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ORIGINAL ARTICLE

**Dinçer Avlan · Kubilay Erdouğan · Burak Çimen
Duygu Düşmez Apa · Ismail Cinel · Selim Aksöyek**

The protective effect of selenium on ipsilateral and contralateral testes in testicular reperfusion injury

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Abstract This study was designed to investigate the effect of selenium on ipsilateral and contralateral testicular damage after unilateral testicular torsion/detorsion (T/D). Thirty-two male rats were divided into four groups, each containing eight rats. Torsion was created by rotating the right testis 720° in a clockwise direction. Group 1 underwent sham operation to determine basal values for biochemical and histopathological evaluation. Sham operation was performed in group 2, and sodium selenate (0.2 mg/kg) was given intraperitoneally. Group 3 served as a T/D group, receiving 4-h torsion and 4-h detorsion. Similarly, in group 4 sodium selenate (0.2 mg/kg) was injected intraperitoneally 20 min before detorsion. Bilateral orchiectomies were performed for measurement of tissue malondialdehyde (MDA) levels and superoxide dismutase (SOD) activities and histopathologic examination. The results were compared statistically. The highest MDA and the lowest SOD values were determined in both testes in group 3. There were statistically significant differences in MDA levels and SOD activities in group 3 compared with group 4. Specimens from group 3 had a significantly greater histologic injury than other groups. These results suggest that ischemia-reperfusion injury occurred in both testes after unilateral testicular T/D and that selenium administration before detorsion prevents reperfusion injury in testicular torsion.

Keywords Testicular torsion · Ischemia-reperfusion injury · Selenium

D. Avlan (✉) · S. Aksöyek
Department of Pediatric Surgery, School of Medicine,
Mersin University, Zeytinlibahçe 33070, Mersin, Turkey
E-mail: dinceravlan@yahoo.com
Fax: +90-324-3374305

K. Erdouğan
Department of Urology, School of Medicine,
Mersin University, Mersin, Turkey

B. Çimen
Department of Biochemistry, School of Medicine,
Mersin University, Mersin, Turkey

D. Düşmez Apa
Department of Pathology, School of Medicine,
Mersin University, Mersin, Turkey

I. Cinel
Department of Anesthesia and Reanimation,
School of Medicine, Mersin University, Mersin, Turkey

Introduction

Testicular torsion or torsion of the spermatic cord is a surgical emergency that requires immediate intervention to untwist the affected gonad. This emergency state can lead to testicular necrosis and decreased fertility [1, 2]. The testicular damage due to torsion and detorsion shares resemblances with the phenomenon of ischemia–reperfusion (I/R) injury observed in other tissues. A possible cause of the testicular injury due to torsion and detorsion is an I/R injury attributed to neutrophil infiltration and oxygen free radicals [3]. These free radicals, including hydrogen peroxide (H_2O_2) or hydroxyl radicals (OH^-), superoxide anions (O_2^-), and nitric oxide (NO) and its toxic products such as peroxynitrite ($ONOO^-$), cause lipid peroxidation in the cellular and mitochondrial membranes. Peroxidation of the lipids in membranes changes membrane permeability or disrupts membrane integrity and thus cell integrity [4, 5].

Institution of correct treatment after the ischemic insult can attenuate or prevent the deleterious effects of ischemia and subsequent reperfusion. One method of effective pharmacological therapy against reperfusion injury is by reestablishing cell membrane integrity via antioxidant therapies such as *N*-acetyl cysteine, vitamins E and C, superoxide dismutase, catalase, lazaroids, allopurinol, and selenium [6]. Selenium is a trace element and may act as a radical scavenger [7]. It is a structural component of glutathione peroxidase (GPx), which is important in providing protection against oxidative damage. It has been demonstrated that selenium supplements increase GPx activity [8]. GPx together with

superoxide dismutase (SOD) and catalase form part of the cellular antioxidant defense system against reactive molecules and free radicals [9]. It has been shown that selenium has beneficial effects in preventing I/R injury in heart [10], lung [11], liver [12], and kidney [13].

It was, therefore, the aim of our study to evaluate the protective effect of selenium on the ipsilateral and contralateral testes after testicular I/R injury.

Materials and methods

This study was approved by the ethics committee of Mersin University School of Medicine. Thirty-two male Wistar rats weighing 200–250 g were used in this study. The animals were housed at room temperature and in a controlled environment of 12-h light–dark periods with free access to water and rat chow.

Animals and experimental design

The rats were randomly divided into four groups each consisting of eight rats. Surgery was conducted under one intramuscular injection of ketamine (80 mg/kg) anesthesia. The testes were exposed through identically opened and closed right-sided midscrotal vertical incisions. In group 1 (sham-control group), the testes were brought out through the incision, a 6–0 silk suture was placed through the tunica albuginea, and the testes were relocated into the scrotum with no additional intervention. The silk suture of the testes was removed 4 h after sham operation with no additional intervention, and the incision was closed. In group 2 (sham + Se group), the same surgical procedure was done as in group 1, but sodium selenate (Aldrich, USA) diluted in 0.9% NaCl (0.2 mg/kg) was injected intraperitoneally 20 min before sham operation. In group 3 (torsion/detorsion [T/D] group), torsions were created by rotating the right testis 720° in a clockwise direction for 4 h. The torsion was maintained by fixing the testis in the scrotum with a 6-0 silk suture, and the incision was closed. After a 4-h torsion period, the incision was entered, the suture was removed, and the right testis was detorted and replaced into the scrotum for 4 h. In group 4 (Se + T/D group), pretreatment with intraperitoneal sodium selenate, 0.2 mg/kg diluted in 0.9% NaCl, was carried out 20 min before the detorsion. The torsion lasted for 4 h followed by a detorsion period of 4 h. At the end of each experiment, bilateral orchiectomies were performed for the tissue biochemical assays and histopathological examinations in all groups.

Biochemical analyses

Tissues were prepared for the metabolic assays as described previously [14]. Superoxide dismutase (SOD) and malondialdehyde (MDA) assays were performed as described previously [15, 16]. SOD enzyme activities were expressed as international units. One unit of SOD activity was defined as the amount of enzyme protein causing 50% inhibition in nitroblue tetrazolium (NBT) reduction rate, and results were expressed as unit/mg protein. The MDA method was based on the spectrophotometric absorbance measurement of the pink product of thiobarbituric acid–malondialdehyde complex formation [16]. MDA levels were expressed as nmol/mg protein. Protein amount was measured by Lowry's method [17]. The spectrophotometer wavelengths for SOD and MDA are 560 nm and 532 nm, respectively.

Histopathological examination

The testicles were removed and fixed in Bouin's solution. After tissue processing, they were placed in paraffin blocks, sectioned at 5 μm, and stained with hematoxylin and eosin. The tissue sections were evaluated under light microscopy by a blinded pathologist according to the classification of Cosentino et al. [18] as follows:

Grade 1 showed normal testicular architecture with an orderly arrangement of germinal cells.

Grade 2 injury showed less orderly, noncohesive germinal cells and closely packed seminiferous tubules.

Grade 3 injury exhibited disordered, sloughed germinal cells with shrunken pycnotic nuclei and less distinct seminiferous tubule borders.

Grade 4 injury defined seminiferous tubules that were closely packed with coagulative necrosis of the germinal cells.

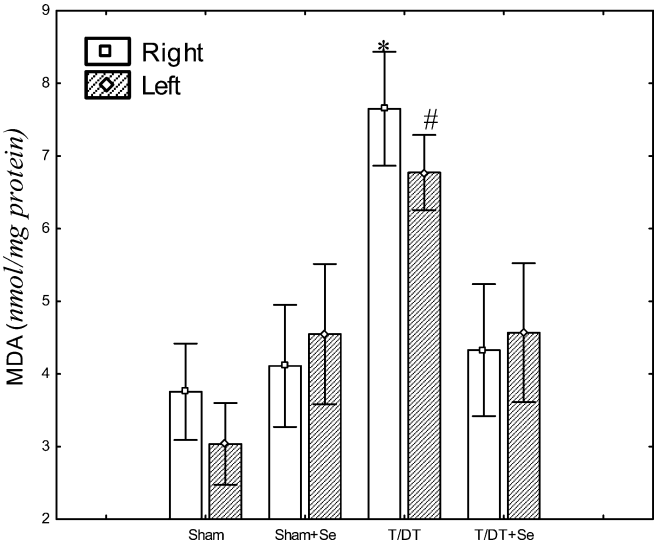

Fig. 1 Mean values of tissue malondialdehyde levels of all groups. Data expressed as mean ± SD. *,# $P < 0.01$ compared with other groups

Statistical analyses

Histopathological findings were compared using a Kruskal–Wallis test for groups. After the measurement of tissue MDA and SOD levels, results were expressed as mean ± standard deviation (SD). Data were analyzed by analysis of repeated measurement variance followed by Student's Newman-Keuls test. P values of less than 0.01 were considered significant.

Results

Biochemical analyses

The results of testicular MDA values and SOD enzyme activities in all groups are shown in Figs. 1 and 2. The highest MDA values and the lowest SOD activities in both testes were demonstrated in group 3. MDA values were significantly reduced in both ipsilateral and contralateral testes in group 4 with selenium supplementation. MDA values of group 4 showed statistically significant differences compared with group 3 ($P < 0.01$; Fig. 1). SOD enzyme activities were increased in both testes in group 4 with selenium pretreatment. There were statistically significant differences in SOD enzyme activities in group 3 compared with other groups ($P < 0.01$; Fig. 2).

Histopathologic examination

The findings of the histopathologic evaluation for each group are shown in Fig. 3. The testes of rats in groups 1 and 2 had normal testicular architecture (Fig. 4). In the T/D group (group 3), most of the testes demonstrated grade 3 (Fig. 5) or grade 4 injury in both testes. These findings were observed more in ipsilateral than in contralateral testes. Selenium-treated animals (group 4) showed an improved histological appearance in both testes compared with group 3 ($P < 0.01$; Fig. 3).

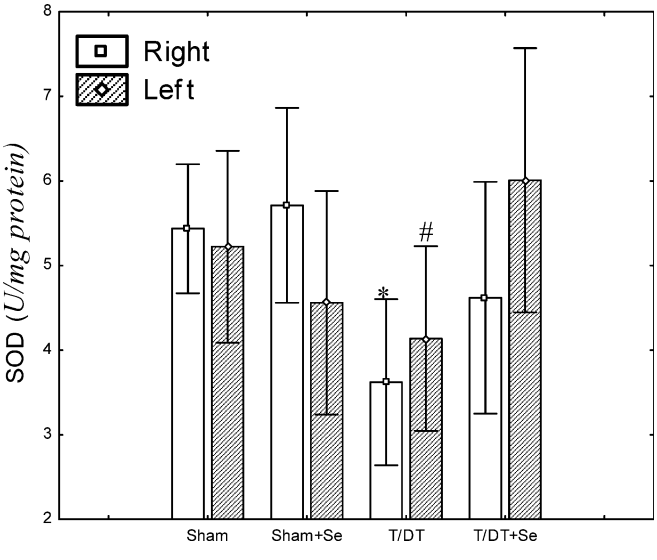

Fig. 2 The results of tissue superoxide dismutase activities in both testes of all groups. Data presented as mean ± SD. *,# $P < 0.01$ compared with other groups

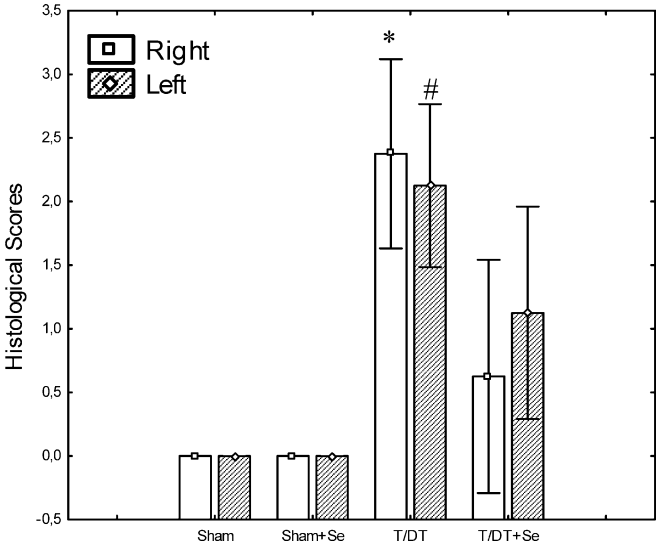

Fig. 3 Histopathologic scores of tissue injury in both testes of all groups. All data expressed as mean ± SD. *,# $P < 0.01$ compared with other groups

Discussion

The present study demonstrates that unilateral testicular T/D causes testicular damage in both testes as evidenced by biochemical and histological changes in tissues and that selenium pretreatment prevents the biochemical changes and protects the morphology in both ipsilateral and contralateral testes after unilateral testicular T/D.

Many studies have shown that testicular tissue injury is related to the degree of rotation of the testicle and the duration of torsion [3, 19]. In general, at least 4 h of 720° unilateral testicular torsion causes enough testicular tissue injury in both ipsilateral and contralateral testes in experimental models [20, 21]. Even though many experimental studies have clearly revealed that the contralateral nontorted testis is damaged after unilateral testicular torsion [22–25], some studies have shown that this phenomenon is not always observed [26, 27]. The mechanisms of contralateral testicular injury remain to be identified. Some authors have suggested that immunological responses play a pivotal role in pathogenesis [22, 23]. Wallace et al. [24] have put forward alternative explanations such as the release of acrosomal enzymes or a neurohumoral mechanism and have also proposed the term "sympathetic orchiopathia" [24]. The most accepted explanation, by Tanyel et al. [25], says that the contralateral testis may be damaged by a reflexive decrease in blood flow from the activated sympathetic system [25]. In our study, 720° and 4-h unilateral tes-

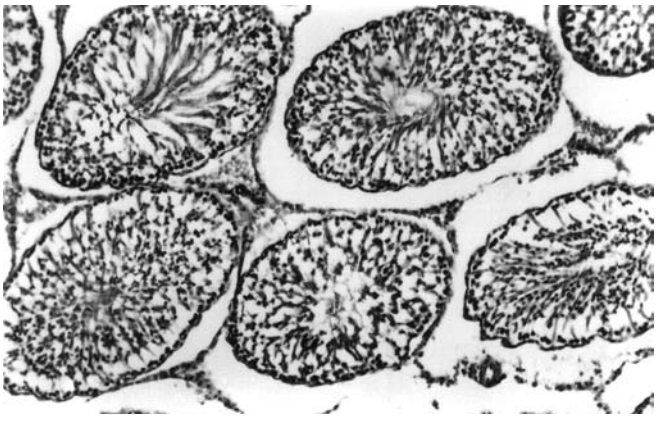

Fig. 4 Orderly arrangement of seminiferous tubules in testes in sham and sham + selenium groups (hematoxylin and eosin, ×200)

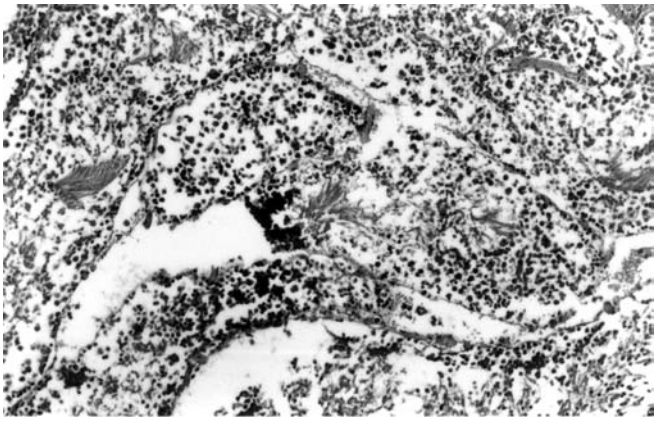

Fig. 5 Disordered, sloughed germinal cells within the seminiferous tubules in the torsion/detorsion group (hematoxylin and eosin, ×200)

ticular T/D caused significant biochemical changes in both testes. Furthermore, histopathologically we observed severe testicular injury such as coagulative necrosis and disarray of germ cell layers in not only ipsilateral testes but also in contralateral testes after unilateral testicular T/D.

The pathophysiological mechanism in testicular damage due to T/D is I/R injury. Ischemia with consecutive reperfusion causes oxidative/nitrosative stress, which is characterized by an imbalance between reactive oxygen species (ROS), reactive nitrogen species (RNS), and the antioxidative defense system. These ROS, such as $H_2O_2^-$, OH^-, and O_2^-, and RNS such as NO and $ONOO^-$ are well known for inducing injury in local and distant tissues [5, 28]. Several studies have shown that ROS cause peroxidation in testis after testicular T/D. Additionally, recent studies have demonstrated the role of NO and adhesion molecules in testicular torsion [29, 30]. In our study, we observed that the level of tissue MDA, which is the product of lipid peroxidation, was significantly increased in both testes in group 3. The increase of MDA levels supports I/R injury on ipsilateral and contralateral testes. Histopathologic examinations were in accordance with elevated testicular tissue MDA levels.

Antioxidant enzymes, also known as free radical scavengers, such as SOD, GPx, and catalase, convert free oxygen radicals to water and oxygen. SOD levels decrease reperfusion injury in a variety of different organ systems [28]. SOD is a key component in testicular cell growth, differentiation, and protection [31]. It has been reported that SOD plus catalase treatment caused significant improvement of testicular function after I/R injury [3]. Saba et al. [32] have shown that levels of SOD decrease in ipsilateral testis and not in contralateral testis. On the contrary, in our study we found that SOD enzyme activities decreased both in ipsilateral and contralateral testis in group 3. Decreased ipsilateral and contralateral tissue SOD activities might have occurred as a result of consumption of the enzyme by oxidative stress. Furthermore, SOD activities were preserved by selenium pretreatment in group 4. These results suggest that selenium might protect against oxidative damage by supporting antioxidant enzyme systems in tissues.

Selenium is an integral component of GPx and plays a vital role in protecting aerobic organisms from oxidative and nitrosative tissue damage. In addition to its known effect on Gpx, selenium is noted to enhance the H_2O_2 scavenging activity of GPx [8]. Also, it may directly neutralize peroxynitrite, which is a strong oxidant and an oxidative and nitrosative stress mediator [33]. It has been demonstrated that selenium has protective effect in I/R injury in many tissues, including neural tissue [34]. In our previous study we showed that selenium pretreatment prevents bacterial translocation in intestinal I/R injury [35]. In this study we demonstrated that selenium prevented ipsilateral and contralateral testes after testicular T/D, as evidenced by biochemical parameters and histopathological changes. According to the results of this study, we believe that contralateral testicular injury after unilateral torsion may result from a reflexive decrease in blood flow by an activated sympathetic system, as the selenium, an antioxidant trace element, protected against testicular tissue injury in both testes in unilateral testicular torsion.

As a transcriptional regulatory protein, nuclear factor-kappa B (NF-kB) plays a central role in regulating cytokines and other mediators involved in tissue injury associated with I/R [36]. Recently, it has been reported that selenium inhibits cytokine-induced NF-kB activation in oxidative stress [37, 38]. Although we did not evaluate NF-kB activity in this study, we believe that selenium supplementation might have suppressed NF-kB activation in our study.

In conclusion, selenium decreased lipid peroxidation and prevented histological damage in both ipsilateral and contralateral testes. The results of this study suggest that selenium treatment decreases reperfusion injury in both testes. Selenium might be a candidate in medical treatment algorithms for clinical states that involve I/R

phenomena. However, further studies are necessary to evaluate the effects of selenium on testicular torsion.

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