IJMS
Vol 45, No 5, September 2020

Original Article

The Impact of Sex Differences on Renal Protective Effects of Lipopolysaccharide Preconditioning in Septic Shock

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Received: 12 August 2018
Revised: 06 January 2019
Accepted: 20 January 2019

What's Known

- Experimental sepsis-induced acute renal failure is caused by lipopolysaccharide (LPS) administration. Exposure to low doses of LPS (LPS preconditioning) causes tissue resistance against a higher dose of LPS.
- Sex differences play an important role in the outcome of septic shock. The impact of sex differences is observed in different kidney diseases.

What's New

- Male rats were more susceptible to renal injury than female rats.
- Pre-treatment with a low dose of LPS in both female and male rats had a protective effect on the subsequent LPS-induced renal injury.

Abstract

Background: Induction of septic shock by lipopolysaccharide (LPS) may lead to acute renal failure. The present study aimed to investigate the impact of sex differences on the effectiveness of low-dose LPS preconditioning (LPS-PC) on LPS-induced acute renal failure in rats.

Methods: This study was conducted at Tehran University of Medical Sciences, in 2017. A total of 48 Wistar rats were equally divided into two groups of male and female rats. The rats in each group were then allocated to three groups (n=8 per group), namely control, septic shock, and LPS-PC group. A high dose of LPS was administered for septic shock induction. LPS-PC was induced by injecting LPS before sepsis induction. The effect of sex differences on renal functional indices, renal oxidative stress markers, plasma tumor necrosis factor-α level, and renal histological changes was evaluated. Data were analyzed using two-way ANOVA followed by Tukey's *post hoc* test.

Results: In the septic shock groups, renal functional parameters (creatinine [Cr] and blood urea nitrogen [BUN]) were increased in both sexes. However, the increase was more significant in male rats (male rats: Cr=2.14±0.13, BUN=81±4.15; female rats: Cr=1.64±0.12, BUN=50±2.7). LPS-PC reduced these indices in both sexes (male rats: Cr=1.24±0.03, BUN=57±4.1; female rats: Cr=0.86±0.02, BUN=30.31±2.25). Renal superoxide dismutase (SOD) activity (male rats: 11.54±1.34, female rats: 24.4±2.04) and catalase (CAT) activity (male rats: 15±1.74, female rats: 25.75±1.97) were significantly higher in the female septic group. LPS-PC significantly increased SOD (male rats: 25.7±2.45, female rats: 42.6±3.31) and CAT (male rats: 37.25±2.34, female rats: 59.21±3.29) activities in renal tissue samples in the LPS-PC group in both sexes compared to the septic groups. In the LPS groups, plasma tumor necrosis factor-α (male rats: 375±25.65, female rats: 285.45±25.94) were significantly higher than in the LPS-PC groups (male rats: 250±21.35, female rats: 121±24.14).

Conclusion: Male rats were more susceptible to sepsis-induced renal damage. LPS-PC had protective effects on the LPS-induced renal injury, and these effects were most prominent in female rats.

Please cite this article as: Kadkhodaee M, Seifi B, Ranjbaran M, Shams S, Delavari F, Najafi A, Sedaghat Z, Khastar H. The Impact of Sex Differences on Renal Protective Effects of Lipopolysaccharide Preconditioning in Septic Shock. Iran J Med Sci. 2020;45(5):383-390. doi: 10.30476/ijms.2020.72461.0.

Keywords ● Renal ● Sex differences ● Septic shock ● Lipopolysaccharides

Introduction

Acute renal failure (ARF) caused by septic shock is a serious complication in clinical settings; accounting for nearly 50% of

all cases of ARF in the intensive care unit.[1-3] The mortality rate of sepsis-induced ARF has been reported to be as high as 75%.[4] Lipopolysaccharide (LPS), a major component in the outer membrane of Gram-negative bacteria, plays a key role in the maintenance of the outer membrane integrity and protects bacteria against bactericidal agents.[5] LPS, also called endotoxin, is well-known as a potent inducer of sepsis and septic shock, which leads to multiple organ failure and death even in the intensive care unit.[6, 7] LPS triggers a strong response from the innate immune system and may lead to local or systemic adverse reactions and substantial inflammatory responses.[5] In rodents, experimental sepsis-induced ARF is caused by LPS administration, which leads to renal dysfunction, alterations in renal blood flow (RBF), and glomerular filtration rate (GFR).[4]

The existence of several different preconditioning stimuli leads to the well-known phenomenon of "cross-tolerance" in which a tolerance-inducing stimulus protects against different types of injury.[8] LPS-induced sepsis is destructive, but exposure to low doses of LPS (LPS preconditioning [LPS-PC]) causes tissue resistance to higher (even lethal) dose of LPS or other major stress conditions (e.g., ischemia-reperfusion).[9] LPS-PC occurs after repeated administration of small or sublethal doses of LPS and is characterized by a reduced systemic response to a subsequent challenge with a large dose of LPS. LPS-PC mechanisms characterize endogenously developed strategies for adaptation to stress. Tolerance or adaptation to these harmful effects of LPS has been detected in humans and experimental animals.[9] Moreover, the effect of sex differences is observed in different kidney diseases. There are some suggestions that the progression of renal disease is faster and more rapid in males. Some studies have reported that males are more susceptible to kidney diseases (membranous nephropathy, IgA nephropathy, and autosomal dominant polycystic kidney disease) and are at a higher risk of developing chronic kidney disease.[10, 11]

The role of sex differences in the renal protective effect of LPS-PC in septic shock is controversial. Therefore, the main objective of the present study was to examine the potential renal protective effect of LPS-PC against the administration of a high dose of LPS in rats and to determine the role of sex differences in this process. To achieve this objective, we evaluated renal functional and histological parameters as well as TNF-α and oxidative stress indices in rats after septic shock and LPS-PC.

Materials and Methods

Animals

This study was conducted at Tehran University of Medical Sciences, in 2017. 48 Wistar rats weighing 250-300 g were used. One week before the experiments, the animals were housed under standard conditions (12 h light-dark cycle, 20-22 °C) and were given ad libitum access to food and water.[12]

Experimental Design

The rats were equally divided into two groups of male and female rats. The rats in each group were then allocated to three groups (n=8 per group), namely control, LPS-PC, and septic shock group.

Induction of LPS-PC

In the tolerant groups, LPS-PC was induced by injecting LPS (0.2 mg/kg i.p.)[13] diluted in 0.5 mL sterile normal saline and administered 24 hours before septic shock induction. In the septic shock groups, animals intraperitoneally received normal saline 24 hours before the administration of a high dose of LPS (Sigma-Aldrich, St. Louis, MO, USA).

Induction of Septic Shock

All animals were intraperitoneally administered a high dose of LPS (8 mg/kg)[14] for the induction of septic shock. Six hours after LPS administration, the animals were anesthetized using sodium pentobarbital (Sigma-Aldrich, St. Louis, MO, USA), 60 mg/kg, i.p., then blood and kidney tissue samples were obtained. Control rats received 0.5 mL normal saline intraperitoneally.

Assessment of Renal Functional Parameters

Blood samples were taken from the inferior vena cava of each rat into heparinized syringes and plasma was subsequently collected by centrifugation (4,000 g for 10 minutes) and stored at -70 °C until further use. Plasma concentrations of creatinine (Cr) and blood urea nitrogen (BUN) were evaluated as indicators of renal injury and function using a fully automated clinical chemistry analyzer (Hitachi 704 auto-analyzer, Japan).

Kidney Oxidative Stress Indices

Kidney tissue samples were obtained and stored at -70 °C after being snap-frozen in liquid nitrogen. Malondialdehyde (MDA) content, superoxide dismutase (SOD) activity, and catalase (CAT) activity were determined in the supernatant of kidney homogenates.

MDA level was assayed by the method described by Esterbauer and Cheeseman.[15] MDA reacts with thiobarbituric acid to form a pink pigment and the optical density was measured at 532 nm. The SOD activity was determined according to the method of Paoletti and Mocali.[16] In this assay, there was superoxide anion generation from oxygen and oxidation of NADPH was related to the availability of superoxide anions in the renal tissue samples. The optical density of supernatants was assessed at 340 nm. The CAT activity was measured based on Aebi's method.[17] In this method, the rate of H_2O_2 decomposition was monitored for 30 seconds and CAT activity was evaluated by the diminished absorbance at 240 nm.

Plasma TNF- α Level

Plasma tumor necrosis factor-α (TNF-α) concentration was assayed by enzyme-linked immunosorbent assay (ELISA). All reagents, standard dilutions, control, and test samples were brought to room temperature and prepared according to the manufacturer's instructions (R&D Systems, Inc, USA). In this assay, the quantitative sandwich enzyme immunoassay technique was employed. Reactions were quantified by optical density using a microplate reader (BioTek Instrument, ELX 800, Inc, USA) at a wavelength of 450-570 nm.

Histopathologic Analysis of the Renal Tissue Samples

Renal tissue samples were fixed in 10% formaldehyde, embedded in paraffin, and cut into 4 μm sections. The sections were stained with hematoxylin and eosin. Histopathologic changes were documented photographically.

Inclusion and Exclusion Criteria

To ensure septic shock induction, we observed changes in the rats' appearance, motor activity, and responses to stimuli.[18] Animals with little or no change in behavior and appearance as well as those expired within the predetermined experimental period were excluded from the study.

Ethical Considerations

Experimental procedures and animal care during the experiments were approved by the Experimental Animal Committee of Tehran University of Medical Sciences, Tehran, Iran (ethical code: IR.TUMS.MEDICINE.REC.1397.605). Prior to the study, all protocols were confirmed to be in accordance with the Guidelines of Animal Ethics Committee of the above-mentioned university. At the end of the study and after sample collection, the animals were sacrificed by deep anesthesia.[19]

Statistical Analysis

Data analysis was performed using SPSS 16.0 for Windows. Data were presented as mean±SEM. Statistical significance of the differences between groups was determined by two-way ANOVA followed by Tukey's *post hoc* test. $P<0.05$ was considered as statistically significant.

Results

The effect of sex differences on renal functional indices, renal oxidative stress markers, plasma TNF-α level, and renal histological changes in rats after septic shock and LPS-PC was evaluated.

Renal Functional Indices

In the male and female septic shock groups, LPS administration significantly increased plasma Cr and BUN levels compared with the control groups. However, as shown in figures 1A and 1B, male rats showed higher values than female rats (male rats: P=0.02 for Cr, P=0.005 for BUN; female rats: P=0.01 for Cr, P=0.003 for BUN). LPS-PC lowered Cr and BUN levels in male and female rats compared with levels in the septic shock groups (male rats: P=0.023 for Cr, P=0.045 for BUN; female rats: P=0.011 for Cr, P=0.021 for BUN). The percentage changes from the septic shock groups were more significant in female rats (48% for Cr and 52% for BUN) than male rats (28% for Cr and 33% for BUN).

Renal Oxidative Stress Markers

As shown in figure 2A, renal MDA content showed a significant increase in male and female rats comparedwith the control groups (male rats: P=0.032; female rats: P=0.001). The MDA level in male rats was significantly higher than in female rats in the septic shock groups. LPS-PC significantly attenuated the rise in the MDA content compared with the septic shock groups in both sexes (male rats: P=0.008; female rats: P=0.002). There was a significant decrease in the percentage change of renal MDA content in the female LPS-PC group (45%) compared to the male LPS-PC group (30%).

As shown in figure 2B, septic shock significantly attenuated renal SOD activity compared with that of the control groups in both sexes (male rats: P=0.001; female rats: P=0.0001). There was a significant decrease

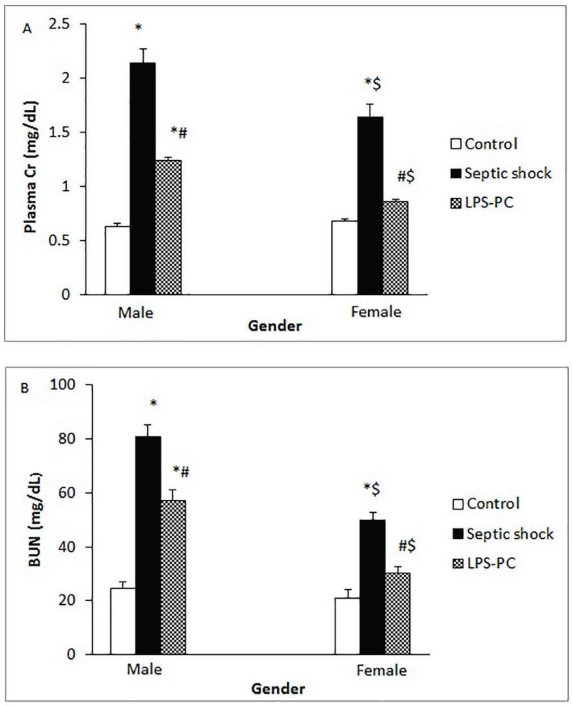

Figure 1: The figure shows the effect of sex differences on renal functional indices (plasma Cr and BUN) in rats following administration of a high dose of LPS injection after LPS preconditioning. Data are presented as mean±SEM (n=8). *LPS-PC groups versus control groups (P<0.05 septic shock), #LPS-PC groups versus septic shock groups (P<0.05), $Female septic shock and LPS-PC groups versus male septic shock and LPS-PC groups (P<0.05), CR: Creatinine; BUN: Blood Urea Nitrogen

in SOD activity in the male septic shock group compared with the female septic shock group. LPS-PC significantly increased SOD activity in renal tissue samples in the LPS-PC groups in both sexes compared with the septic shock groups (male rats: P=0.003; female rats: P=0.001). However, this increase was more significant in female rats (2.3-fold) compared with male rats (1.6-fold).

As shown in figure 2C, septic shock resulted in a significant decrease in renal CAT activity in both sexes compared with the control groups (male rats: P=0.005; female rats: P=0.002). The activity of CAT was significantly lower in male rats compared to female rats. LPS-PC significantly prevented the reduction in renal CAT activity in both sexes compared with the septic shock groups (male rats: P=0.04; female rats: P=0.035). There was a significant increase in CAT activity in the female LPS-PC group (2.5-fold) compared with the male LPS-PC group (1.5-fold).

Plasma TNF-α Level

In the male and female septic shock groups, LPS administration significantly increased plasma TNF-α compared with the control

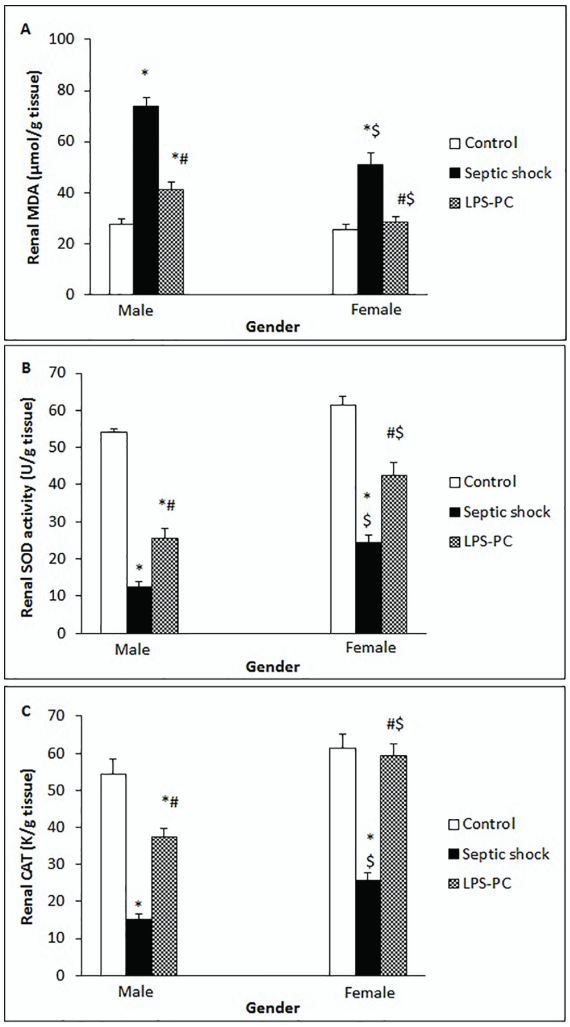

Figure 2: the figure demonstrates the effect of sex differences on renal oxidative stress markers (MDA, SOD, and CAT) in rats following administration of a high dose of LPS injection after LPS preconditioning. Data are presented as mean±SEM (n=8). *LPS-PC groups versus control groups (P<0.05 septic shock), #LPS-PC groups versus septic shock groups (P<0.05), $Female septic shock and LPS-PC groups versus male septic shock and LPS-PC groups (P<0.05), MDA: Malondialdehyde; SOD: superoxide dismutase; CAT: catalase

groups. However, as shown in figure 3, male rats showed higher values than female rats (male rats: P=0.005; female rats: P=0.002). LPS-PC lowered TNF-α level in both male and female rats compared with the septic shock groups. This reduction was more significant in female rats than male rats (male rats: P=0.045, female rats: P=0.03). The percentage change from the septic shock groups was more significant in female rats (59%) than the male rats (33%).

Renal Histological Changes

In the kidneys of the male LPS group, there was attenuation of tubular lumen and loss of tubular cells into the lumen (figure 4). Less structural damage, although not significant,

was observed in the LPS-PC male group. In the renal tissues of this group, the tubules appeared slightly healthier and cells were thicker and less attenuated. In the renal tissues of the female LPS group, tubular lumen attenuation and shedding of cells were present. However, although not significant, less damage was observed in the LPS-PC female group.

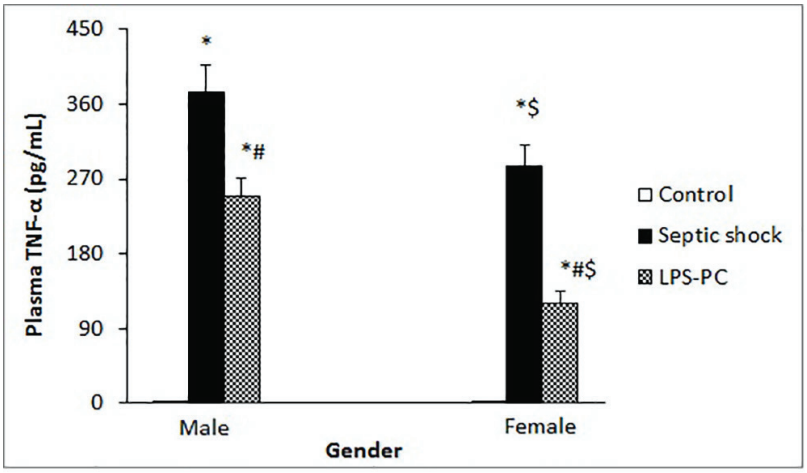

Figure 3: The effect of sex differences on the plasma TNF-α level in rats following administration of a high dose of LPS injection after LPS preconditioning is demonstrated. Data are presented as mean±SEM (n=8). *LPS-PC groups versus control groups (P<0.05 septic shock), #LPS-PC groups versus septic shock groups (P<0.05), $Female septic shock and LPS-PC groups versus male septic shock and LPS-PC groups (P<0.05), TNF-α: Tumor Necrosis Factor- α

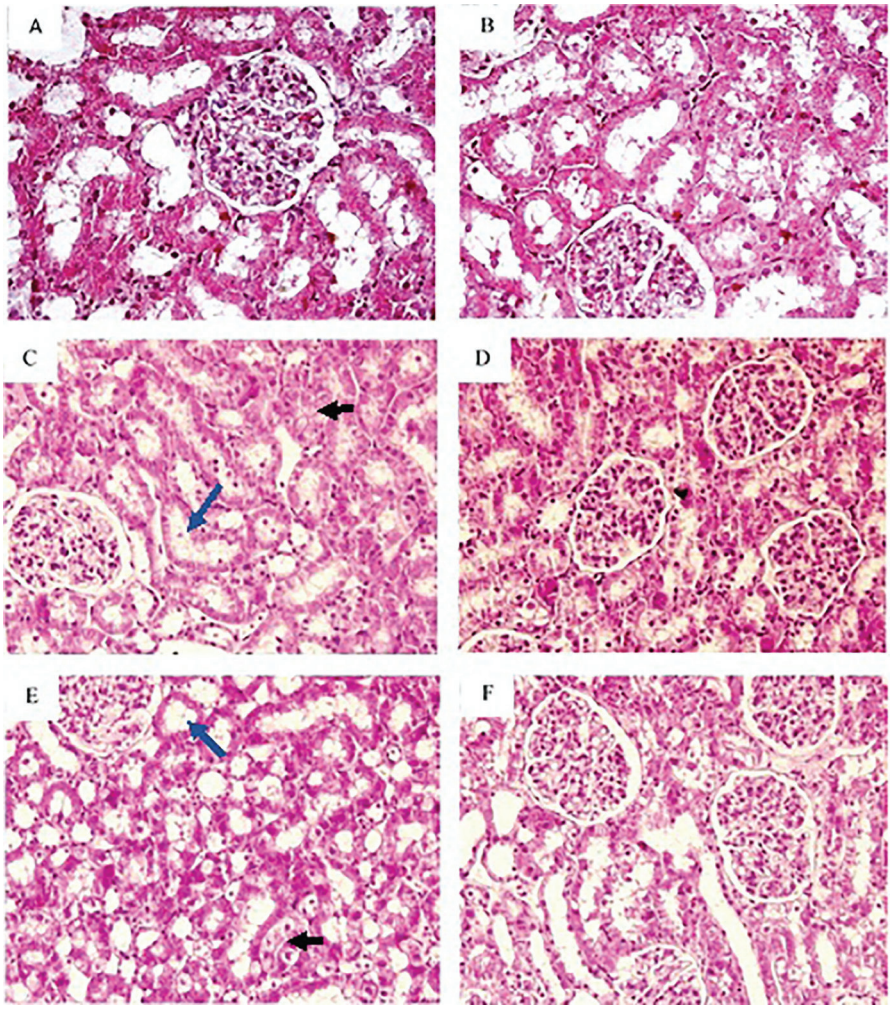

Figure 4: The effect of sex differences and a high dose of LPS injection after LPS preconditioning on the renal histological changes in rats is demonstrated. (A) Male control, (B) Female control, (C) Male septic shock, (D) Male LPS-PC, (E) Female septic shock, (F) Female LPS-PC. The blue and black arrows indicate attenuation and shedding, respectively.

Discussion

In the present study, we demonstrated that a high-dose LPS administration resulted in higher levels of plasma Cr and BUN in both sexes, especially in male rats more than the treated female rats. Some studies have indicated that sepsis-induced ARF is associated with renal dysfunction, reduction in RBF, and alteration in GFR.[2, 4] Tran and colleagues have also reported a reduction in RBF during sepsis. However, they reported that tissue oxygenation showed no change despite reduced RBF.[20] We demonstrated that LPS-PC reduced the increase in plasma Cr and BUN levels compared with the levels in the septic shock groups. Female rats were much more resistant to LPS-induced renal injury than male rats. In the LPS-PC female group, a more significant decrease in these parameters was observed. Our findings are consistent with a previous study that demonstrated male rats exhibited significantly higher levels of renal injury than the female rats following LPS exposure.[21]

LPS, as a potent activator of macrophages during sepsis, generates reactive oxygen species.[22, 23] Previous studies have reported that a reduction in RBF and alteration in GFR during LPS exposure reduced blood circulation in the capillaries surrounding the tubules that exhibited oxidative stress.[2, 4] To investigate whether LPS-PC reduces susceptibility to the subsequent high-dose LPS-induced oxidative stress, we measured MDA content, SOD and CAT activity in the renal tissue samples. The results showed a significant increase in MDA content and a reduction in SOD and CAT activity in renal tissue samples in both sexes. However, these changes were significantly greater in male rats treated with a high dose of LPS than the female rats. When LPS-PC was applied 24 hours before high-dose LPS administration, renal lipid peroxidation levels significantly reduced and interestingly, this reduction was more significant in females than in male rats. It should be noted that low-dose LPS pretreatment induces LPS tolerance and in fact, female rats showed a higher tolerance against high-dose LPS-induced lipid peroxidation in the renal tissue samples. Moreover, there were higher renal SOD and CAT activity in the LPS-PC groups than the septic shock groups. A previous study reported that LPS-PC activated protein syntheses, including antioxidant enzymes.[9]

During sepsis, Nuclear Factor kappa-light-chain-enhancer of activated B cells activation and the formation of free radicals stimulated the inflammatory cascade and produced substantial amounts of TNF-α. Godet and colleagues showed that administration of low doses of LPS in rats had a protective effect on the renal reperfusion injury and reduced renal TNF-α production.[24] Similarly, our results indicated that the TNF-α levels, as an inflammatory response marker, increased during septic shock in both sexes, especially in male rats. LPS-PC significantly attenuated the rise in TNF-α, while this reduction was more significant in female rats.

We found that female rats were more resistant to a high-dose of LPS (septic shock) than male rats. Recent studies have suggested that male hormones may also play an important role in disease susceptibility. Park and colleagues reported that female mice were much more resistant to ischemia-reperfusion-induced renal injury than males.[25] It seems that the presence of testosterone plays a critical role in the sex differences and susceptibility of the kidneys to ischemic injuries.[26] In contrast, Takaoka and colleagues reported that estrogen exhibited protective effects against ischemic ARF in animal models, possibly through the suppression of endothelin-1 overproduction.[27] Marriott and colleagues demonstrated that the overproduction of inflammatory mediators in males, as a result of higher levels of both CD14 and Toll-like receptor 4 (TLR4) on male macrophages, may be responsible for the lethal nature of the septic shock. CD14 and TLR4 on macrophages are likely to render these cells more sensitive to LPS exposure and contribute to the production of inflammatory mediators and thereby favoring male's lesser resistance to bacterial sepsis.[21] Campesi and colleagues identified a significant role of estrogen receptors α in LPS-mediated inflammatory responses in male blood monocytes-derived macrophages.[28] Several other mechanisms were suggested for female resistance to LPS exposure.

It is generally accepted that LPS-PC protects organs against the subsequent injurious stimulus. In the present study, when we applied low-dose LPS 24 hours before high-dose LPS, both sexes showed protection against high-dose LPS, but these responses were more prominent in female than in male rats. These results may explain why female rats were able to react better to pre-treatment than male rats. It is suggested that LPS-PC is neutralized by protein synthesis inhibition. Antioxidant enzymes and nitric oxide appear to be important mediators of these protective effects.[9] Some studies reported that the endotoxin tolerance phenomenon of LPS was associated with the up-regulation of several pro-inflammatory and anti-inflammatory mediators and signal transduction intermediates.[29] The mechanisms mediating the beneficial effects of

LPS-PC are not well-understood and warrant further research. The mortality rate during the experiments and the absence of the underlying mechanism of LPS-PC were the limitations of the present study.

Conclusion

Sex differences played a significant role in the outcomes of septic shock. We confirmed that male rats were more susceptible to kidney disease than female rats. This finding supports the hypothesis that sex difference plays a key role in the response of the rats' body to renal injury. Pre-treatment with low-dose LPS in female and male rats had a protective effect on the subsequent LPS-induced renal injury.

Acknowledgment

The authors would like to thank the Vice-Chancellor for Research Affairs of Tehran University of Medical Sciences (Tehran, Iran) for financial support (grant number: 14906).

Conflict of Interest: None declared.

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