Original Article

Effect of Γ-aminobutyric acid on kidney injury induced by renal ischemia-reperfusion in male and female rats: Gender-related difference

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Abstract

Background: The most important cause of kidney injury is renal ischemia/reperfusion injury (IRI), which is gender-related. This study was designed to investigate the protective role of Γ-aminobutyric acid (GABA (against IRI in male and female rats.

Materials and Methods: Thirty-six female and male wistar rats were assigned to six experimental groups. The IRI was induced by clamping renal vessels for 45 min then was performed reperfusion for 24 h. The group sex posed to IRI were pretreated with GABA and were compared with the control groups.

Results: Serum levels of creatinine and blood urea nitrogen, kidney weight, and kidney tissue damage score increased in the IRI alone groups, ($P < 0.05$), while GABA decreased these parameters in female significantly ($P < 0.05$), but not in male rats. Uterus weight decreased significantly in female rats treated with GABA. Testis weight did not alter in male rats. Serum level of nitrite and kidney level of malondialdehyde (MDA) had no significant change in both female and male rats. Kidney level of nitrite increased significantly in female rats experienced IRI and serum level of MDA increased significantly in males that were exposed to IRI ($P < 0.05$).

Conclusion: GABA could ameliorate kidney injury induced by renal IRI in a gender dependent manner.

Key Words: Γ-aminobutyric acid, gender, rat, renal ischemia-reperfusion

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INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome with high morbidity and mortality.[1,2] Kidney ischemia/reperfusion injury (IRI) is one of the most common cause of AKI,[3] by which complex events related to kidney damage and cell death occur.[4,5]

Several causal factors contribute to the pathogenesis of this renal damage.[6,7] However, the mechanisms underlying IRI are not fully understood,[2] but sex hormones have been reported to play an important role in kidney injuries induced by inflammatory processes.

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In this regard, the incidence of acute renal failure in surgical patients is significantly higher in men than women.[8] Males exhibit rapid progression of nondiabetic kidney diseases.[9] In this regard, male sex hormone, testosterone, increases the susceptibility to ischemic renal injury when compared with estrogen depletion or absence of male sex hormones, which induce a reduction in the levels of markers of postischemic oxidative stress in the kidney.[10-12]

Γ-aminobutyric acid (GABA) is a major neurotransmitter that is largely found in the central nervous system.[13-15] The peripheral GABAergic system is one of the major inhibitory neurotransmitters in the brain.[16,17] The peripheral organs including the kidneys are known to possess various subtypes of GABAA receptors.[18] Moreover, it is reported that GABA has neuroprotective effects in the brain ischemic injury, which is one of the serious complications of atherosclerosis.[19]

Although, some reports indicated that GABA provides a powerful protective mechanism against ischemic injury in a variety of organ systems including kidneys,[14] and on conditions including acute renal failure,[20] endocrine disorders,[16] hypertension,[13] and IRIs;[21] still its effect on IRI between males and females has not been compared.

Studies have shown that GABA has renoprotective effects against glycerol-induced AKI and administration of GABA ameliorates renal dysfunction, and a longer administration period of GABA increases its protective effect.[20] The results also indicate that GABA may play a protective role against chronic renal failure through improvement of the serum lipid profile.[22] Therefore, we hypothesized that GABA may affect renal ischemia in a gender-related manner.

MATERIALS AND METHODS

Animals

Eighteen adult female (weight: 191 ± 4 g) and 18 adult male (weight: 216 ± 5 g) wistar rats were used in this study. The rats were housed at a temperature of 23–25°C with a 12-h light/12-h dark cycle and the experimental procedures were in advance approved by the Isfahan University of Medical Sciences Ethics Committee.

Drugs

Γ-aminobutyric acid (code A2129-10G) was provided from Sigma (St. Louis, MO, USA).

Experimental protocol

The animals were randomly divided into six experimental groups as follows:

- Group 1 (n = 7, named MG), male rats exposed to surgery without IRI and 15 min before ischemia received GABA (50 μmol/kg; intravenously)
- Group 2 (n = 5, named MI), male rats treated as Group 1 except saline instead of GABA
- Group 3 (n = 6, named MIG), male rats exposed to IRI and treated with GABA 15 min before the surgery
- Groups 4 (n = 7), 5 (n = 6), and 6 (n = 6) named as FG, FI, and FIG, female rats treated as Groups 1–3, respectively.

Ischemia/reperfusion injury

To induce the IRI model, the animals in Groups 2, 3, 5, and 6 were anesthetized by chlorohydrate (450 mg/kg). Two small incisions were made on the skin to expose kidneys and vessels. Both the renal artery and vein were clamped simultaneously in each kidney for 45 min. then, the clamps were removed to initiate reperfusion. The skin was sutured, and the animals were returned back to the cages under direct observation.

After 24 h, the animals were anesthetized again, blood samples were taken via heart puncture and the animals were sacrificed. The samples were centrifuged to obtain serum sample for measuring the serum level of blood urea nitrogen (BUN), creatinine (Cr), nitrite, and malondialdehyde (MDA). The kidneys were also removed. The right kidney was homogenized and centrifuged at 15,000 g for 2 min, and the supernatant was used for MDA and nitrite measurements. The left kidney was fixed in formalin for histopathological investigation.

Measurements

Serum levels of Cr and BUN were measured using quantitative kits (Pars Azmoon, Iran). Serum and kidney levels of nitrite (stable metabolite of nitric oxide [NO]) were measured using a colorimetric kit (Promega Corporation, USA) that involved the Griess reaction. Assessment of MDA levels in the serum and kidney was performed by the manual method.[23,24]

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. The intensity of tubular lesions was scored by a pathologist who was blind to the study protocol. The score was considered from 1 to 4, while score zero was assigned to normal tissue.

Statistical analysis

Statistical data were presented as mean ± standard error of the mean. The quantitative data between

the groups were compared by the one-way analysis of variance, followed by the least significant difference. The Mann–Whitney or Kruskal–Wallis tests were used to compare the pathological damage score among groups. $P < 0.05$ were considered statistically significant.

RESULTS

Effect of ischemia/reperfusion injury on serum levels of blood urea nitrogen and creatinine

The serum levels of Cr and BUN increased significantly in male and female rats exposed to IRI ($P < 0.05$). Administration of GABA ameliorated the increased levels of BUN and Cr induced by IRI in female ($P < 0.05$), but not in male rats [Figure 1].

Effect of ischemia/reperfusion injury on kidney tissue damage score

Ischemia/reperfusion injury increased kidney tissue damage score (KTDS) significantly in male and female rats ($P < 0.05$) [Figure 2]; however, GABA could attenuate KTDS in female rats underwent IRI.

Effect of ischemia/reperfusion injury on bodyweight and kidney weight changes

Total kidney weight/100 g body weight (KW) increased significantly in male groups exposed to IRI ($P < 0.05$) and decreased significantly in the female groups exposed to IRI pretreated with GABA ($P < 0.05$).

In male rats, BW in the group exposed to IRI decreased significantly compared to other groups ($P < 0.05$). However, this was not the case in female rats [Figure 1].

Effect of ischemia/reperfusion injury on uterus weight and testis weight

The uterus weight in rats exposed to IRI and treated with GABA decreased significantly ($P < 0.05$) [Figure 1], while no significant differences in testis weight were detected between the groups.

Effect of ischemia/reperfusion injury on kidney and serum nitrite and malondialdehyde levels

Kidney nitrite level in female rats exposed to IRI increased significantly when compared with the FG group ($P < 0.05$) [Table 1], while MDA level in the MG group was greater than that in the MI group ($P < 0.05$) [Table 1].

DISCUSSION

Our objective was to determine the effects of GABA on IRI in male and female rats. The findings indicate that GABA has protective effects on renal IRI, although it is more efficient in female rats. Different reports have described that renal IRI affects serum biochemical factors such as Cr, BUN, nitrite, and MDA levels; as well

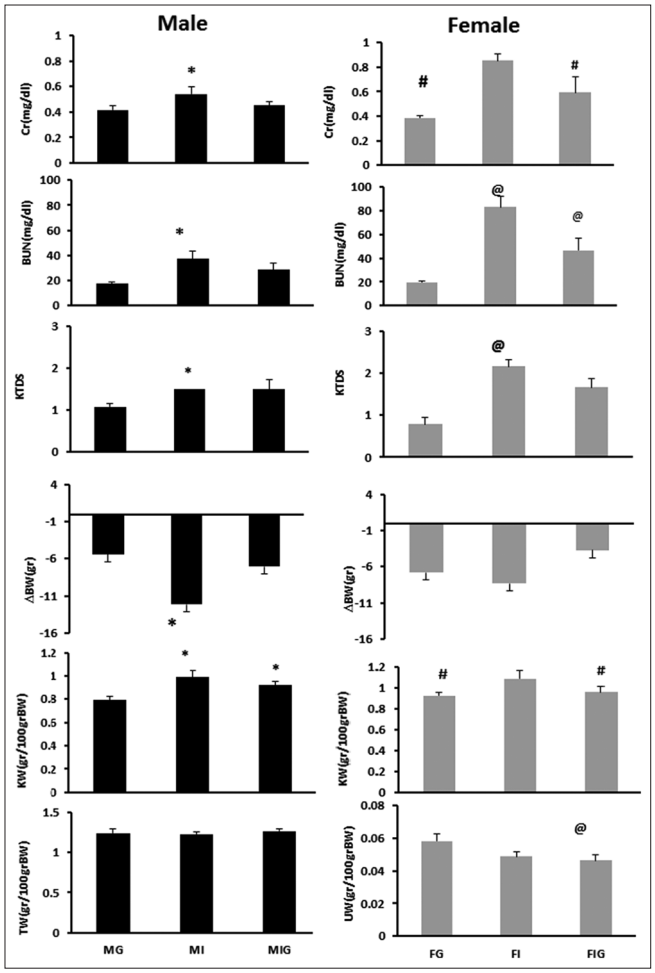

Figure 1: Serum levels of blood urea nitrogen (BUN) and creatinine (Cr); kidney weight (KW); body weight change (BW), kidney tissue damage score (KTDS), uterus weight (UW) or testis weight (TW) in female and male rats, respectively. The *, #, and @ indicate significant difference from the MG, FI, and FG groups, respectively

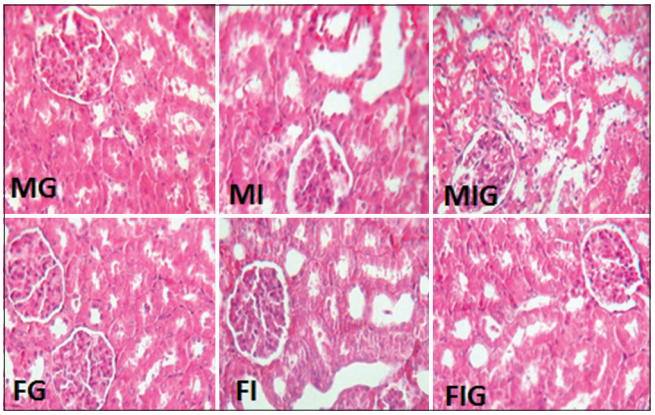

Figure 2: Images (magnification × 400) of kidney tissue. MG = Male + Γ-aminobutyric acid (GABA), MI = Male + Ischemia, MIG = Male + Ischemia + GABA, FG = Female + GABA, FI = Female + Ischemia, FIG = Female + Ischemia + GABA. Higher damage scores were observed in the FI and MI groups

Table 1: Level of nitrite and MDA in serum and kidney in all experimental groups

Group	Serum nitrite (μmol/L)	Kidney nitrite (μmol/g tissue)	Serum MDA (μmol/L)	Kidney MDA (nmol/g tissue)
MG, Group 1	22.16±1.98	0.25±0.02	25.37±0.75	11.41±1.20
MI, Group 2	24.29±6.93	0.24±0.02	17.76±2.42*	10.02±0.75
MIG, Group 3	22.87±2.13	0.26±0.02	21.71±1.25	10.02±0.88
FG, Group 4	18.33±4.94	0.15±0.01	22.92±1.59	12.31±0.72
FI, Group 5	23.95±3.60	0.27±0.04#	24.84±0.58	8.94±1.62
FIG, Group 6	26.73±5.83	0.19±0.02	22.20±1.57	11.62±1.24

#Significant difference from Group 5, *Significant from Group 1, $P<0.05$. MDA: Malondialdehyde, MG: Male + GABA, MI: Male + ischemia, MIG: Male + ischemia + GABA, FG: Female + GABA, FI: Female + Ischemia, FIG: Female + ischemia + GABA, GABA: Γ-aminobutyric acid

as weight and histology of the kidney.[23-28] We found that the functional markers of kidney, BUN and Cr, increased significantly after renal IR in the female and male rats while GABA reduced these parameters in male and female rats with renal IRI. The ischemia-reperfusion causes increase in some serum parameters and markers of oxidative stress and histopathological changes in renal tissues.[29,30] It appears that reduction of BUN and Cr levels in the current study and other studies is probably associated with alteration of glomerular filtration rate,[7,31] since obstruction and destruction of tubules together with a renal artery stenosis lead to changes in glomerular filtration rate.[32]

Body weight loss and KTDS increase were observed after renal IRI.[27,33] BW loss in rodents after kidney IRI is most likely due to the inability of the kidney to retain salt and water, and this effect causes polyuria.[34]

Γ-aminobutyric acid did not significantly ameliorate kidney damage, but its improvement in female rats was clear. Renal sympathetic nerve activity plays an important role in IRI.[21] GABA inhibits the development of ischemia by suppressing sympathetic nerve activity in excessive norepinephrine secretion from sympathetic nerve endings of the kidney.[35] The recovery may be related to the inhibitory effect of GABA on sympathetic nerve activity and its suppression.[22] The sex difference in ischemic injury of likely may be related to sex hormones[11] and pretreatment with the antioxidants abolished sex differences.[8] In addition, the sex hormone status may be important for initiation of the inflammatory response following renal IRI.[36]

Malondialdehyde, as the final product of lipid peroxidation, is one of the biomarkers of oxidative stress.[37] Several studies have reported that IRI increases the MDA level.[38,39] In the present study, the serum and tissue levels of MDA increased insignificantly after renal IR, but GABA did not decrease the MDA levels except the serum MDA level in female rats. This may be related to the female sex hormones because estrogen has antioxidant properties and reduction of free radicals will decrease the MDA level.[11] Although administration of GABA improved renal dysfunction through regulation of blood pressure and improved oxidative stress induced by nephrectomy, this effect is probably due to the increased activity of superoxide dismutase or other antioxidant enzymes.[22,40] According to the scavenging effects of GABA on protecting against renal failure progression[22] and it the antioxidant properties,[40] possibly administration of GABA with estrogen in the female have a positive effect on sex difference in renal ischemia.

The serum and tissue levels of nitrite were increased by IRI. GABA did not decrease the nitrite levels in the male rats. NO is produced by endothelial NO synthase and inducible NO synthase increased after renal IRI in response to the oxidative stress.[23] The vasodilator action of NO on vascular smooth muscle cells is well known.[41] The major new finding of this study was that GABA improved postischemic structural recovery in kidney in both female and male rats, but this recovery was more significant in females. Sex difference in the IRIs has been studied in diverse organs and in most organs such as brain, heart, and gastrointestinal system, females are more resistant to the injuries.[42] Individual published reports have presented conflicting result.[43] Gasbarrini *et al*. reported that the liver is more vulnerable to IRI in females.[44] Müller *et al*. revealed that IRI in the kidney is less severe in female than male rats.[45] It has been shown that progression of renal diseases in males is much rapider than that in females. Furthermore, in chronic renal diseases, the outcome is worse in males.[5,45-47] This might be due to the differences in kidney structure, glomerular hemodynamic responses to stress, and the direct cellular effects of sex hormones.[45,48] The difference is due to the activity of the sympathetic nervous system, leading to increase in norepinephrine level after reperfusion and is significantly higher in male rats than in female rats.[35] This decline may be related to the estrogen hormone system.[47]

In the present study, GABA administration ameliorated renal functional loss in the treatment group. According to the results obtained, there was no significant difference between male and female groups in the kidney function tests. However, we found that IRIs were less in males and also the protective effects of GABA were more pronounced in females.

CONCLUSION

Although GABA improved IRI in male and female rats, its improvement was more pronounced in females.

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