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Evaluation of the antioxidant effects of zolpidem in the rat model of cisplatin-induced nephrotoxicity

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ABSTRACT

Introduction: Nephrotoxicity is one of the most important side effects of cisplatin which has limited its use. Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) plays a significant role in the pathogenesis of this drug.
Objectives: The aim of this study was to evaluate the antioxidant effect of zolpidem on the reduction of nephrotoxicity associated with cisplatin.
Materials and Methods: In this study, 40 adult male rats were divided into 4 groups; 1) healthy group, 2) control group, 3, 4) cisplatin-induced nephrotoxicity + different doses of zolpidem. After a certain period of time, the urine, spinal cord and kidney samples of rats were collected. Then, urine levels of functional factors including urea, creatinine and albumin/creatinine ratio, antioxidant enzymes and malondialdehyde (MDA) levels were estimated. Consequently, histological studies were conducted with the collected samples.
Results: Zolpidem reduced levels of urea, creatinine, albumin/creatinine ratio, and MDA. It also increased the amount of antioxidant enzymes of the kidney including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and moderated the tubular damage caused by the use of cisplatin.
Conclusion: Zolpidem is able to improve the nephrotoxicity by reducing oxidative stress.

Original

Implication for health policy/practice/research/medical education:
Zolpidem is a nonspecific hypnotic drug that has antioxidant and neuroprotective properties. Cisplatin is mainly secreted through the kidneys, and its accumulation in tubular tuberculosis is more than 5 times greater than other tissues. Zolpidem exhibits this effect by reducing oxidative stress, increasing the activity of the antioxidant system, including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), and preventing apoptosis in renal cells. The present study has approved, the antioxidant effect of zolpidem on the improvement of the defect of antioxidant system of the kidney in nephrotoxicity.
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Introduction

The cisplatin alkylating agent is one of the most widely used chemotherapy drugs for treating a variety of cancers including lung, testicular, ovarian, breast, head, neck and bladder (1,2). This drug stops the replication and transcription of the genetic material and ultimately inhibits the growth and proliferation of cancer cells by binding to DNA and proteins (1,3,4). It also increases the oxidative stress by stimulating the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), causing damage to subcellular structures and macromolecules such as DNA, proteins and lipids (5,6). Cisplatin is mainly secreted through the kidneys, and its accumulation in tubular tuberculosis is more than 5 times greater than other tissues (7,8). Previous studies have indicated that the clinical use of cisplatin in 25%-35% of

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hospitalized patients undergoing chemotherapy leads to nephrotoxicity, which limits the dose and duration of use of the drug (9). In many studies, the mechanism of the occurrence of this complication includes the induction of free radicals' generation, oxidative damage, and lipid peroxidation in the kidney (10-12). Therefore, researchers have evaluated the role of antioxidant agents in improving renal injury caused by cisplatin. Zolpidem is a nonspecific hypnotic drug that has antioxidant and neuroprotective properties (13). Studies have shown that zolpidem and its derivatives can trap free radicals, and reduce lipid peroxidation, protein oxidation and carbonyl formation (14). Nonetheless, its effect on kidney tissues has not been studied yet.

Objectives

In the present study, the antioxidant role of this substance in the improvement of cisplatin nephrotoxicity will be investigated.

Materials and Methods

This experimental study was performed on 40 adult male rats weighing 250-230 g purchased from Razi Herbal Medicines Research Center, Lorestan, Iran. Water and food were provided freely, and the rats were kept at a 12-hour light and 12-hour dark cycle at an appropriate temperature. Animals were randomly divided into four groups of ten rats before each experiment as follows;

Group 1: healthy group which did not receive any medication.

Group 2: control group which received cisplatin (2.5 mg/kg) daily for intraperitoneal injection for 3 weeks for the purpose of the induction of nephrotoxicity.

Group 3: nephrotoxicity group treated with zolpidem (2 mg/kg) for 11 days from the 10th day.

Group 4: nephrotoxicity group treated with zolpidem (4 mg/kg) for 11 days from the 10th day.

Evaluation of the renal structure and level of oxidative stress factors

In order to evaluate the renal structure and the level of oxidative stress factors such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) Malondialdehyde (MDA) on the last day (21st day), the first urine samples were prepared and the levels of creatinine, urea and the ratio of albumin to urine creatinine were measured by the use of commercial kits (prepared by Diasis Diagnostic System Istanbul/Turkey). Following the collection of urine samples, we anesthetized the animals using pentobarbital, and then, dorsal area of rats and subsequently the muscles were separated from the spinal column and finally the spinal column was removed completely. Then, the spinal cord was completely removed using distilled water injection into the spinal canal. After separating the spinal cord, it was lysed with buffer (Tris HCl, pH 7.5 Sucrose, 0.01 M 5 mM MgCl2, 1% Triton X100 0.3M) and eventually the supernatant was isolated in order to evaluate the oxidative stress level with ELISA kits (Sigma Aldrich, USA).

Kidney histology

After urine collection and spinal separation on the last day, the left kidneys were removed in order to evaluate kidney tissue. Subsequently, the paraffin blocks of samples were prepared and cut using a micrometer with a thickness of 3 μm. The samples were stained with H&E staining and then the severity of tissue necrosis and degeneration of the epithelial cells of the kidneys were assessed microscopically. (No damage = 0, mild = 1; unicellular patchy isolated, moderate = 2; damage less than 25%, severe = 3; damage between 25%-50%, very severe = 4; more than 50% damage).

Ethical issues

The research followed the tenets of the Declaration of Helsinki. The protocol of this study is designed in accordance with the ethical principles of the International Committees for the Protection of Animal Rights Laboratory. This project was approved by Ethics Committee of Lorestan University of Medical Sciences.

Statistical analysis

Appropriate central and diffusion indices were calculated for data analysis using one-way ANOVA or Kruskal-Wallis test. SPSS version 16 was also used for data analysis. The significance level was considered as $P<0.05$. Hormonal and enzymatic data were analyzed using GraphPad software and one-way ANOVA data.

Results

Effect of zolpidem on functional and structural parameters of the kidneys

According to our results, cisplatin administration causes impaired kidney function. This dysfunction was evaluated by measuring urine levels of urea, creatinine, and albumin/creatinine.

In all three cases, there was a significant increase in their levels in group 2 (control) compared to group 1 ($P<0.05$) (Figure 1A-C), in which the amount of albumin/creatinine ratio was doubled. The intraperitoneal injection of zolpidem to groups 3 and 4 resulted in a significant decrease in the values of functional parameters of the kidneys, in a dose-dependent manner, which means that with an increase in the injectable dose of zolpidem in group 4 in comparison with group 3, the level of urea, creatinine and albumin/creatinine ratio significantly decreased. The chart shows a slower rate of decrease in albumin/creatinine ratio evaluation compared to urea and creatinine.

Effect of zolpidem on oxidative stress and anti-oxidant system deficiency by cisplatin

Our results indicated that cisplatin significantly reduced the antioxidant enzymes of the kidney, including GPx,

SOD and CAT in group 2, and at the same time remarkably increased the MDA (oxidative stress marker). The administration of zolpidem in dose-dependent manner led to a significant increase in antioxidant enzymes in groups 3 and 4 ($P<0.05$) (Figure 1D-G). Furthermore, a comparing between the amount of MDA in groups 2 and 4 indicated that zolpidem could reduce the lipid peroxidation induced by cisplatin by half.

Effect of zolpidem on cisplatin-induced kidney tissue damage

Cisplatin causes apoptotic and necrotic death in kidney tubular cells. We clearly observed the cisplatin-induced renal injury in H&E stained tissue sections. Under the influence of zolpidem, this damage was reduced. Moreover, tubular death, necrosis and renal cell degeneration significantly decreased.

Discussion

The most important side effect of dose-dependent administration of cisplatin is nephrotoxicity (2). This drug has been associated with the induction of multiple signaling factors and pathways, including one of the most important mechanisms for stimulating oxidative

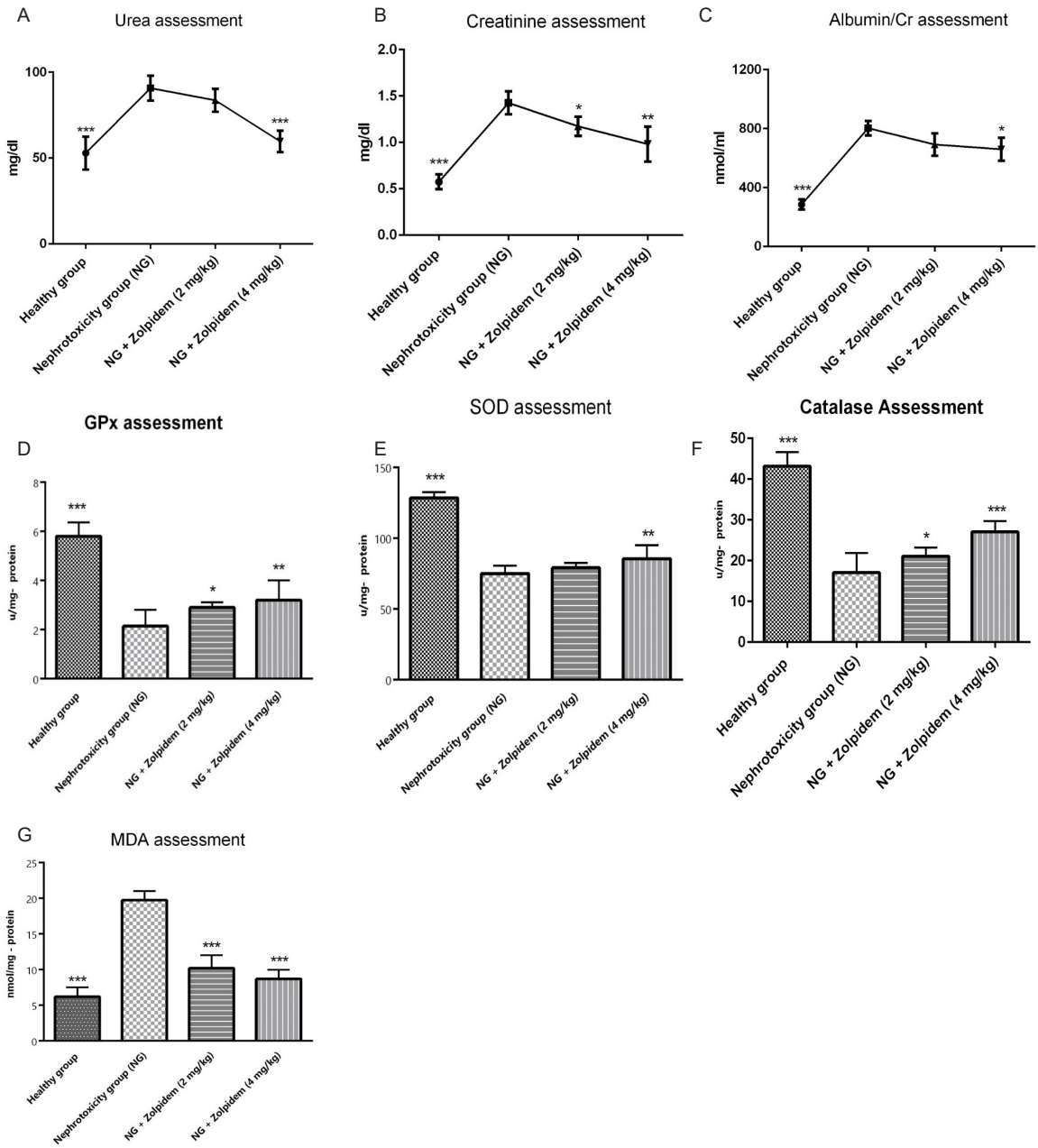

Figure 1. The effects of zolpidem on the level of (**A**) urea, (**B**) creatinine, (**C**) albumin/creatinine ratio, (**D**) GPx, (**E**) SOD, (**F**) CAT and (**G**) MDA tests in different groups. * $P<0.05$, ** $P<0.01$, *** $P<0.001$ versus NG.

stress, reducing or malfunctioning of antioxidant agents, and increasing the production of free radicals (9,12,15). Following these disorders, apoptosis and necrosis of the tubular cells will occur (16-18), which will, in turn, change various parameters of the kidney including urea, creatinine, MDA, CAT, GPx, and SOD (19-22). We also observed urine level changes of these parameters in group 2. To date, the only strategy used to limit nephrotoxicity is the hydration of patients during the course of treatment (23). However, some complications are relatively irreversible, and defects in tubular renewal capacity can lead to the onset and spread of kidney fibrosis (16). Hence, there is an urgent need to develop methods to prevent or treat this complication. Cisplatin, by binding to the mitochondrial DNA and causing impairment in its transcription, reduces the synthesis of proteins and subsequently reduces the components of the electron transport chain. Consequently, oxidative phosphorylation has a defect that ultimately stimulates ROS production (24). Nephrotoxicity caused by cisplatin is induced by oxidative stress (25). Many studies, including the present research, have indicated the oxidative damage and production of free radicals in the kidney brought about by cisplatin (10,11). These factors can alter the structure of cellular macromolecules, including DNA, proteins and lipids, and can also lead to cell death (16). Regarding the mechanisms of cisplatin in the development of nephrotoxicity, several studies have investigated the role of various bioactive compounds with antioxidant properties in protecting the kidneys against the damage caused by oxidative cisplatin effects. Substances with antioxidant properties, such as daidzein in soybeans (3), fisetin (2), green tea (26), honey (27), ferulic acid (1), ellagic acid (19) and other antioxidants, have increased the levels of antioxidant system of the kidney tissues that greatly reduces nephrotoxicity. The results of this study confirmed a significant positive effect of zolpidem on the improvement of renal damage caused by cisplatin. The zolpidem imitates its antioxidant property by having a structure similar to melatonin (13,14). According to our results, zolpidem exhibits this effect by reducing oxidative stress, increasing the activity of the antioxidant system, including SOD, CAT and GPx, and preventing apoptosis in renal cells. This feature was confirmed by measuring the amount of MDA in tissue in 2004, which indicated the inhibitory effect of zolpidem on the induction of lipid peroxidation in the liver and brain of rats (13).

Conclusion

The present study has approved, for the first time, the antioxidant effect of zolpidem on the improvement of the defect of antioxidant system of the kidney in nephrotoxicity. Based on the results, pre-treatment with zolpidem causes the balance of the oxidant/antioxidant system to improve the function of the tubular cells of the kidneys. Hence, there will occur the adjusting of the urine level of its functional indices. Adjustment of the balance of the production of ROS and antioxidant defense enzymes is essential in order to control or reduce the nephrotoxicity caused by cisplatin. Further studies are recommended to explore other mechanisms involved in the improvement process and how they are induced by zolpidem.

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Authors' contribution

PN, AF, SM, PAB, MG, RMK and AK conducted the research. AH designed and supervised the study, analyzed the data and prepared the final draft of the article.

Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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