Interleukin 10 Reduces Testicular Damage in Experimental Testicular Ischemia/Reperfusion Injury

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OBJECTIVE To evaluate the protective effect of interleukin 10 (IL-10) on biochemical and histopathologic changes in experimental testicular ischemia or reperfusion injury (RI) in rats.

METHODS Sprague-Dawley rats were randomly divided into 3 groups, each containing 7 rats; sham-control, I-R/untreated group, and I/R treated with IL-10. The ischemia period was 6 hours, and orchiectomy was performed after 1 hour of detorsion. IL-10 was given intraperitoneally in a period of 10 minutes before reperfusion. In all groups, ipsilateral orchiectomies were performed to make histologic examination and biochemical analysis such as malondialdehyde, glutathione peroxidase, and myeloperoxidase (MPO).

RESULTS IL-10 treatment significantly decreased the I-R–induced elevation in testes malondialdehyde levels. In the I-R/IL-10–treated group, testes glutathione peroxidase levels were increased compared with the I-R/untreated group rats. MPO activities were significantly increased in the testes tissues of the I-R/untreated group. However, in the I-R/IL-10–treated group, MPO levels significantly decreased. Histopathologically, in the I-R/untreated group rats, edema, congestion, hemorrhage among seminiferous tubules, and necrosis of the germinal cells were predominant features in sections. The testicular injury score was lower in the IL-10–treated group rats compared with the I-R/untreated group.

CONCLUSION IL-10 might play a protective role in reducing reperfusion injury. UROLOGY 83: 508.e1–508.e6, 2014. Published by Elsevier Inc.

Testicular torsion is a urologic emergency resulting in permanent testicular damage, loss of spermatogenesis, and necrosis. Therefore, it requires early diagnosis and surgical intervention to prevent subfertility and infertility.[1] Although the basic pathologic mechanism underlying testicular injury is not completely understood, reperfusion injury (RI) is responsible for a pathophysiological cascade, including an activation of neutrophils, inflammatory cytokines and adhesion molecules, and oxygen-derived free radicals.[2]

Reactive oxygen species (ROS), such as superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (HO), occur from oxygen species formed by the partial reduction of oxygen.[3] ROS after reperfusion might cause cellular devastation when they are generated excessively and hazardous to lipids, proteins, carbohydrates, and nucleic acids.[4] Oxidative stress means a change in the critical balance between free radicals and the scavenging capacity of antioxidant enzymes. Antioxidants enzymes catalyze the conversion of ROS into less reactive species.[5]

Glutathione (GSH) is an important antioxidant, and the increased concentration of GSH in cells might be useful in the prevention of oxidative damage of endothelial cells.[5] Malondialdehyde (MDA), the end product of lipid peroxidation, is a good marker of free radical–mediated damage and oxidative stress.[6] Infiltration of neutrophils into tissues is commonly assessed by changes in activity of myeloperoxidase (MPO), which is an enzyme found primarily in neutrophils.[7] In addition, it has been reported that the activated neutrophils located in the inflammatory foci and secreting MPO into the extracellular space can convert hydroperoxides into free radicals, triggering lipid peroxidation.[8]

Inflammatory cytokines such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1, and IL-8 accelerate inflammatory processes by inducing inflammatory molecules. These inflammatory modulators will cause more inflammatory leukocytes to infiltrate into the ischemic region.[9] Whereas, anti-inflammatory cytokines suppress the production of proinflammatory cytokine in protection

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of damaged tissues after ischemic.[10] IL-10 is a Th2 type cytokine and known to modulates inflammatory responses by inhibiting the production of proinflammatory cytokines, such as TNF-α, IL-1, IL-6, and IL-8. Furthermore, IL-10 has a role in upregulating monocyte production of soluble TNF-α and IL-1 receptor antagonist.[11] Recent data provided direct evidence that IL-10 protects endothelial function after an acute inflammatory stimulus by limiting local increases in superoxide anion.[12] In the present study, we aimed to evaluate the effects of IL-10 on testicular damage in a rat testicular torsion model.

MATERIALS AND METHODS

All experimental protocols were approved by the Abant Izzet Baysal University School of Medicine Animal Care and Use Committee. Twenty-one prepubertal (35-days old) male Sprague-Dawley rats, weighing 130-140 g, were randomly separated into 3 groups, each containing 7 rats. All animals were housed at a temperature- and light-controlled room with ad libitum access to water and rat chow.

Surgical procedures were performed under ketamine (50 mg/kg, i.p.) and xylazine (20 mg/kg, i.p.) anesthesia using sterile conditions. The scrotum was entered through a midline incision. The tunica vaginalis was opened, and the right testis was delivered to the surgical field. Torsions were created by rotating ipsilateral testes 720° in a clockwise direction for 6 hours and maintained by fixing the testes medially and laterally to the scrotum using 4/0 silk suture. After 6 hours of testicular torsion, detorsion procedure was performed again under sterile conditions. Right femoral vein was cannulated for administration of drugs and saline. The rats were divided into 3 groups according to the procedure performed. Sham group: a sham procedure was performed to determine the biochemical and histopathologic basal values. The right testis was brought out through the incision and then replaced with a fixation to the scrotum without torsion by 4/0 silk suture. Ischemia-reperfusion (I-R)/untreated group: after 6 hours of unilateral testicular torsion, detorsion of the twisted testis was performed and detorsed for 1 hour thereafter. Saline (NaCl at 0.02%, 10 mL/kg/min) was administered during the procedure. I-R/IL-10—treated group: the same surgical procedure (torsion and detorsion) as in group 2 was performed. The rats were treated with IL-10 (50 μg/kg; i.p., Sigma, St Louis, MO) intraperitoneally in a period of 10 minutes before reperfusion. In all groups, ipsilateral orchiectomies were obtained for biochemical and histologic investigations.

Analytical Procedures of Oxidative Stress-associated Parameters

All tissues were homogenized in ice-cold buffer (0.25 M sucrose, 10 mM Tris-HCl, and 0.25 mM phenylmethylsulfonyl fluoride; pH 7.4), and a portion of the homogenate was measured immediately for MDA (nmol/g) using a commercial kit (Calbiochem, San Diego, CA). Another portion of the homogenate was centrifuged at 10,000 ×g for 20 minutes at 4°C, and glutathione peroxidase (GPx; μmol/mg protein) activities in the supernatant were measured using commercial kits (Randox Laboratories Ltd, Crumlin, UK). MPO activities of all tissues were determined as a marker enzyme for measuring neutrophil accumulation in tissue samples, because it is closely correlated with the number of neutrophils present in the tissue. The homogenates were then centrifuged at 17,000 ×g at 4°C for 15 minutes, and MPO activity (U/g protein) in the supernatant was measured.

Table 1. Histologic grading system developed by Cosentino et al[18]

Grade I	Showed normal testicular architecture with an orderly arrangement of germinal cells.
Grade II	Injury showed less orderly, noncohesive germinal cells, and closely packed seminiferous tubules.
Grade III	Injury exhibited disordered sloughed germinal cells with shrunken pyknotic nuclei and less distinct seminiferous tubule borders.
Grade IV	Injury defined seminiferous tubules that were closely packed with coagulative necrosis of the germinal cells.

Histologic Examination

The extracted testes were immediately placed into 10% formalin solution. The tissue specimens were placed in paraffin blocks, sectioned at 5 μm, and stained with Hematoxylene & Eosine. The sections were examined under light microscope (Olympus BH-2; Olympus Corporation, Tokyo, Japan) by 2 investigators blindly. The histologic parameters were scored by Cosentino et al[13] classification (Table 1).

Statistical Analysis

All values were expressed as mean ± standard deviation. The significance of the data obtained from oxidative stress-associated parameters was evaluated using analysis of variance. Differences between means were analyzed using the post analysis of variance (Tukey's b) test. The Mann-Whitney *U* test and Kruskal-Wallis test were used to compare statistical analysis of histologic data. *P* values of $<.05$ were considered significant.

RESULTS

The MDA, GPx, and MPO values for the different groups are shown in Figure 1A, B, and C, respectively. The testes MDA levels in the I-R/untreated group were elevated by RI ($P < .05$); however, IL-10 treatment significantly decreased the RI-induced elevation in testes MDA levels ($P < .05$). RI caused a significant decrease in testes GPx levels ($P < .5$) when compared with sham group. However, in the IL-10—treated group, testes GPx levels were increased compared with the I-R/untreated group. The levels of MPO activity were significantly increased in the testes tissues of the I-R/untreated group ($P < .001$) compared with the sham group. In the I-R/IL-10—treated group, MPO activity significantly decreased ($P < .05$) compared with the I-R/untreated group.

The testicular injury score increased in the I-R/untreated and I-R/IL-10—treated group rats compared with sham group ($P < .05$, $P < .05$, respectively). However, the testicular injury score in I-R/IL-10—treated group was decreased compared with the I-R/untreated groups ($P < .05$; Fig. 2).

Histopathologically, the rats in the sham group had essentially normal seminiferous tubule morphology