and interferon-γ.

Furthermore, there is an increase expression of endothelial Cell Adhesion Molecule (CAM), which facilitate infiltration of leukocytes and T lymphocytes into kidney parenchyma [4]. The kidneys are the main organ which concentrate and retain cisplatin and excrete it predominantly via glomerular filtration and tubular excretion [5]. Organic Cation Transporter (OTC) plays an important role in cellular uptake of cisplatin. Cisplatin toxicity can be increased in OTC2 (Organic Cation Transporter 2) overexpressing in human proximal tubular cells. Cisplatin is trapped mainly in proximal tubular cells and concentrated in renal cortex 5 times greater than serum cisplatin concentration. It seems that accumulation of cisplatin in a dose-dependent manner is the basis for cisplatin nephrotoxic side effects [1].

Antunes et al. study shows that cisplatin increases serum BUN and creatinine level up to 65% of normal level and administration of vitamin C as a protective agent leads to significant decrease in serum BUN and creatinine level. Glutathione is one of the important compounds of cellular integrity and kidney glutathione contents change after cisplatin injection. The amount of glutathione decreases in kidney in response to induced oxidative stress after cisplatin administration. There are many similarities in glutathione contents changes induced by cisplatin in rat and human [3].

Cisplatin in the kidney induces stress-related protein c-Jun and D-Jun. Cellular stress activates 2 related signaling pathways of Jun N-terminal kinase and extra-cellular regulated kinase. Cisplatin induces apoptosis and necrosis in proximal and convoluted tubules and also significantly up-regulates several chemokines. Furthermore, cisplatin induces increased expression of cyclin-dependent kinase inhibitor [5]. The present study aimed to find quantitative and qualitative changes of renal parenchyma and distribution pattern of Gal-GalNAc containing glycoconjugates in rats after double IP injection of cisplatin.

2. Material and Methods

In this experimental study, 45 adult male Sprague Dawley rats were randomly divided into control (n=15) and experimental (n=30) groups. Experimental group 1 (E1) received two repeated IP injection of 2.5 mg/kg of cisplatin while experimental group 2 (E2) received two injection of 5 mg/kg of the drug at the beginning of the first week and fifth week of the experiment. At the end of the experiment (the eighth week), under deep anesthesia all rats were killed. Blood samples were directly collected from heart for biochemical analysis to determine serum levels of BUN and creatinine.

After dissection, kidneys were removed and fixed in formalin buffer, embedded in paraffin and 5 µm tissue sections were processed for H&E, PAS, trichrome Masson stainings, and PNA lectin histochemistry. The epithelial thickness of proximal and distal convoluted tubules and collecting ducts as well as the diameter of
vasa recta were measured morphometrically. Lectin histochemistry was carried out according to the Spicer method [7]. Briefly, lectin was diluted up to 5 µg/mL in PBS at pH=6.8, where DAB was used as the chromogen. The weights of rats of control and experimental groups were recorded before and after the experiment. Furthermore, the amount of used water and food were measured daily. Data were analyzed by ANOVA using SPSS. Data were expressed as mean±SD and P<0.05 was considered as statistically significant. The study protocol was approved (Registered No. 94-7261) by Ethics Committee of Zahedan University of Medical Sciences.

3. Results

Statistical data on measured parameters showed a significant difference for urinary space diameter between experimental and control groups (P<0.001, Figure 1). There was also a significant increase in diameter of vasa recta in experimental groups, compared to the control group (P<0.001, Figure 1). Statistical analysis also demonstrated a significant decrease in epithelial thickness of proximal, distal, and collecting tubules in experimental groups (P<0.001, Figure 1). Statistical analyses for creatinine and BUN serum levels confirmed a significant difference between control and experimental groups (P<0.001, Figure 2).

On the other hand, evaluation of inflammatory process in cortex and medulla revealed no significant difference between control and experimental groups. Furthermore, analysis of data for the amount of consumed water and food showed a significant difference between control and experimental groups (P<0.001, Table 1). Paired t-test revealed a significant difference for the weight of rats before and after the experiment (P<0.01). Analyses of data for the kidney weight and ratio of kidney weight/whole weight showed that there was a significant difference between control and experimental groups (P<0.01, Table 1).

Histological studies revealed these findings. Acute tubular necrosis and glomerular distension which lead to urinary space obstruction were the main histological findings in experimental groups, in comparison to the control group. Histological examination of medullary region showed the presence of cast in experimental groups. The presence of inflammatory cells in the cortical region was more prominent in comparison to the medullary regions in the experimental groups. PAS and Masson staining demonstrated severe staining intensity of mesangium in glomerulus of experimental groups which shows induction of fibrosis in mesangium and interstitial tissue of rat kidney (Figure 3).

4. Discussion

Our results demonstrated a variety of quantitative and qualitative changes in renal parenchyma after cisplatin injection, including glomerular distention, urinary space obstruction, vasodilation of vasa recta, as well as a significant decrease in the epithelial thickness of proximal convoluted tubules, distal convoluted tubules, and collecting ducts. Cisplatin also induced many structural changes in glomerulus which is the basis for urinary space obstruction. It seems that this histological changes may be the main reason for acute renal failure in human and experimental animals such as rat [8]. Studies showed that mesangium hypertrophy and vascular congestion induced by cisplatin is the structural basis for renal failure [8-10].
Experimental studies showed that cisplatin induce tubular cystic distention with a flat epithelium in cortical tubules and also an increase in fibrous and inflammatory elements of renal interstitium in rats [9]. Our results showed an increase in fibrous element and inflammatory cells in experimental groups which is in accordance with the results of Yao and associates [9]. After entrance of cisplatin into cancer cells, the labile chloride ions are replaced by water molecules which produced highly reactive electrophilic products, intrastand and interstand binding of cisplatin to purine bases alter secondary structure of DNA and inhibiting

<table>
<thead>
<tr>
<th>Groups Variables</th>
<th>Control Mean±SD</th>
<th>Experimental 1 Mean±SD</th>
<th>Experimental 2 Mean±SD</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat weight before experiment (g)</td>
<td>214.73±12.07</td>
<td>222.36±17.7</td>
<td>212.13±9.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Rat weight after experiment (g)</td>
<td>306.53±32.04</td>
<td>328.4±23.82</td>
<td>311.33±31.53</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Consumed water</td>
<td>56.72±1.83</td>
<td>47.41±2.86</td>
<td>56.14±2.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Consumed food</td>
<td>19.13±0.18</td>
<td>17.62±0.23</td>
<td>15.17±0.05</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Kidney weight</td>
<td>1.2±1.01</td>
<td>1.12±1.04</td>
<td>1.02±1.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Kidney/total weight×1000</td>
<td>3.96±0.63</td>
<td>3.93±0.26</td>
<td>3.23±0.4</td>
<td>&lt;0.01</td>
</tr>
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</table>

Figure 3. Normal appearance of urinary space (arrow), glomerulus and Bowman capsule in control group (a). Obstructive changes (thin arrow) in urinary space and tubular necrosis in Group E1 (IP injection of 2.5 mg/kg of cisplatin) (b) are shown (a, b H&E×40). Positive reaction of mesangium matrix to PAS staining (arrow) in Group E1 (c) (PAS×40). Positive reaction of striated border (arrow) to PNA for demonstrating the Gal-GalNAc containing glycoconjugates in PCT and negative reaction of a rare case of non-affected glomerulus (d) are shown in group E2 (d) (IP injection of 5 mg/kg of cisplatin) (PNA×40).
DNA replication and transcription; whether the cisplatin nephrotoxicity depends on such mechanisms is unknown [5].

Cisplatin induced increased expression of cyclin-dependent kinase inhibitor p21 and hence mice with deletion of p21 gene are more susceptible to cisplatin nephrotoxicity. Cisplatin also decrease significantly glomerular filtration rate due to afferent arteriole vasoconstriction even after a single dose injection [5]. Renal hemodynamic changes induced by cisplatin are one of the most important physiological changes of cisplatin nephrotoxicity [11]. Our results showed glomerular distension and vasa recta dilation after cisplatin injection as a vascular changes which may be induced by cisplatin. Induction of severe inflammatory reaction and increase in fibrous elements may be manifestation of renal hemodynamic changes. Although the exact mechanism of cisplatin nephrotoxicity is not known yet, it seems that after cross link of DNA with cisplatin, caspase increase activity due to mitochondrial changes, increase production of Reactive Oxygen Species (ROS), and decrease in glutathione content may be part of complex mechanisms responsible for cisplatin nephrotoxicity [11].

Our results showed an increase in the content of inflammatory cells in experimental group in comparison to control group. The severity of inflammation in cortex was more prominent than medulla, although there were no any significant difference between control and experimental groups. The study of Ortega showed that endothelial cell injury induced by cisplatin play an important role in acute tubular necrosis after cisplatin injection [12]. Our results showed that there was significant decrease in the amount of used water and food between control and experimental groups, which may be a reason for difference of weights of rats in experimental and control groups. Impairment of kidney function induced by cisplatin were evaluated by changes in urine volume, creatinine clearance, glutathione status and increase in the products of lipid peroxidation and also the degree of Reactive Oxygen Species (ROS), and decrease in glutathione content may be part of complex mechanisms responsible for cisplatin nephrotoxicity [3].

Our results showed significant difference between control and experimental groups for creatinine and BUN, which clearly shows impairment of kidney function. Our results showed different pattern of Gal-GalNAc containing glycoconjugates in cortex and medulla of rat kidneys, although there was no any prominent different between control and experimental group after cisplatin injection. The more staining intensity to PNA lectin was shown in apical plasmalemma of proximal convoluted tubules and there was not any reaction of glomerulus to PNA. Glycoconjugates are a class of glycoprotein or glycolipid of cell surface and extracellular matrix which play an important role in so many physiological reactions [7]. It seems that cisplatin induces so many quantitative and qualitative changes in kidney parenchyma, including urinary space obstruction in up to 95% of nephrons and acute tubular necrosis, which may be the basis for cisplatin nephrotoxicity.

In conclusion, cisplatin seems to induces many quantitative and qualitative changes in kidney parenchyma, including urinary space obstruction in up to 95% of nephrons and acute tubular necrosis, which may be the basis for cisplatin nephrotoxicity.

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Conflict of Interest

All authors certify that this manuscript has neither been published in whole nor in part nor being considered for publication elsewhere. The authors have no conflicts of interest to declare.

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