Original Article

Protective Effect of Zinc Supplementation on Renal Ischemia/Reperfusion Injury in Rat: Gender-related Difference

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Abstract

Background: Zinc (Zn) knows as essential microelement which prevents oxidative stress. The effect of Zn supplement on renal function parameters in rats subjected to renal ischemia-reperfusion (IR) injury was investigated. **Methods:** Male and female rats were subjected to renal IR with and without Zn sulfate (10 mg/kg/day for 5 days) supplementation. The kidney function markers and histology findings in Zn-treated group were compared with sham and control groups. **Results:** The serum levels of blood urea nitrogen and creatinine (Cr) and kidney tissue damage score were increased significantly after renal injury ($P < 0.05$) gender dependently, but no alterations were observed for these markers in Zn-treated animals after renal IR injury. Cr clearance was significantly different between genders ($P < 0.05$); however, Zn supplementation increased the Cr clearance and kidney nitrite level significantly in male rats ($P < 0.05$). Zn also increased urine flow in female ($P < 0.05$), but it did not alter urine load of Na ($U_{Na}V$) and percentage of Na excretion (E_{Na}%). **Conclusions:** Zn may improve renal function after IR injury gender dependently.

Keywords: *Gender, rats, renal ischemia-reperfusion, zinc*

Introduction

Kidney ischemia is the most common disturbance in clinic which is accompanied with acute renal failure (ARF). ARF may occur in condition of transplantation, renal vascular surgery, and shock.[1] The membrane injury through production of oxygen-free radicals is the side effect of ischemia-reperfusion (IR) injury.[2] It is also demonstrated that gender is an important parameter in pathophysiology of ARF and inflammatory processes in the kidney.[3] It is known that male was more susceptible than female in acute renal injury in mice.[4] Due to the formation of stress oxidative during IR injury, usually, antioxidant agents' potentially are candidate to protect the kidney against IR injury. Previously, we reported that the agents such as Γ-aminobutyric acid, diminazene aceturate, and estrogen as antioxidants could protect the kidney against IR injury.[5-7] Trace elements play an important role in body metabolism, and some of them perform antioxidant properties. Among them, zinc (Zn) is a trace element and antioxidant prevents oxidative stress. Zn is known as essential microelement for growth, metabolism, and it is active part of superoxide dismutase.[8] The protective effect of Zn against IR injury in some organs such as spinal cord and myocardium was reported.[9,10] The antioxidant effect of Zn mediated through the induction of metallothionein.[11] The protective role of Zn and its antioxidants effect on renal IR injury was investigated before;[11-13] however, the role of gender was not reported. It is proposed that the protective role of Zn against renal IR is gender related, and this study was designed to investigate the effect of Zn supplement on renal function parameters in male and female rats subjected to renal IR injury.

Methods

Forty-eight adult male and female rats (180 ± 10 g) were randomized into six groups. All experiments were approved by the Isfahan University of Medical Sciences' Ethics Committee.

Groups 1 and 2: male (n = 8) and female (n = 8) rats as sham groups were anesthetized with chloral hydrate injection (450 mg/kg; ip) and subjected to surgery without renal IR injury. Groups 3 and 4: male (n = 8) and female (n = 8) rats as control groups received vehicle (saline) for 5 consecutive days, and 2-h post

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last injection, the rats were anesthetized and subjected to surgery and renal IR injury. The renal IR injury was induced by clamping both left and right kidney vascular for 45 min followed by 24-h reperfusion. Groups 5 and 6: male (n = 8) and female (n = 5) rats as Zn groups received the same regimen and procedures as Groups 3 and 4, respectively, except Zn sulfate (10 mg/kg/day; ip) instead vehicle. Next day after surgery (18 h after reperfusion), all animals were kept in metabolic cage for next 6 h for urine collection.

Finally, blood samples were obtained through heart puncture and the animal was sacrificed humanly. The left kidney was fixed in 10% formalin solution and stained (H and E method). Kidney tissue damage score (KTDS) was graded from 1 to 4 while score 0 was assigned to normal kidney tissue. The right kidney was homogenized and centrifuged. The levels of serum creatinine (Cr), blood urea nitrogen (BUN), and urine Cr were determined using quantitative diagnostic kits (Pars Azmoon, Iran). The serum and kidney levels of nitrite (stable nitric oxide metabolite) were measured using Griess method. The levels of sodium (Na) in serum and urine were measured using flame photometer assay.

Statistical analysis

Data were presented as the mean ± standard error of mean. Comparison between the groups in each gender was tested by ANOVA and Tukey test. Comparison between each parameter in two genders was done by independent sample Student's t-test. KTDS between the groups was compared by Kruskal–Wallis or Mann–Whitney test. $P \leq 0.05$ indicates a statistically significant.

Results

The results indicated that after renal IR injury (24-h postreperfusion), the serum levels of BUN and Cr and KTDS were increased significantly ($P < 0.05$) in both male and female rats while these increased parameters were statistically different between genders ($P < 0.05$) [Figure 1]. In addition, no alterations were observed for the serum levels of BUN and Cr and KTDS in Zn-treated animals, after renal IR injury. Cr clearance was significantly different between male and female in all the groups ($P < 0.05$); however, Zn supplementation increased the Cr clearance and kidney nitrite level significantly in male rats at 24-h postreperfusion ($P < 0.05$) when compared with control and sham groups, and such observation was not seen in

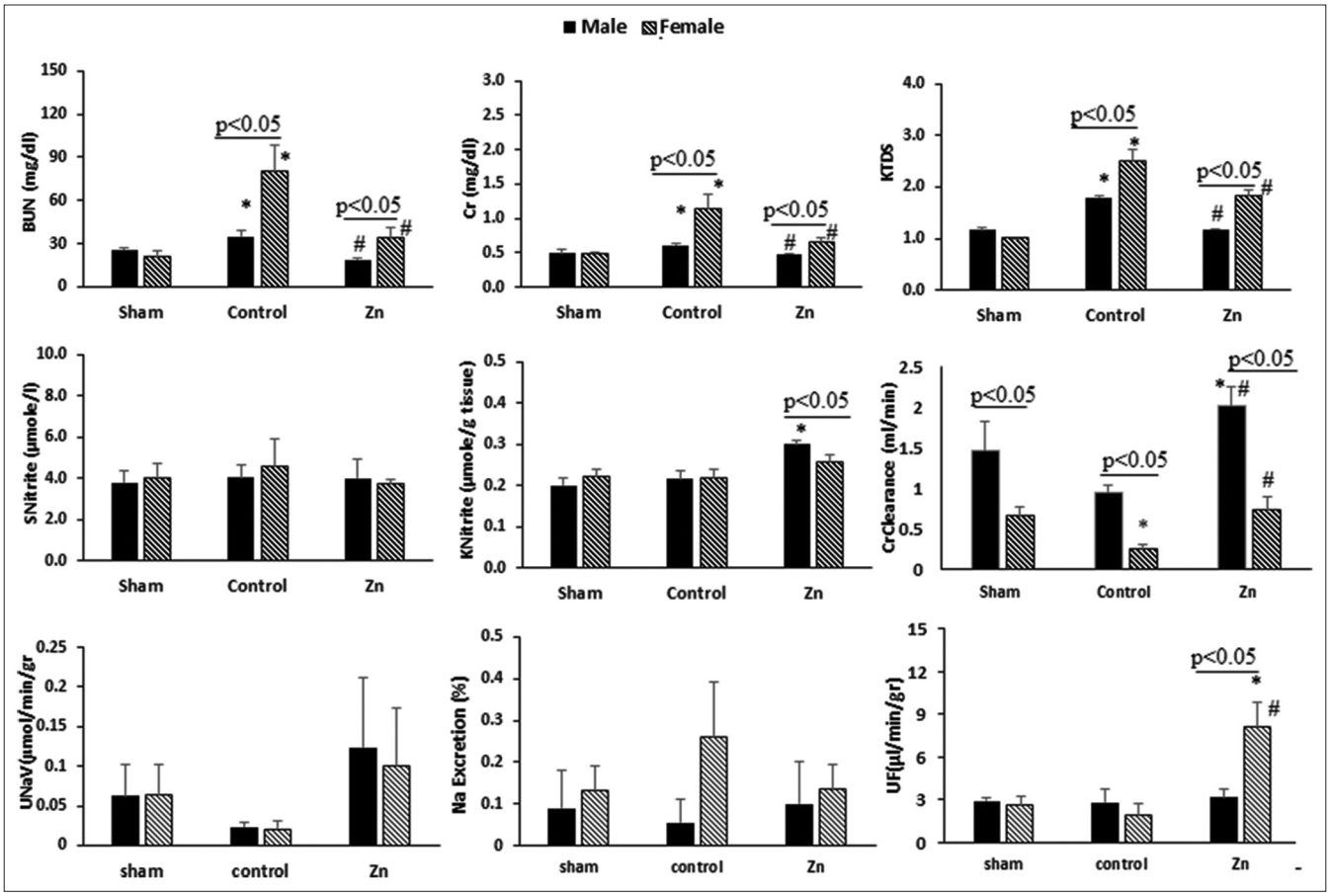

Figure 1: The serum levels of blood urea nitrogen and creatinine and creatinine clearance, urine flow, sodium urinary output per g kidney tissue ($U_{Na}V$), and percentage of sodium excretion (E_{Na} %) in experimental groups. The star (*) indicates a significant difference from other groups in the same gender ($P < 0.05$). The sign (#) shows a significant difference from control group ($P < 0.05$)

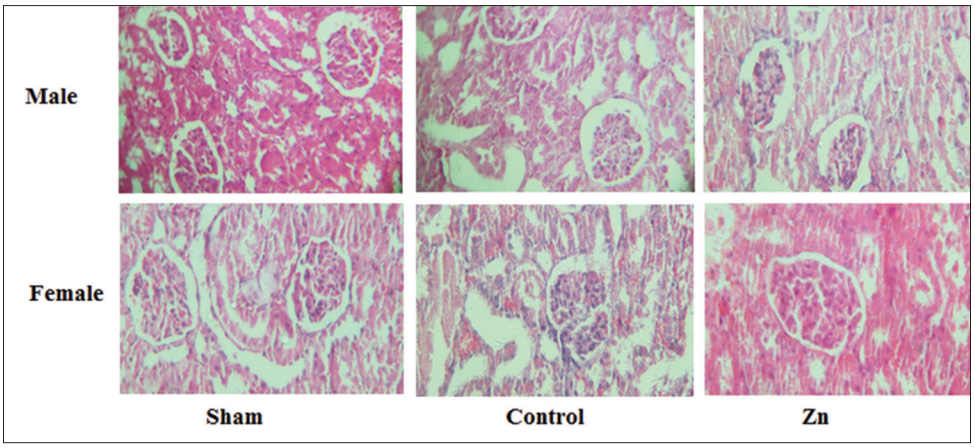

Figure 2: The tissue images of kidney for all the groups of experiment. More damages were seen in control groups

female rats [Figure 1]. On the other hand, Zn increased urine flow (UF) significantly in female at 24-h postrenal IR injury ($P < 0.05$), but it did not alter urine load of Na ($U_{Na}V$) and percentage of Na excretion ($E_{Na}\%$). Tissue nitrite level increased in male Zn group compared to the other groups in the same gender while such observation was not seen in female. The UF in female control group showed significant difference ($P < 0.05$) when compared with female sham and Zn groups. The tissues images of kidney for all the groups of experiment are demonstrated in Figure 2.

Discussion

The present study showed that renal IR injury decreased renal function in male and female rats due to increase of serum BUN and Cr levels, KTDS and decrease of Cr clearance. However, administration of Zn improved renal function in both genders. Zn alteration was reported in hemodialysis patients,[14] and it may prevent the progression of the injury and improves the healing process in injured testes in rats by its antioxidant effect.[15] It is observed that patients with chronic failure have lower level of serum Zn.[16,17] Some studies also showed that Zn had protective effect on renal IR injury.[11,18,19]

Yilmaz and Mogulkoc indicated that supplementation of Zn and melatonin causes a protective effect in renal IR by inhibiting the oxidant systems and activating the antioxidant system in the kidney.[12] Zn also inhibits apoptosis and plays a protective role on renal IR injury possibly due to decreasing of oxidative stress and reducing caspase-3 activity.[17]

Regarding the gender and based on our data, it seems that Zn is more protective in male than female rats against renal IR injury because the serum levels of BUN and Cr and KTDS were lower, and Cr clearance and tissue level of nitrite were higher by Zn in male than female. One reason may be related to sex hormones. Sexual hormones were reported to be protected the kidney against IR injury.[7,20]

Conclusions

According to our finding, Zn may improve renal function after IR injury, possibly to its antioxidant effects as previously reported,[6,7,11,18,19] and its protective role is gender related due to sex hormone and maybe other unknown factors.

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Conflicts of interest

There are no conflicts of interest.

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