Sex Differences in Protective Effect of Recombinant Human Erythropoietin Against Cisplatin-induced Nephrotoxicity in Rats

Fatemeh Eshraghi-Jazi,[1] Mehdi Nematbakhsh,[1,2,3]
Zahra Pezeshki,[1] Hamid Nasri,[1,3] Ardeshir Talebi,[1,4] Tahereh Safari,[1,2]
Azam Mansouri,[1] Safoora Mazaheri,[1] Farzaneh Ashrafi[1,5]

[1]Water and Electrolytes Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[2]Department of Physiology, Isfahan University of Medical Sciences, Isfahan, Iran
[3]Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
[4]Department of Clinical Pathology, Isfahan University of Medical Sciences, Isfahan, Iran
[5]Department of Internal Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

Introduction. The protective role of recombinant human erythropoietin (RHE) against cisplatin-induced nephrotoxicity has been reported, but the role of sex differences is not clearly known. The aim of this study was to determine the sex-based difference in the protective effect of RHE against cisplatin-induced nephrotoxicity. **Materials and Methods.** Thirty-three Wistar rats were divided into 6 groups. According to protocol l, male and female rats were treated with RHE (100 IU/kg/d) for 3 days and then received a single dose of cisplatin (7 mg/kg). According to protocol 2, the rats received the same single dose of cisplatin and then were treated with RHE for 7 days. Two other groups of male and female rats received a similar regimen of protocol 2 except for saline instead of RHE. All the animals were sacrificed 1 week after cisplatin administration. **Results.** All of the experimental animals experienced weight loss. The percentage change of weight in male rats with protocol 1 was significantly less than that in male rats in protocol 2 and control groups. However, in female groups, the percentage of change in weights was slightly higher with protocol 2 than with protocol 1 and control treatment. Administration of RHE significantly decreased changes in serum creatinine, BUN, and malondialdehyde levels in male rats, but not in females. No significant difference was observed in serum nitrite level, kidney weight, and kidney damage score between the groups.
Conclusions. This study suggested that erythropoietin may lead to different responses against cisplatin-induced nephrotoxicity in male and female rats.

Original Paper

INTRODUCTION

Erythropoietin, as a growth factor, is essential for cell proliferation and angiogenesis. Recombinant human erythropoietin (RHE) is widely used for treatment of anemia in patients with end-stage renal disease and those with a kidney allograft,[1-3] and to protect renal tissue against reperfusion injury.[4] Nephrotoxicity on the other hand, is one of the most important side effects of antitumor drugs such as cisplatin, and is characterized by reduction in glomerular filtration rate and renal blood flow.[5-10] Different antioxidants such as vitamins E and C were used in animal experiment to prevent nephrotoxicity after cisplatin administration,[11-15] and the protective effect of RHE against cisplatin-induced nephrotoxicity in female rats was reported

previously.[16] In addition, RHE ameliorates cisplatin-induced kidney dysfunction in male rats.[17]

The role of sex differences in kidney function and circulation has been subjected to new research projects.[18-20] Clinical studies have also shown that progress of kidney disease is more rapid in men than women,[21,22] and male animals are experimentally more vulnerable than females against cyclacillin-induced and chloroform-induced kidney damage.[23,24] Likewise, sex-related factors play an important role in the effectiveness of erythropoietin in reperfusion-induced renal injury in rats.[25]

Nematbakhsh and coworkers showed evidence for sex-based differences in cisplatin-induced nephrotoxicity,[26] while the different nephroprotective effects of L-arginine and losartan were reported by others in male and female rats against cisplatin toxicity.[27,28] Cisplatin also increases urinary sodium excretion in animal model, which was reported to be sex related.[29] The nephroprotective effect of RHE against cisplatin was reported widely by others in animal models,[17,30-33] but no report is found related to its sex-based differences. Therefore, the main objective of in this study was to show that whether sex differences can modulate the protective role of RHE in cisplatin-induced nephrotoxicity in rats.

MATERIALS AND METHODS
Animals

Seventeen adult female Wistar rats (mean weight, 162.0 ± 2.6 g) and 16 male Wistar arts (mena weight, 188.0 ± 6.3 g) were obtained from the animal center of Isfahan University of Medical Sciences, Isfahan, Iran. The animals were housed under standard conditions with 12-hour light and 12-hour dark cycles and were given ad libitum access to water and food. The experiments were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences.

Experimental Design

Blood samples were obtained from the animals, and then the animals were randomly divided into the following groups: groups 1 and 2 (5 males and 6 females) were treated by intraperitoneal RHE (100 IU/kg/d) for 3 days and then received a single dose of intraperitoneal cisplatin, 7 mg/kg (protocol 1). On day 7, after cisplatin administration, blood samples were obtained from the animals, and then they were sacrificed by overdose of anesthetic drugs. Groups 3 and 4 (5 males and 7 females) received a single dose of cisplatin (7 mg/kg) and then were treated by RHE (100 IU/kg/d) for 7 days. On day 7, blood samples were obtained from these animals and then they were sacrificed by overdose of anesthetic drugs (protocol 2). Groups 5 and 6 (6 males and 4 females) received a regimen similar to groups 3 and 4 except for saline instead of RHE. Cisplatin (cis-Diammineplatinum (II) dichloride, code P4394) and RHE were purchased from Sigma (St Louis, MO, USA) and Janssen-Cilag (Prague, Czech Republic), respectively.

The body weight of animals was recorded on a daily basis. When the animals were sacrificed, the kidneys were immediately removed and weighed for histopathological investigations.

Measurements

Serum creatinine and blood urea nitrogen (BUN) levels were determined using quantitative kits (Pars Azmoon, Tehran, Iran). Serum level of nitrite (stable nitrite metabolite) was measured using a colorimetric assay kit (Promega Corporation, USA). Serum malondialdehyde level was quantified according to the manual method. Briefly, 500 μL of the serum sample was mixed with 1000 μL trichloroacetic acid 10%. The mixture was vigorously shaken and centrifuged at 2000 g for 10 minutes; 500 μL of the supernatant was added to 500 μL thiobarbituric acid 0.67%. Then, the solution was incubated for 10 minutes in warm water bath at the temperature of 100°C. After cooling, the absorbance was measured at 532 nm. Concentration of malondialdehyde was reported in μmol/L. For all measurements, the difference between concentrations 7 days after cisplatin administration and before cisplatin injection) were calculated and compared between the groups.

Histopathological Procedures

The removed kidneys were fixed in 10% formalin solution, embedded in paraffin for histopathological staining. Hematoxylin and eosin staining was applied to examine the tubular injury. Presence of acute tubular damage such as tubular dilation and simplification, tubular cell swelling and necrosis, tubular casts and intra luminal cell debris with inflammatory cell infiltration were considered. The intensity of tubular lesions as mentioned above, were

scored from 1 to 4, while score zero was assigned to normal tissue without damage (Table 1).

Statistical Analysis

Data were presented as mean ± standard error. To compare the weight change among groups, repeated measures analysis was applied. The groups were compared with regard to changes in BUN; serum creatinine, malondialdehyde, and nitrite levels; and kidney weight, using the 1-way analysis of variance, followed by the LSD test. The Mann-Whitney and Kruskal-Wallis tests were employed to compare the pathological damage

scores among the groups. P values less than .05 were considered significant.

RESULTS

All of the experimental animals experienced weight loss. The percentage change of weight in group 3 was significantly less than that in groups 1 and 5 ($P < .05$). However, in female groups, the percentage of change in weights in group 4 was slightly higher than that in groups 2 and 6 ($P < .1$, Figure 1).

Administration of RHE significantly decreased changes in serum creatinine, BUN, and malondialdehyde levels in male rats ($P < .05$), but not in females (Figure 2 and Table 2). No significant difference was observed in serum nitrite level, kidney weight, and kidney damage score between the groups (Table 2 and Figure 3).

Table 1. Pathologic Scoring for Kidney Tissue Damage

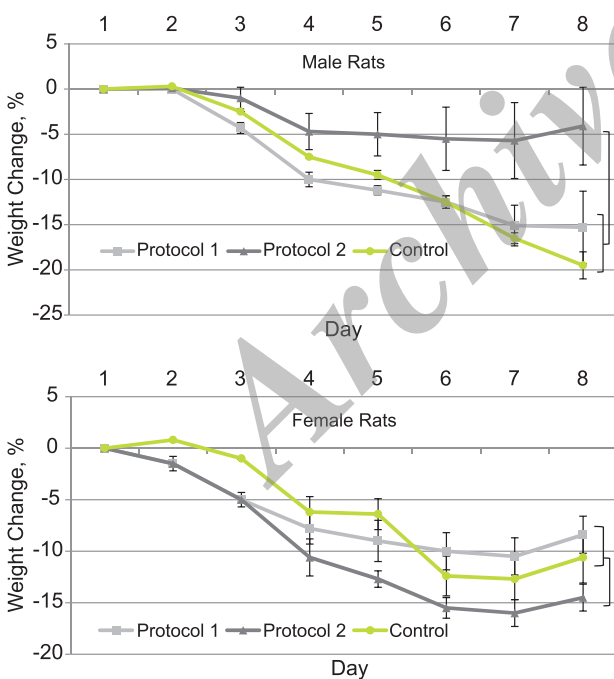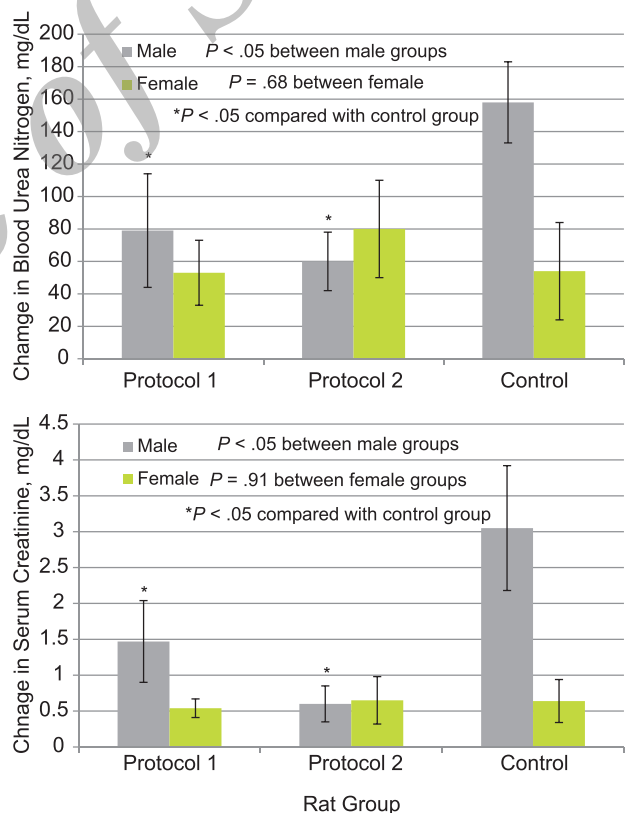| Score	Kidney Tissue Condition	Intensity of Tissue Damage
0	Normal	No tissue damage
1	Low damage	Up to 25% of tissue damage
2	Mild damage	Between 26 to 50% of tissue damage
3	Moderate damage	Between 51 to 75% of tissue damage
4	Sever damage	More than 75% of tissue damage

Figure 1. Percentage of change in animals' body weight after cisplatin injection in all experimental groups. Protocol 1 was treatment with recombinant human erythropoietin (100 IU/kg/d) for 3 days and then a single dose of cisplatin (7 mg/kg) in two groups of male and female rats. Protocol 2 was treatment with the same single dose of cisplatin, preceding administration of recombinant human erythropoietin (100 IU/kg/d) for 7 days. Rats in the control group received cisplatin and saline (vehicle).

Figure 2. Percentage of changes in serum creatinine, and blood urea nitrogen levels after cisplatin injection in all experimental groups. Protocol 1 was treatment with recombinant human erythropoietin (100 IU/kg/d) for 3 days and then a single dose of cisplatin (7 mg/kg) in two groups of male and female rats. Protocol 2 was treatment with the same single dose of cisplatin, preceding administration of recombinant human erythropoietin (100 IU/kg/d) for 7 days. Rats in the control group received cisplatin and saline (vehicle).

Table 2. Mean Changes in Kidney Markers and Pathology Features 7 Days After Cisplatin Administration*

Mean Change in Parameter	Male Rats			Female Rats		
	Protocol 1	Protocol 2	Control	Protocol 1	Protocol 2	Control
Malondialdehyde, μmol/ L	6.76 ± 2.60†	-0.91 ± 0.88†	25.44 ± 12.56	5.42 ± 4.03	1.34 ± 3.51	0 ± 8.04
Nitrite, μmol/ L	22.63 ± 11.79	16.79 ± 8.67	19.59 ± 6.23	24.28 ± 15.20	20.68 ± 10.68	-5.08 ± 13.25
Kidney weight, g/100 g BW	1.11 ± 0.01	1.00 ± 0.10	1.14 ± 0.07	1.15 ± 0.01	1.16 ± 0.08	1.03 ± 0.06
Damage score	2.60 ± 0.29	2.00 ± 0.42	1.91 ± 0.23	2.41 ± 0.08	2.42 ± 0.17	1.75 ± 0.32

*Protocol 1 was treatment with recombinant human erythropoietin (100 IU/kg/d) for 3 days and then a single dose of cisplatin (7 mg/kg) in two groups of male and female rats. Protocol 2 was treatment with the same single dose of cisplatin, preceding administration of recombinant human erythropoietin (100 IU/kg/d) for 7 days. Rats in the control group received cisplatin and saline (vehicle).
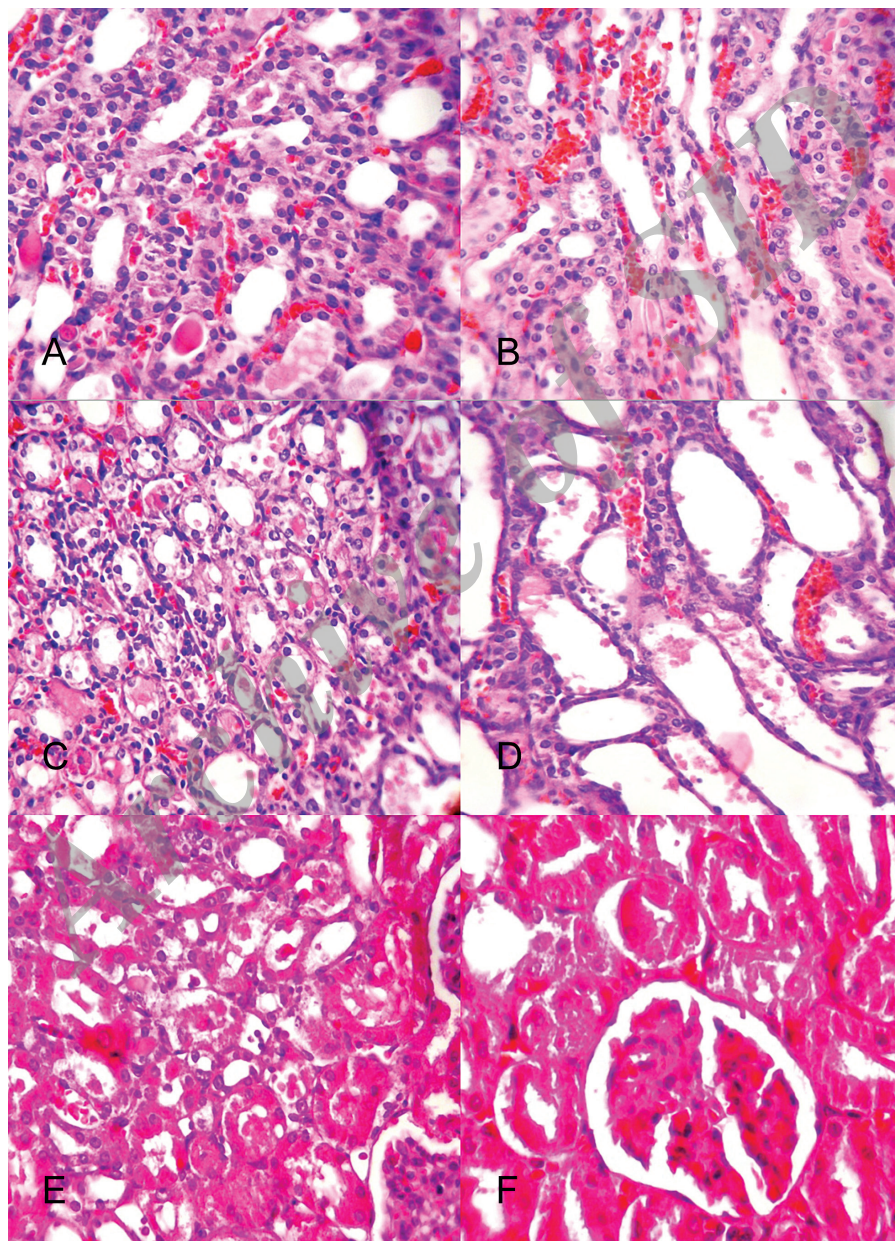†Significantly different from control (P < .05)

Figure 3. Kidney tissues in male and female rats. **A** and **B**, specimens from male (left) and female (right) rats in the protocols 1 groups. **C** and **D**, specimens from male (left) and female (right) rats in the protocol 2 groups. **E** and **F**, specimens from male (left) and female (right) rats in the control groups. The tissue damage in male rats is more severe than that in their female counterparts for the protocol 1 and control pair of groups. On the contrary, the tissue damage in female rats on protocol 2 is more than that in the male rats. According to the mean pathological damage score (Table 1), no significant differences were detected (hematoxylin-eosin, × 100).

DISCUSSION

We investigated the role of sex of the rats in protective effect of RHE against cisplatin-induced nephrotoxicity in rats. Recombinant human erythropoietin ameliorated the percentage change in body weight induced by cisplatin administration in the treated male group (protocol 1) in comparison with other male groups, whereas this observation was reversed indicating more weight loss in females treated according to protocol 2. The weight loss difference in male and female rats treated with cisplatin were demonstrated in our previous studies,[26-28] while some reports indicated that cisplatin reduced body weight due to gastrointestinal disturbances.[34-36] Disturbance of renal function by cisplatin may also be the other reason for body weight loss caused by tubular damage.

Administration of cisplatin disturbs kidney function by increasing serum levels of creatinine, BUN, and malondialdehyde in male animals. Such findings were not obtained for female animals. These results in male rats were in agreement with other studies.[17,31,33] It is documented that oxidative stress is one of the major mechanisms involving cisplatin-induced nephrotoxicity,[33] which leads to production of free radicals and lipid peroxidation in tubular cells.[37] In this study, erythropoietin (especially when administered according to protocol 2) ameliorated serum creatinine, BUN, and malondialdehyde levels in the male groups. In agreement with our study, Rjiba-Touati and coworkers reported that administration of erythropoietin (as pre-, co-, or post-administration with regard to cisplatin) improved cisplatin-induced kidney dysfunction.[33] Likewise, other studies reported results similar to ours in male animals.[17,30,31] The effect of RHE in reducing the BUN, serum creatinine, and malondialdehyde concentrations increased by cisplatin could be related to antioxidant properties, as RHE acts as a general radical scavenger during nephrotoxin-induced nephrotoxicity.[38] Recombinant human erythropoietin attributes in activation of antioxidant enzymes and acts as an inhibitor of nitric oxide production and decreases lipid peroxidation.[39,40] Moreover, it has anti-inflammatory properties and reduces expression of pro-inflammatory mediators,[1] activates anti-apoptotic pathways,[41] modulates expression of anti-apoptotic proteins such as Bcl-2,

and decreases cell death in tubular epithelium.[42] Vesey and colleagues suggested that erythropoietin prevents apoptotic cell death, increases tubular epithelial regeneration, and elicits renal functional recovery in hypoxic or ischemic models.[4]

To consider the female gender, our results did not show significant different in serum levels of BUN, serum creatinine, and malondialdehyde between the groups. Our findings are consistent with the data reported in other studies. Yalcin and colleagues showed that administration of RHE does not affect the serum levels of creatinine and BUN in female albino rats.[16] Also, Prokai and colleagues indicated that administration of erythropoietin diminishes post-ischemic renal failure that is indicated by lower BUN and serum creatinine levels in male animals, but not in female animals.[25] This sex difference in erythropoietin effect may not be related to erythrocyte response to erythropoietin,[43] but possibly it may be related to sex-based differences in kidney function and circulation,[44,45] which is influenced by rennin-angiotensin system[28] or sex differences in renal brush border membrane binding affinity for certain drugs.[46]

We had some limitations for this study. First, gastrointestinal problem with toxic drug that cause poor appetite is a confounding factor for this study. Second, we did not hydrate the animals, but since all groups received cisplatin, the possible dehydration was similar for all groups, although the animals had free access to water and food. Third, we did not control sexual cycle in female animals and also we did not detect serum level of sex hormones.

Results of the present study did not show significant differences in serum level of nitrite, kidney tissue damage score, and kidney weight between the groups. In contrast with our results, Prokai and coworkers demonstrated that in kidney reperfusion injury, erythropoietin improved pathological parameters in both sexes without sex differences.[25] The concentration of erythropoietin is higher in males,[25,47] and males are more susceptible to renal damage.[23,24]

CONCLUSIONS

We conclude that erythropoietin ameliorates nephrotoxicity induced by cisplatin in male animals, but not in females, possibly due to sex-

based differences in renal circulation and rennin-angiotensin system.[20,28]

CONFLICT OF INTEREST

None declared.

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Correspondence to:
Mehdi Nematbakhsh, MD
Water and Electrolytes Research Center, Kidney Diseases Research Center, Department of Physiology, Isfahan University of Medical Science, Isfahan, Iran
E-mail: nematbakhsh@med.mui.ac.ir