Original Article

Testosterone and Zinc Supplementations on Renal Ischemia-Reperfusion Injury in Orchiectomized Rats

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Abstract

Background: Renal ischemia-reperfusion (IR) injury has numerous deleterious effects on the kidney function. An experimental investigation was conducted to determine the possible protective role of testosterone (TES) and zinc (Zn) supplementations on the kidney function after IR injury in orchiectomized rats. **Methods:** Orchiectomized rats (n = 32) were divided into the five groups as sham operated (Group 1), IR (Group 2), IR pretreatment with TES (IR + TES, Group 3), Zn (IR + Zn, Group 4), and TES + Zn (IR + TES + Zn, Group 5). Twenty-four hours' post-IR injury, the animals were sacrificed and the required parameters were measured. **Results:** The results revealed that there were not any significant difference in serum levels of creatinine (Cr), nitrite and malondialdehyde (MDA), Cr clearance (ClCr), renal sodium (Na) load, and percentage of Na excretion (ENa%) between sham and IR groups. The pretreatment with TES and Zn either alone or combine did not alter the serum levels of Cr, nitrite and MDA, and ClCr, Na load, and ENa%. However, pretreatment with Zn, TES, or combined altered kidney weight, kidney tissue levels of nitrite and MDA, and urine flow in IR groups. **Conclusions:** The orchiectomy itself performed protective effect against renal IR injury. However, pretreatment with Zn or TES may not alter kidney function against renal IR in orchiectomized rats.

Keywords: *Orchiectomy, renal ischemia, testosterone, zinc*

Introduction

The restriction of blood flow to vital organs followed by reperfusion is characterized as ischemia-reperfusion (IR) injury.[1] The renal IR injury usually is accompanied with formation or activation of many substances such as reactive oxygen species, cytokines, and chemokines.[2] In clinic, acute kidney injury and chronic kidney disease usually are resulted in renal IR injury.[3] Gender also plays a pivotal role in the outcome of kidney IR injury, and the sex hormones are highlighted while males compared with females are considered to be more susceptible to renal IR injury.[4,5] Some controversial studies indicated that orchiectomy has a protective role in renal IR injury and testosterone (TES) supplementation could reverse it while others' experimental studies proved otherwise.[6-8]

On the other hand, the antioxidant therapy by zinc (Zn) supplementation may protect the kidney against IR injury.[9] Zn inhibits the apoptosis process after renal IR injury,[10] and the protective role of Zn is reported to act in a dose-dependent manner.[9,11] Previously, we reported that Zn could protect the kidney against renal IR injury gender dependently.[12] In the current study, the roles of TES and Zn supplementations were considered in orchiectomized rats.

Methods

Orchiectomy

Thirty-two adult male (200 ± 20 g) Wistar rats (Animal Center, Isfahan University of Medical Sciences, Isfahan, Iran) were housed under standard conditions. The protocol of this study was confirmed to be in accordance by Ethics Committee of the Isfahan University of Medical Sciences.

Male rats were anesthetized with chloral hydrate (450 mg/kg, ip), and a midline abdominal incision was made and the testicles were removed and the skin was sutured.

Experimental protocol

One week after orchiectomy, the animals were randomly assigned into five

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experimental groups, and they received one of the following treatments:

- Group 1 (n = 6, sham operated): Rats received sesame oil alone intramuscularly once a week for 3 weeks and 1 week later underwent renal IR injury procedure without clamping renal vessels
- Group 2 (n = 5, IR group): Rats received sesame oil alone intramuscularly once a week for 3 weeks and 1 week later underwent renal IR injury surgery
- Group 3 (n = 6, IR + TES group): Rats received TES (10 mg/kg dissolved in sesame oil) intramuscularly once a week for 3 weeks and 1 week later underwent renal IR surgery
- Groups 4 (n = 7, IR + Zn group): Rats received sesame oil alone intramuscularly once a week for 3 weeks and 1 week later underwent renal IR surgery, but 5 consecutive days before surgery, they received Zn supplement (10 mg/kg/day, ip)
- Group 5 (n = 8, IR + TES + Zn group): Rats received TES once a week for 3 weeks and 1 week later underwent renal IR surgery, but 5 consecutive days before surgery, they received Zn supplement. The summary of the group's treatments is shown in Table 1.

Renal ischemia-reperfusion injury

The animals were anesthetized by chloral hydrate (450 mg/kg, ip). Two small incisions were made on the flanks, and the renal artery and vein on both sides were clamped. After 45 min, the clamps were removed and the kidneys were allowed to reperfuse. Eighteen hours later, the animals were placed in metabolic cages to collect the urine for the next 6 h. Twenty-four hours' postrenal IR injury, blood samples were obtained through heart puncture. Finally, all animals were sacrificed and kidneys were removed and weighed immediately. The right kidney was homogenized and centrifuged. The Cr clearance (ClCr) was calculated based on clearance formula as ClCr = urine flow (UF) × Ucr/Pcr, where UF, Ucr, and Pcr stand for UF, urine Cr level, and serum Cr level.

Table 1: The summary of the groups' treatments. The experiment was designed in five steps

Steps of experiment		1	2	3	4							5
Group	Name	ORC	RW	SO/ TES	V/Zn (day)							IR
					1	2	3	4	5	6	7	
1	Sham	+	+	SO	V				V			-
2	IR	+	+	SO	V				V			+
3	IR + TES	+	+	SO + TES	V				V			+
4	IRI + Zn	+	+	SO	V				Zn			+
5	IRI + TES + ZN	+	+	SO + TES	V				Zn			+

IR=Ischemia-reperfusion; ORC=Orchiectomy; RW=1 week recovery; SO=Sesame oil administration for 3 weeks; TES=Testosterone administration for 3 weeks; V=Vehicle infusion; Zn=Zinc treatment, IRI=Ischemia-reperfusion injury. +: Yes, -: No

Measurements

The levels of Cr in serum and urine were determined using quantitative diagnostic kits (Pars Azmoon, Iran). Assessments of malondialdehyde (MDA) level in the serum and kidney tissue were performed by the manual method. The serum and kidney levels of nitrite (stable nitric oxide [NO] 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 reported as mean ± standard error of mean. The exact statistic method (Kruskal–Wallis H-test and Mann–Whitney U-test) was applied to compare the parameters between the groups. $P < 0.05$ was considered statistically significant.

Results

The serum levels of Cr, nitrite and MDA, ClCr, filtrate Na load, and percentage of Na excretion (ENa%) were not significantly different between the groups [Figure 1]. The total kidney weight (KW) per 100 g body weight in IR, IR + Zn, and IR + TES + Zn groups was decreased significantly when compared with sham group [$P < 0.05$, Figure 1]. In addition, KW in IR + TES group was increased statistically when compared with IR group ($P < 0.05$). The tissue level of nitrite in IR + TES, IR + Zn, and IR + TES + Zn groups was greater than sham group ($P < 0.05$) while this marker in IR + TES + Zn group was increased when compared with IR groups significantly [$P < 0.05$, Figure 1]. The tissue MDA level in IR + Zn and IR + TES + Zn groups were lower than IR group statistically [$P < 0.05$, Figure 1]. In the all IR group (Groups 2–5), the mean levels of UF were increased; however, statistically, a higher UF was detected in IR + TES, IR + Zn, and IR + TES + Zn groups when compared with sham group [$P < 0.05$, Figure 1].

Discussion

The present study was designed to evaluate the effect of TES and Zn supplementations on renal IR injury in orchiectomized rat. The findings suggested that orchiectomy alone has a protective role in renal IR injury. However, although administration of TES and Zn altered some of the parameters, it seems that their certain protective roles may be failed in this model.

Gender plays a critical role in renal IR outcomes, and males are more susceptible to the renal IR and also have a delayed repair in comparison to females.[4] Kang *et al*. reported that orchiectomy decreased the inflammatory response, and TES replacement enhanced renal injury.[6] Furthermore, Park *et al*. showed that administration of TES in female mice increases renal inflammatory response in renal IR injury.[8] Actually, the presence of TES rather

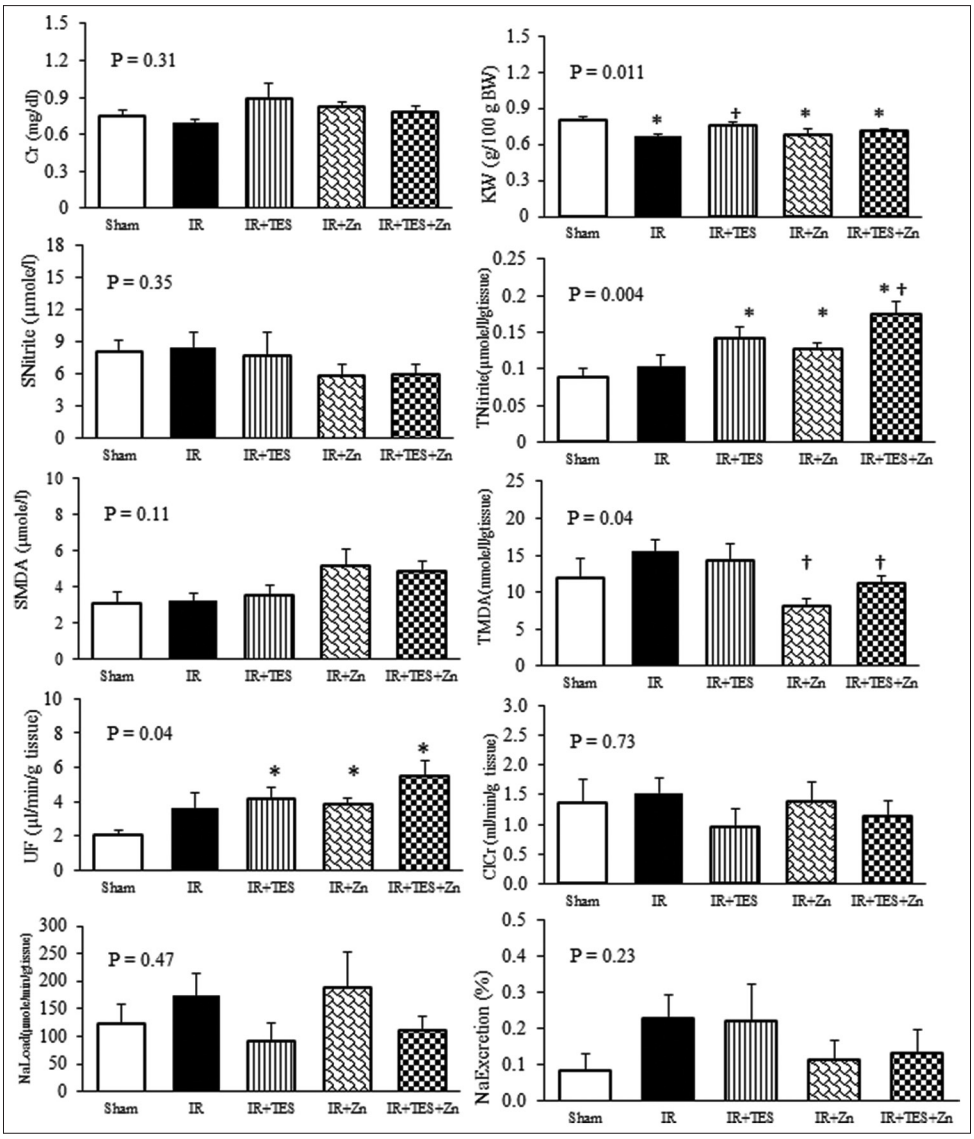

Figure 1: The serum (S) levels of creatinine, nitrite, and malondialdehyde and kidney tissue (T) levels of malondialdehyde and nitrite and kidney weight, Cr clearance, urine flow, renal Na load, and percentage of Na excretion (ENa%) in the all experimental group. The *P* value in each panel was obtained by Kruskal–Wallis H-test. The data in ischemia-reperfusion + testosterone, ischemia-reperfusion + zinc, and ischemia-reperfusion + testosterone + zinc were compared with sham or ischemia-reperfusion groups using Mann–Whitney U-test, and the symbols indicate significant difference from (*) sham or (†) ischemia-reperfusion groups (*P* < 0.05)

than the absence of estrogen cause inflammation through inhibition of NO synthase activation.[5,6]

There are some studies against our findings. Soljancic *et al*. concluded that TES level in serum was seriously reduced after renal IR injury in normal rats, and the kidney could be protected against IR injury by the reduced TES while castration also promoted kidney injury.[7] They also found that intravenous administration of TES (20 µg/kg/min) reduced the kidney IR injury.[6] Other study indicated that reduction of TES level is associated with undesired outcome in coronary heart diseases.[13] Moreover, TES exhibits a protective effect against spinal cord IR.[14] Albayrak *et al*. data indicated that administration of TES protected intestinal IR injury, but such observation was not detected in the absence of testes.[15] However, the exact mechanism by which TES protects the kidney is not documented yet.[6] On the contrary, the TES treatment in renal IR injury did not protect the kidney while orchiectomy alone showed a protective effect.[16] In addition and similar to our study, Park *et al*. found that orchiectomy itself could protect the kidney against IR injury,[16] and the protective role of orchiectomy is related to the expression of heat-shock proteins.[8] One possibility for these controversies is speculated that this difference may be due to the injection protocol. In this study, TES was injected once a week; however, other studies injected TES daily or more frequently.

Guo *et al*. found that Zn has a protective effect on renal IR by antiapoptotic and antioxidant capacity,[11] and the protective effect of Zn on renal IR is dose dependent.[9]

Therefore, although Zn performed some protective effect against renal IR, it may fail in orchiectomized model.

Conclusions

It seems that orchiectomy itself with uncertain mechanism performed some protective effect against renal IR injury. In such condition, no protective role from TES or Zn supplementations was observed. According to other studies, the protective effect of TES or Zn against renal IR injury was reported[6,12] in normal animals. Orchidectomy disturbs many hormonal and nonhormonal pathways,[17,18] and TES also may affect Zn through glucose cycle.[19] Therefore, exogenous administration of TES and Zn may not perform an effective role against renal IR injury in orchiectomized rats due to complex pathways disturbances.

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Conflicts of interest

There are no conflicts of interest.

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