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Preventive Role of Endothelin Antagonist on Kidney Ischemia: Reperfusion Injury in Male and Female Rats

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ABSTRACT

Background: Renal ischemia/reperfusion injury (RIRI) is the most common cause of acute kidney injury. We tested the protective role of endothelin-1 receptor blocker; bosentan (BOS) in animal model of RIRI in two different genders.

Methods: Male and female Wistar rats were assigned as sham operated (sham), control group (ischemia), and case group (ischemia + BOS) treated with BOS (50 mg/kg) 2 h before bilateral kidney ischemia induced by clamping renal vessels for 45 min followed by 24 h of renal reperfusion.

Results: The RIRI significantly increased the serum levels of blood urea nitrogen and creatinine in both genders ($P < 0.05$). These values were significantly decreased by BOS in both genders. In male rats, the serum levels of malondialdehyde in the ischemia + BOS group were decreased significantly when compared with ischemia group ($P < 0.05$).

Conclusions: BOS can be used in both genders to attenuate kidney ischemia injury possibly due to its effect in the renal vascular system.

Keywords: Bosentan, endothelin, gender, ischemia-reperfusion, kidney

INTRODUCTION

Acute renal failure (ARF) or acute kidney injury includes a wide range of disturbance in kidney function.[1] Renal ischemia/reperfusion injury (RIRI) which occurs mainly in different clinical circumstances such as renal transplantation[2] is responsible for high morbidity and mortality rate.[3,4] Reactive oxygen species in RIRI increase renal vascular resistance and reduce renal blood flow.[5] Endothelin-1 (ET-1) as one of the most known potent vasoconstrictors[6] is up-regulated during renal ischemia,[7] so it increases renal vascular resistance and causes reduction in renal blood flow after reperfusion. ET-1 seems to exacerbate RIRI, because administration of bosentan (BOS) - a dual ET-1 receptor antagonist[8] demonstrated protective effects on experimental RIRI.[9] ET-1 and its receptors are effected by sex hormones. Therefore, ET-1 is probably a key mediator in the maintenance of gender-mediated differences after RIRI.[10] In this short study, we tested the role of BOS RIRI in both male and female rats.

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METHODS

Thirty-six age-matched male and female Wistar anesthetized rats were used. Groups 1 (male, $n = 6$) and 2 (female, $n = 6$) as sham-operated were subjected to surgery without ischemia. The groups 3 (male, $n = 6$) and 4 (female, $n = 6$) received saline as vehicle 2 h before induction of ischemia. Groups 5 (male, $n = 6$) and 6 (female, $n = 6$) were treated similar to groups 3 and 4 except they received BOS (50 mg/kg) instead of vehicle. Bilateral kidney ischemia was induced by clamping renal vessels for 45 min. Then, the clamps were removed to induce renal reperfusion. Twenty-four hours postreperfusion, the animal was anesthetized again, and blood samples were obtained via heart puncture. After sacrificing the animals, the kidneys were removed and weighted. Right kidneys were homogenized and centrifuged to obtain supernatant for the measurement.

Measurements

The serum levels of creatinine (Cr) and blood urea nitrogen (BUN) were measured by quantitative diagnostic kits (Pars Azmoon, Tehran, Iran). Serum and tissue levels of nitrite were measured by an assay kit (Promega Corporation, USA) that involves the Griess reaction. Malondialdehyde (MDA) levels in serum and tissue were also measured manually.

Statistical analysis

The data were expressed as mean ± standard error of mean The groups were compared in terms of the serum levels of BUN, Cr, nitrite, and MDA; and tissue nitrite and MDA levels and kidney weight (KW) using the one-way analysis of variance followed by the least significant difference test.

RESULTS

Effects of bosentan on serum levels of blood urea nitrogen and creatinine

RIRI significantly increased the serum levels of BUN and Cr in both genders when compared with the sham operated groups. These observations were significantly decreased by BOS in both genders [Figure 1].

Effects of bosentan on kidney and body weight

KW was significantly increased by the RIRI, and it was significantly decreased by BOS in female rats [Figure 1]. Body weight change indicated no significant difference between the males and females groups [Table 1].

Effects of bosentan on serum and kidney levels of nitrite and malondialdehyde

There were significant differences in serum and kidney levels of MDA in male but not those of female. Neither male nor female groups demonstrated no significant changes in serum and kidney levels of nitrite [Table 1].

DISCUSSION

RIRI is the first most common cause of inpatient's ARF.[11,12] Over the past years, studies have identified a variety of methods to treat renal RIRI.[13,14] In the present study, we attempted to investigate the gender-related effect of BOS as ET-1 blocker on renal RIRI. Our results showed that renal ischemia-reperfusion induced renal failure that characterized by increasing BUN and Cr as well as KW in both genders. These observations were in agreement with others.[15-18] There are some reports suggesting that ET-1 is involved in the development of postischemic ARF in clinical transplantation.[19,20]

Recent studies suggest the importance of enhanced renal production of ET-1 in the pathogenesis of ischemic ARF. For example, plasma ET-1 levels are elevated in ARF, and renal ischemia increases renal ET-1 content[21,22] and ET receptor affinity.[23,24] Infusion of ET-1 decreases glomerular filtration rate, renal blood flow, sodium excretion and increases filtration fraction and renal vascular resistant while co-injection of VML 588 (ET-A antagonist) will reduce all sequences except glomerular filtration rate.[25] Herrero *et al*. showed that ET-1 contributes to experimental renal cold ischemia-reperfusion injury, and BOS can attenuate this injury.[26] The present study showed that pretreatment with BOS as a nonselective dual ETA/ETB receptor antagonist could attenuate post-ischemic renal injury in both genders. Moreover, several investigators have noted that exogenous monoclonal or polyclonal antibody to ET,[22,27-29] ETA receptor antagonists,[21,26,30-32] or ETA/ETB dual receptor antagonists[33-35] ameliorated declines in glomerular filtration rate and tubular damage in ischemia-reperfusion injury. BOS was shown to have a beneficial effect on experimental ischemia/reperfusion injury in the spinal cord,[36,37] testis,[38] heart[39] and kidney. It was showed that BOS treated rats had higher renal blood flow, Cr clearance, glomerular filtration rate and lower plasma Cr after RIRI.[9] Also, administration of an ET receptor antagonist 24 h after the ischemic damage was highly effective in reversing ARF.[34,40] Despite sexual dimorphism in the majority of physiological and pathophysiological conditions, the majority of experiments on ischemia-induced ARF have been conducted in male animals only. The course of post-ischemic renal failure has not been systematically compared between males and females. Therefore in this study to gain further insights into the role of gender in ischemic renal damage, we compared this process in male and female rats.[10] There is identified that female mice were more resistant to renal insulin receptor (IR) injury compared with male mice.[41] Furthermore, the prevalence of ARF is noticeably greater in males than females.[42-45] Williams *et al*. measured the serum level of BUN and Cr levels for 0, 0.5, 1, 2, 4, 6, 9, and 24 h and 1

Table 1: SN (μmole/l) and SMDA (μmole/l), KN (μmole/g tissue) and KMDA(nanomole/g tissue), and ΔW (g) in three experimental groups of sham, ischemia and ischemia treated with BOS in male and female rats

Group	SN		KN		SMDA		KMDA		ΔW	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Sham	6.65±1.9	5.56±1.2	0.28±0.06	0.31±0.02	4.50±0.32	4.73±0.41	9.02±1.3	10.40±1.5	7.33±2.4	7.33±0.6
Ischemia	5.38±0.8	5.25±1.5	0.22±0.03	0.25±0.06	5.40±0.57	5.03±0.5	4.27±1.0*	6.24±0.7	10.83±1.8	2.17±3.5
Ischemia + BOS	9.14±2.1	4.57±0.7	0.27±0.04	0.36±0.03	3.84±0.29#	4.95±0.65	5.94±1.2	10.83±1.8	12.83±4.2	6.0±2.5
P	0.33	0.84	0.34	0.17	0.05	0.92	0.04	0.07	0.44	0.35

*,#Significant differences (*P*<0.05) from sham or ischemia group respectively. *n*=6 in each group. SN=Serum nitrite, SMDA=Serum malodialdehyde, KN=Kidney nitrite, KMDA=Kidney malodialdehyde, ΔW=Body weight change, BOS=Bosentan

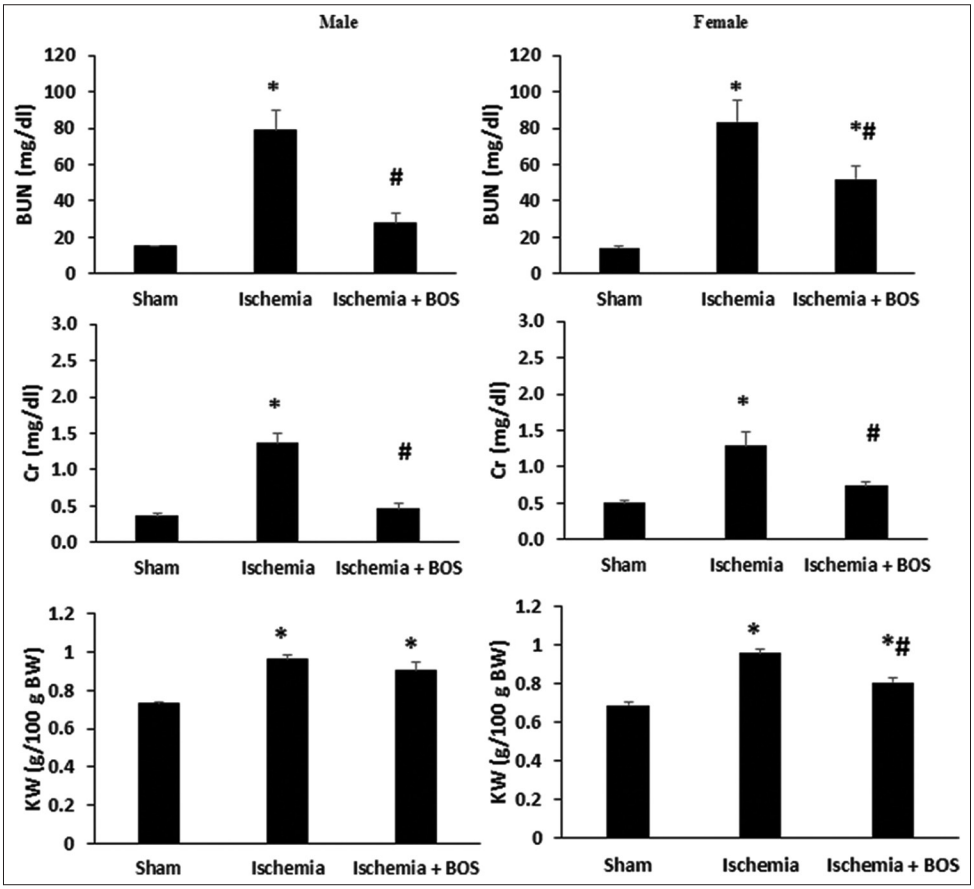

Figure 1: Serum levels of blood urea nitrogen and creatinine, and kidney weight per 100 g body weight in three experimental groups of sham, ischemia and ischemia treated with bosentan in male and female rats. Star (*) and (#) indicate significant differences (*P* < 0.05) from sham or ischemia group, respectively. *N* = 6 in each group

week post-RIRI, and reported that the earliest renal injury started at the 4th h following ischemia and peaked at the 1st day.[46] Commonly used definitions of ARF include an increase in serum Cr, a reduction in the calculated Cr clearance of 50%, or a decrease in renal function that results in the need for dialysis.[47-49] However, serum Cr production changes significantly according to age, sex, muscle mass and dietary intake.[50] The possible effect of sex on serum Cr level might be one of the reasons that we did not infer any significant difference in Cr level among different genders. By contrast, Müller *et al.* suggested that gender has a major impact on ischemia-induced renal damage and sex hormones play a crucial role in this difference.[10] Sex hormones have been reported to have an important role in I/R-induced inflammatory processes in the kidneys.[51] Previous studies have demonstrated that testosterone has an important role in increasing the susceptibility to ischemic renal injury.[52,53] By contrast, other experimental results suggest that estrogen has a protective effect in ischemic renal injury in female[18] via suppression of ET-1 production and activation of the phopsphatidylinositol-3 kinase/protein kinase B signaling pathway.[54,55] Our result showed no difference in nitrite level between the male and female groups, and kidney

MDA level reduced only in male, but other study showed that kidney MDA level decreased in male and female ischemic animals,[17] and IR decreased serum and kidney level of nitrite.[15]

CONCLUSIONS

We conclude that BOS can be used in both genders to attenuate injury induced in kidney ischemia possibly due to its effect in the renal vascular system.

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