Research Article

Analysis of Tissue Cytokine Levels in Rats with Cisplatin-Induced Experimental Nephrotoxicity

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Abstract

Objectives: Analysis of tissue cytokine levels in rats with cisplatin-induced experimental nephrotoxicity.

Methods: Twenty Sprague – Dawley male rats were included in the study and assigned to two groups of 10. The Sham group (Group I) received a single intraperitoneal (ip) dose of 1 cc saline solution every 12 hours. The cisplatin group (Group II) received a 10 mg/kg ip dose of cisplatin to induce nephrotoxicity. Each group was placed in a metabolic cage for one week. Salin solution was administered every 12 hours, starting 36 hours before inducing nephrotoxicity with cisplatin. Body weight and 24-hour urine of rats were followed up in the metabolic cage. The rats were anesthetized with a 5 mg/kg dose of rompun and 100 mg/kg dose of ketamine 84 hours after the cisplatin administration; then, they were sacrificed. Then blood samples were collected from the abdominal aorta and kidney samples were collected to test the levels of tissue antioxidant enzymes. Kidney tissue samples were assigned to two groups. The samples in one group were fixed in formalin and the samples in the other group were fixed in glutaraldehyde. These two group were separately stored in the refrigerator at -80ºC until the time of analysis.

Results: The comparison of Group I and two Group II revealed that the levels of urea and creatinine increased significantly (p=0.001 for both parameters), indicating cisplatin-induced nephrotoxicity. Tissue TNF-α levels of Group I and Group II were significantly different (p=0.049); however, there were no significant differences in the tissue levels of IL-1β, IL-10 and IL-6 (p=0.151, p=1, p=0.545; respectively).

Conclusion: Cisplatin is one of the most important antineoplastic drugs used for treating solid tumors. Nephrotoxicity is the most important dose-limiting side effect. According to the data obtained from this study, a single high dose of cisplatin in cancer therapy cause kidney injury. It is known that inflammation is one of the majör mechanisms in cisplatin nephrotoxicity and that TNF-α plays a central role in this development. In the group with cisplatin-induced nephrotoxicity, there has been a significant increase in the tissue levels of TNF-α, which is an inflammatory cytokine. However, there have been no significant changes in the tissue IL-1β and IL-6, both of which are inflammatory cytokines, too. Also, there has not been an increase in the tissue levels of IL-10, which is an anti-inflammatory cytokine.

Keywords: Cisplatin, cytokine, nephrotoxicity

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Cisplatin is one of the most important antineoplastic drugs used for treating solid tumors. Cytotoxic side effects are caused by connecting to the nuclear DNA transcription, disrupting replication and stimulating various signal transduction pathways.[1] There are multisystemic toxic effects of cisplatin, and nephrotoxicity is the major dose-

limiting side effect.[2] The pathogenesis of nephrotoxicity is not fully elucidated, but is thought to be due to apoptosis, necrosis, oxidative stress, inflammation, fibrogenesis, hypoxia, and mitochondrial damage. After the drug enters the cell signaling pathway, cells go to apoptosis or necrosis.[3] Oxidative stress is one of the most important mechanisms playing an active role in cisplatin nephrotoxicity. Cisplatin leads to deformations in renal tubule cell interactions resulting in reactive oxygen species, can exert effects on lipids, proteins and DNA, and consequently detorioration in cell structure.[4] As a result of these events, cisplatin causes impair of renal function and may lead to the development of acute renal failure. Many strategies have been developed to reduce this serious side effect of cisplatin.[3]

In this study, it was thought that it would be helpful to show the level of cytokines and to find new treatments for the prevention of cisplatin nephrotoxicity.

Inflammation in Cisplatin Nephrotoxicity

Recent studies have shown that inflammation plays an important role in cisplatin-induced renal injury. Cisplatin increases the renal expression of TNF-α, Transforming growth factor β (TGF-β), monocyte chemoattractant protein-1 (MCP-1), intercellular adhesion molecule (ICAM), hemoxygenase-1 are also found to be increased in the kidneys. TNF-α also plays a central role in renal injury; induces apoptosis, contributes to free oxygen radical production, coordinates the activation of many chemokines and cytokines in the kidney. Previous Studies have shown that TNF-α inhibitors reduce cisplatin-induced renal dysfunction and structural damage.[5] Rats which have low TNF-α were significantly protected from cisplatin nephrotoxicity.[6]

Methods

Rat Selection and Groups

20 Sprague - Dawley male rats, each of which were six to eight week old in between weight of 190-330 g, were obtained from Gulhane Military Medical Faculty Chairman of Research and Development Center. Rats were kept at room temperature (20±2 ºC) in metabolic cages and 12-hour day light/dark environment, fed with standard rat chow pellets, and provided free access to water during the study period. Exclusion criteria were the mortality, sepsis, local infection and proven trauma.

Rats were randomly selected and two groups, each with 10 rats, were formed.

Group 1 [control (sham) group]: The rats were administered 1 cc saline as a single dose intraperitoneally (ip).

Group 2 (Cisplatin control group): the rats were treated with 10 mg/kg of cisplatin i.p.

The drug applications started 36 hours before cisplatin administration until 72 hours passed, for every 12 hours. Eightyfour hours after treatment with cisplatin, rats were anesthetized and sacrificed by using 5 mg/kg of Rompun and 100 mg/kg of ketamine, kidney tissue samples was taken. Kidney tissues were prevented in three ways until analysis; one in formaldehyde, the other in glutaraldehyde and the last one in the refrigerator at -80 °C.

After the Experiment is Terminated

1. Biochemical analysis: Serum urea, creatinine, sodium, potassium, magnesium, calcium were determined.
2. Inflammatory and Anti-inflammatory Cytokine Analysis: Changes in inflammatory cytokines (TNF-α, IL-1β, IL-6) and anti-inflammatory cytokine (IL-10) levels in tissue samples were analyzed with therapeutic agents.

In the study, cisplatin (50 mg/100 ml Vial® Ebewa cisplatin-Lib Pharmaceutical) was used. To form cisplatin nephrotoxicity, rats were administered a dose of 0.5 mg/ml at a concentration of 10 mg/kg.[7] Cisplatin application time was defined as zero, and 10 mg/kg cisplatin was administered intra peritoneally in a 5 ml syringe.

Measurement of Serum Creatinine and Serum Electrolyte Levels in Rats

Serum creatinine level was measured kinetically by Jaffe method on DP modular device (Roche®). In this method, creatinine and picric acid complex formed in alkaline medium were measured photometrically. Creatinine results were expressed as mg/dL. In addition, serum urea, calcium, magnesium, sodium and potassium levels were measured by the same method.

Determination of Levels of Inflammatory and Anti-Inflammatory Cytokines in Rat Kidney Tissue

Cytokine levels of inflammatory TNF-α, IL-1β, IL-6 and anti-inflammatory IL-10 were measured by Bendermed Systems brand elisa tissue kit.

Statistical Evaluation

Statistical evaluation of the data was performed by the "SPSS 15.1 for Windows" computer based statistics program. Comparison of two groups were performed by using "Mann-Whitney U test (two samples from the same distribution, nonparametric tests)" and comparision of all groups data "Kruskal wallis [non-parametric statistical tests, sorting one-way analysis of variance]" test was used for.

Results

The comparison of Group I and two Group II revealed that the levels of urea and creatinine increased significantly

(p=0.001 for both parameters), indicating cisplatin-induced nephrotoxicity. Sodium and potassium values decreased significantly (p values 0.001) and magnesium values did not change significantly (p=0.238). Cisplatin-induced nephrotoxicity and hyponatremia, hypokalemia developed, but had no effect on magnesium levels (Table 1).

Tissue TNF-α levels of Group I and Group II were significantly different (p=0.049); however, there were no significant differences in the tissue levels of IL-1β, IL-10 and IL-6 (p=0.151, p=1, p=0.545; respectively) (Table 2).

Discussion

Cisplatin is one of the most important antineoplastic drugs used for treating solid tumors. Nephrotoxicity is the major dose-limiting side effect. Dose restriction is applied against this undesirable side effect and this causes treatment disruptions.

The pathogenesis of nephrotoxicity is not fully elucidated, but is thought to be due to apoptosis, necrosis, oxidative stress, inflammation, fibrogenesis, hypoxia, and mitochondrial damage. After the drug enters the cell signaling pathway, cells go to apoptosis or necrosis.[3] Oxidative stress is one of the most important mechanisms playing an active role in cisplatin nephrotoxicity. Cisplatin leads to deformations in renal tubule cell interactions resulting in reactive oxygen species.

In addition to these mechanisms, cisplatin can cause ischemia, renal tissue damage and GFR (glomerular filtration rate) reduction by causing vascular damage to the kidney and causing acute renal failure.[3] Studies aimed at elucidating the pathogenesis of nephrotoxicity will help to provide new solutions for the prevention of cisplatin nephrotoxicity. Related to the prevention of nephrotoxicity; Many ways have been tried considering the mechanism of action and pathogenesis of the drug. Since oxidative damage plays an important role in pathogenesis,

many antioxidant agents have been studied to prevent this.[5] GFR may decrease with cumulative doses after cisplatin administration or may decrease after single dose administration.[8] 25% of patients treated with cisplatin in the subsequent two week period of treatment is known reversible azotemia detected.[9] In animal studies on nephrotoxicity of the drug, nephrotoxicity indexes were found to be elevated and serum creatinine rates were found to be elevated.[10] In this study, urea and creatinine values were significantly increased in cisplatin group. Many patients may experience loss of sodium, potassium and magnesium in the urine due to cisplatin, and some may develop orthostatic hypotension.[5] In this study, while hyponatremia and hypokalemia were observed due to cisplatin, no change in magnesium level was observed.

It is known that inflammation is one of the important mechanisms in cisplatin nephrotoxicity and TNF-α plays a central role in its development. In addition, macrophage and lymphocyte infiltration around the affected tubules are also seen in cisplatin nephrotoxicity. When the effects of melatonin on inflammatory and antiinflammatory cytokines were compared because of their effects on cisplatin nephrotoxicity and antioxidant effects, melatonin was found to have a significant decreasing effect on tissue TNF-α levels, but not on tissue IL-1β, tissue IL-10 and tissue IL-6. This main difference was thought to be due to the fact that melatonin is a physiological TNF-α inhibitor.

Further investigations for cisplatin nephrotoxicity will not only provide a better understanding of nephrotoxicity mechanisms that cannot be fully resolved, but will also provide new insights for the use of other active substances that may inhibit nephrotoxicity. Every new substance and every new attempt to reduce the side effects of anticancer drugs without disturbing the antineoplastic efficacy is important for the success of the treatment and consequently it provides a better quality of life for the patients.

Table 1. Comparison of serum urea, creatinine, sodium, potassium and magnesium levels of the groups

Groups	Urea (mg/dl)	p	Creatinin (mg/dl)	p	Sodium (mg/dl)	p	Potassium (mg/dl)	p	Magnesium (mg/dl)	p
Group I	54.20±13.02	0.001	0.59±0.03	0.001	138.27±3.76	0.001	7.15±0.71	0.001	3.24±0.39	**0.238**
Group II	323.97±109.16		2.92±1.0 6		126.89±4.08		4.88±0.62		3.41±0.31	

Table 2. Renal tissue inflammatory and antiinflammatory cytokines results

Groups	Tissue TNF-α (pg/mg protein)	p	Tissue IL- 1β (pg/mg protein)	p	Tissue IL-10 (pg/mg protein)	p	Tissue IL-6 (pg/mg protein)	p
Group I	29.90	0.049	29.80	0.151	25.20	1.0	31.10	0.545
Group II	39.20		36.40		25.50		27.30	

Disclosures

Ethics Committee Approval: The Ethics Committee of Gulhane Military Medical Academy approved the study with 830-82-09 ethical comatitte number at 04 December, 2009. All procedures were carried out according to the Helsinki Declaration of 1975 (revised in 2008) and all procedures adhered to the ethical standards of the responsible committee on animal experimentation (institutional and national).

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