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The protective roles of zinc and estradiol in renal ischemia/reperfusion injury in ovariectomized rats

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ABSTRACT

Background: One of the main events associated with ischemia/reperfusion injury (IRI) is excessive production of reactive oxygen species (ROS). Zinc (Zn) and estradiol are considered as antioxidants that scavenge free radicals.
Objectives: The aim of this study was to compare the protective effect of Zn and estradiol against renal IRI in ovariectomized rats.
Materials and Methods: Ovariectomized Wistar rats were randomly divided into five experimental groups including control (sham operated), IRI, estradiol treated +IRI, estradiol and Zn treated +IRI, Zn treated +IRI groups. The IRI was induced by clamping renal vessels for 45 minutes followed by 24 hours reperfusion. During the last 6 hours of reperfusion, urine output was collected and the measurements were performed.
Results: IRI caused an increase in kidney tissue damage score (KTDS) significantly ($P<0.05$). The serum levels of blood urea nitrogen (BUN) and the creatinine (Cr) also elevated by IRI, but theses parameters attenuated by Zn treatment significantly ($P<0.05$). Cr-clearance and urine flow were increased by Zn, and the percent of sodium excretion was increased by estradiol significantly ($P<0.05$). The kidney tissue level of malondialdehyde was decreased by co-treatment of Zn and estradiol statistically ($P<0.05$).
Conclusions: Zn protected the kidney against IRI with an evident improvements of serum BUN and Cr levels, Cr clearance and tissue damage.

Implication for health policy/practice/research/medical education:
Administration of zinc (Zn) as an antioxidant agent demonstrated an efficient role in preventing renal dysfunction induced by renal ischemia/reperfusion injury (IRI) in female rats. It seems the overproduction of reactive oxygen species (ROS) during renal IRI may inhibit by Zn; however more studies may clarify the exact mechanism.
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1. Background

Ischemia/reperfusion injury (IRI) is un avoidable in kidney transplantation that results acute kidney injury (AKI) (1). The pathogenesis of IRI induced AKI may aggravate by many factors such as reactive oxygen species (ROS), neutrophil infiltration, vasoactive peptides and ATP depletion (2). IRI can lead to an imbalance between the productions of oxygen free radicals (ROS) and antioxidant (3), and produce kidney inflammation (4). It is also known that sex hormones play an important role in the inflammatory process. Pretreatment with antioxidants almost protects kidneys against IRI (5), and it may reduce apoptosis and lipid peroxidation by pre-ischemic activation of adenosine receptors (6). Zinc (Zn) as an antioxidant is one of the most important elements in physiological processes while it is reported that Zn deficient diet enhances antioxidant capacity and decreases lipid peroxidation in rats damaged tissues (7,8).

2. Objectives

This study was planned to investigate the effects of Zn and estradiol (Es) on renal IRI in ovariectomized rats.

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Original Article

3. Materials and Methods

3.1. Ovariectomy

Thirty-two adult female (150 ± 5.2 g) Wistar rats (Animal Center, Isfahan University of Medical Sciences, Isfahan, Iran) were used in this study.

The rats were anesthetized, (ketamine 75 mg/kg, i.p. and xylazine 10 mg/kg, i.p.), and an incision was made in the abdominal middle line above the urinary outlet, and the ovaries were removed.

3.2. Experimental protocol

The five experimental groups were assigned as following: animals were randomly divided into;

Group 1 (n = 8, control group): rats received sesame oil intramuscularly during the study, and underwent the surgical procedure without IRI induction.

Group 2 (n = 6, IRI group): rats were treated as group 1, and underwent IRI induction.

Group 3 (n = 6, IRI +Es group); rats received single dose of estradiol (250 µg/kg dissolved in sesame oil) intramuscularly and 5 days later underwent IRI induction.

Groups 4 (n = 7, IRI+Es+Zn); rats received single dose of estradiol (250 µg/kg; dissolved in sesame oil,) intramuscularly plus Zn (10 mg/kg/d for 5 days), and underwent IRI induction.

Groups 5 (n = 5, IRI+Zn group); rats received sesame oil and Zn (10 mg/kg/d for 5 days) and underwent IRI.

3.3. Renal ischemia-reperfusion injury

The animals were anesthetized by chloral hydrate. Two small incisions were made on the flanks and the kidneys vessels were clamped to induce renal ischemia. After 45 minutes the clamp was removed to recirculate renal blood flow. Around 18 hours later, the animals put in metabolic cages to collect the urine for during next 6 hours. Therefore 24 hours post-IRI, blood samples were obtained via heart puncture. Serum samples were removed and stored at −20°C until measurement. Finally after sacrificing the animals the kidneys and uterus were removed and weighed immediately. The left kidney was fixed in 10% formalin solution for pathological assessments, and the right one was homogenized and centrifuged.

3.4. Measurements

The levels of creatinine (Cr), blood urea nitrogen (BUN) and urine Cr were measured by commercial kit (Pars Azmoon, Iran). Assessments of malondialdehyde (MDA) level in the serum and kidney tissue were performed by the manual method using10% trichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA). The serum and kidney level of nitrite as a nitric oxide (NO) metabolite were measured using Griess method, and the levels of sodium (Na) in serum and urine were measured using flame photometer assay.

3.5. Histopathological procedures

The kidneys were fixed in 10% neutral formalin solution and embedded in paraffin. The tissue sections were stained with H&E and examined. Kidney tissue damage score (KTDS) was graded from 1 to 4, while score zero was assigned to normal tissue.

3.6. Ethical issues

This project was approved by Ethics Committee of Isfahan University of Medical. Prior to the experiment, the protocols were confirmed to be in accordance with the guidelines of Animal Ethics Committee of Isfahan University of Medical Sciences (code# IR.MUI.REC.1394.2.236).

3.7. Statistical analysis

Data were reported as mean ± SEM. To compare the parameters between the groups, one-way analysis of variance (ANOVA) followed by LSD as post hoc was applied. The Kruskal-Wallis or Mann-Whitney U tests was applied to compare KTDS between the groups. Values of $P \leq 0.05$ were considered statistically significant.

4. Results

4.1. Effect of ischemia-reperfusion injury

The serum concentrations of BUN and Cr, and urine flow (UF) rate and KTDS increased, and Cr-clearance decreased significantly by IRI ($P < 0.05$) (Figures 1 and 2).

4.2. Effect of Zn and estradiol on ischemia-reperfusion injury

The results indicated that estradiol in IRI+Es group did not alter the serum level of BUN and Cr, and UF, Cr-clearance, Na excretion load ($U_{Na}V$) and KTDS, but increased the percentage of Na excretion (ENa%) when compared with those of the control group rats (Figure 1). Co-administration of Es and Zn in IRI+Es+Zn group caused significant decrease in the serum level of BUN and Cr, and KTDS, and significant increase in UF ($P < 0.05$), while Zn alone (in IRI+Zn group) also performed similar results (Figures 1 and 2).

The data related to MDA and nitrite levels are tabulated in Table 1. The serum levels of nitrite and MDA were not altered significantly between the groups. The kidney nitrite level in IRI group was greater than the control ($P < 0.05$). The kidney nitrite level in the IRI+Es group was less than the IRI group ($P < 0.05$), but MDA level in IRI+Es and IRI+Es+Zn groups were lower than IRI group ($P < 0.05$). No kidney weight and uterus weight changes were detected between the groups.

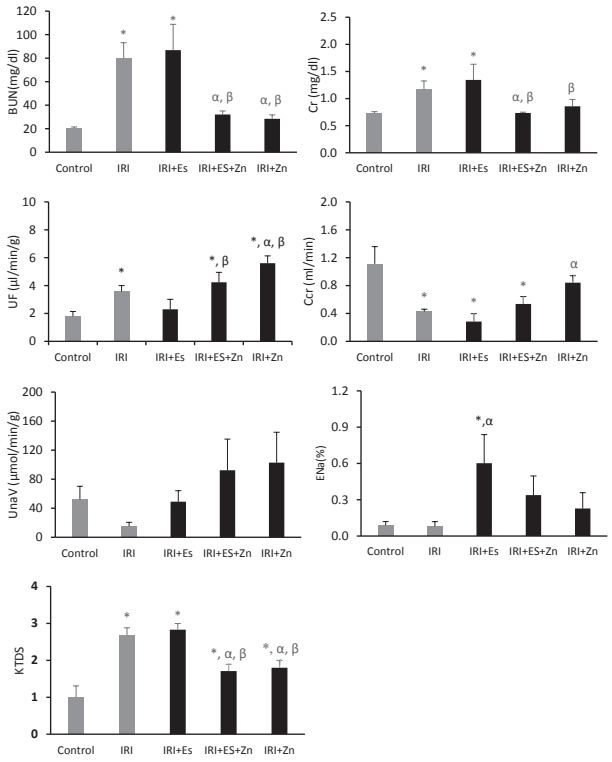

Figure 1. Serum levels of BUN, Cr, KTDS, urinary Ccr levels, UF, urine load of sodium ($U_{Na}V$) and percentage of sodium excretion (E_{Na}%) in all experimental groups. * , α and β indicate significant difference ($P \leq 0.05$) from the control, IRI and IRI+Es groups, respectively.
Abbreviations: IRI: Ischemia/reperfusion injury, Es=Estradiol, Zn=Zinc sulfate; BUN, blood urea nitrogen; Cr, creatinine; KTDS, kidney tissue damage score; Ccr, Cr clearance; UF, urine flow;

5. Discussion

Acute renal failure (ARF) is a common complication of kidney IRI which may lead to free formation and tubular cells damage. One of the key events in the pathology of renal damage after IRI is overproduction of ROS and/or decrease the antioxidant capacity (9). Free radical production promotes kidney injury via lipids peroxidation and proteins and DNA oxidative damage (10). The kidney injury may also increase by inflammatory cascade via releasing of the ROS, cytokines, and leukocytes activation (11,12). Experimental data from the present study showed that IRI increased BUN, Cr and KTDS as shown before (13). The reduction of Cr-clearance after IRI also is associated with reduction of glomerular filtration rate (GFR) as demonstrated in acute kidney injury (AKI) (14). However Zn either alone or accompanied with estradiol has protective effect against IRI as improves the kidney functional indexes. It is reported that Zn administration improved the reduced GFR which usually seen after IRI (15). Yonova et al reported that plasmatic Zn decreased in patients suffering from renal failure (16).

IRI was found to be associated with increased apoptosis in experimental models of kidney failure due to renal lipid peroxidation (17,18). Zn is a part of superoxide dismutase (SOD), and it has the ability to replace the metal ions (iron and copper, redox active metals) and may prevent the production of high ROS (7,8). Zn potentially may attenuate renal injury via inhibition of apoptosis and neutrophils infiltration (19,20). Estradiol as a sex hormone has major protective roles in renal damage, but the exact mechanisms are not fully clear. However, it may have protective effect on renal system by stimulating the function of the glomerular mesangial cells in the kidneys (21).

In our study, the nitrite and MDA levels decreased in IRI+Es+Zn group insignificantly compared to the IRI+Zn group. The anti-inflammatory and antioxidant potential of Zn stabilize the cellular membrane and inhibit inflammation factors production by special mechanisms (22,23). The tumor necrosis factor alpha (TNF-a) and interleukin 1 beta (IL1β) play an important role in renal dysfunction of IRI via ROS generation (24). Zn deficiency leads to exacerbate the detrimental effects of inflammatory cytokines such as TNF-α and damaging effects on the vascular endothelial functions (25). The possible protective effect of Zn during IRI-induced injury exposure may be related to its antioxidative properties and reducing damage induced kidney histological changes while the increased ROS production induced by IRI and may be implicated in tubular injury and contribute to renal damage (26). The fractional excretion of sodium (FE_{Na}%) indicates that the kidney is being under perfused. Hypoperfusion causes tubular

Table 1. The MDA and nitrite levels, kidney weight and uterus weights per 100 g body weight in all experimental groups

Group	Serum MDA (µmol/l)	Kidney MDA (nmol/g tissue)	Serum nitrite (µmol/L)	Kidney nitrite (µmol/g tissue)	Kidney weight (g)/100 g body weight	Uterus weight (g)/100 g body weight
Control	8.03±2.16	21.90±2.14	6.50±1.25	0.19±0.01	1.17±0.06	0.17±0.01
IRI	8.66±3.73	22.44±6.58	9.67±5.22	0.22±0.05*	1.19±0.09	0.27±0.10
IRI+Es	8.96±2.88	9.33±1.56*	9.39±4.37	0.17±0.01α	1.32±0.08	0.24±0.04
IRI+Es+Zn	5.80±0.97	6.84±0.51*	4.38±0.33	0.19±0.01	1.18±0.05	0.19±0.03
IRI+Zn	11.34±6.85	10.54±2.00	7.19±2.05	0.25±0.04	1.02±0.06	0.12±0.04

The symbols (*, α) indicate significant difference from the control (*) or IRI (α) groups. $P < 0.05$.
Abbreviations: MDA, malondialdehyde; IRI, Ischemia/reperfusion injury; Es, estradiol; Zn, Zinc sulfate.

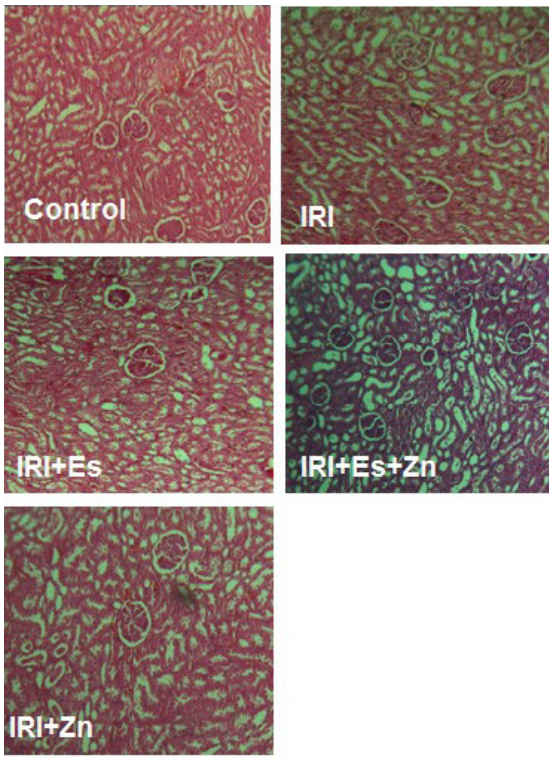

Figure 2. Sample images (magnification ×100) of kidney tissue in 5 experimental groups.

cell necrosis, and the tubules are no longer able to retain sodium and concentrate the urine, leading to an increase in the fractional excretion of sodium (27). The significant reduction of FE_{Na}% in Zn sulfate pretreatment suggests that Zn has an antioxidant effect mediated through the induction of metallothionein, but appears only to have a minor protective effect on renal function induced by renal IRI (15). It is demonstrated that Zn could prevent IRI-induced apoptosis by the activation of caspase 3 in a kidney cortex (28). It also protects the traumatic kidney against healing (29).

6. Conclusions

Administration of Zn as an antioxidant agent demonstrated an efficient role in preventing renal dysfunction induced by IRI in female rats. It seems the overproduction of ROS during IRI may inhibit by Zn; however more studies may clarify the exact mechanism.

Authors' contribution

FB conduced the experimental procedures and helped to prepare the first draft of article. AT analyzed the pathology data. MN designed, supervised and analyzed the research and completed the manuscript. All authors read and signed the paper.

Conflicts of interest

None to be declared.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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