

The Effect of L-Carnitine on Testicular Ischemia-Reperfusion Injury due to Testicular Torsion in Rats

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ABSTRACT

This experimental was study designed to examine the efficacy of L-carnitine in preventing reperfusion injury following testicular torsion. We aimed to evaluate the effects of L-carnitine, administered during the reperfusion period, on the torsed and contralateral non-torsed testes in cases of unilateral testicular torsion in rats. This experimental study was performed in the research laboratory Dicle University, School of Medicine, between March 1, 2007 and May 31 2007. The study included 20 adult male rats those 6 months old that were divided into 3 groups: a sham group (group 1), ischemia/reperfusion group (group 2), and ischemia/reperfusion + L-carnitine group (group 3). In group 2 one ml of serum physiologic was injected intraperitoneally versus 500 mg/kg of L-carnitine in group 3 immediately after detorsion following a 4-h torsion period; the doses were repeated after 8 h later. Histologically, Sections of the left testes in group 2 showed irregularities in germinal epithelial cell configuration and degeneration in several cells, and all the samples of the right testes in group 2 had arrested sperm maturation and primary spermatocytes had a dusky appearance in some tubules, while all remaining groups were normal. L-carnitine had a positive effect on ipsilateral and contralateral testes. The positive effect of L-carnitine in reducing reperfusion damage in ipsilateral and also in contralateral testis was histopathologically observed. We think L-carnitine should always be applied immediately after reperfusion period and should be repeated after 8 hours in testicular torsion cases.

Key words: L-carnitine-ischemia-reperfusion-testis-torsion

Farelerde Testis Torsiyonu Nedeniyle Ortaya Çıkan İskemi-Reperfüzyon Hasarı Üzerine L-Karnitin'in Etkisi

ÖZET

Yapılan bu deneysel çalışmada testis torsiyonu sonucu gelişen iskemi ve detorsiyon sonrasında gelişecek olan reperfüzyonun testiste neden olacağı hasarı önlemede L-Karnitin'in etkinliği araştırılmıştır. Bu çalışmanın amacı, ratlarda oluşturulan tek taraflı torsiyon ve detorsiyonu takiben, torsiyone testiste ve karşı testiste gelişebilecek olan reperfüzyon hasarı üzerine L-Karnitin'in etkilerini araştırmaktır. Toplam 20 yetişkin erkek rat kontrol grubu, (Grup 1) iskemi / reperfüzyon grubu (Grup 2) ve iskemi / reperfüzyon + L-Karnitin grubu (Grup 3) olmak üzere 3 gruba ayrıldı. Dört saatlik torsiyon periyodundan sonra yapılan detorsiyondan hemen sonra ikinci gruba 1 ml serum fizyolojik, 3. gruba ise 500 mg/kg L-Karnitin intraperitoneal olarak verildi. Ve bu dozlar 8 saat sonra tekrarlandı. Histolojik olarak L-Karnitin'in torsiyone testiste ve karşı testiste gelişen iskemi / reperfüzyon hasarını düzelttiği görülmüştür.

Anahtar kelimeler: L-Karnitin, iskemi-reperfüzyon, testis, torsiyon

INTRODUCTION

Testicular torsion is a urologic emergency commonly seen in males, which may cause loss of the gonad. If this emergency is not treated. While it could be encountered in all age groups, because testicular volume increases at a faster rate than the mesenteric structure during puberty, the risk of torsion increases during this period (1). Testicular edema and pain occur as a result of the initial

venous congestion, and venous congestion disrupts arterial circulation. As in other tissues, reduced blood flow leads to hypoxia. Spermatogonium and spermatocytes are the cells most sensitive to testicular ischemia (2). The main principle behind the treatment of damage associated with ischemia is the establishment of reperfusion; therefore, cases with testicular torsion should be treated immediately and reperfusion must be achieved.

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During the reperfusion period various reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, and hydrogen radicals, increase in number in testicular tissue and cause testicular destruction (3). ROS lead to protein denaturation and lipid peroxidation in cell membranes, which results in Deoxyribonucleic acid (DNA) damage (3-6). In other words, after the reperfusion that follows ischemia, damage occurs in the torsed testicle and even in the contralateral one. Experimental studies have used various drugs in order to prevent the damage that can occur in the ipsilateral and contralateral testes after detorsion (7,8) L-carnitine, a vitamin-like compound biosynthesized from amino acids, is used to prevent ischemia and reperfusion injury. It is synthesized endogenically from lysine and methionine in skeletal and cardiac muscle, the liver, kidneys, and brain⁹. L-carnitine facilitates passage of fatty acids from mitochondrial membranes during β -oxidation during fatty acid metabolism. Moreover, it contributes to transportation of the end products of peroxisomal fatty acid oxidation and α -ketoacids, which are composed of branched-chain amino acids, has antiradical and antioxidant functions, and acts as an ROS scavenger (10-13). As such, the present study aimed to histopathologically evaluate the effects of L-carnitine on ischemia-reperfusion damage.

MATERIAL AND METHODS

This experimental study was performed in the research laboratory Dicle University, School of Medicine, between March 1, 2007 and May 31, 2007. After obtaining the approval of the Dicle University School of Medicine Ethics Committee, the study began with 20 adult male Wistar albino rats those 6 months old weighing between 250 and 300g. The experimental animals were housed at $22\pm 10^{\circ}\text{C}$ under a 12 h light/12 h dark cycle, with ad libitum access to a standard pellet diet for rats and tap water. The L-carnitine used in this study was manufactured by Santa Farma. Each 5-ml Carnitene[®] ampoule contained 1 g of L-carnitine.

The rats were divided into the following 3 groups.

Group 1: Sham group (n = 4). Under ketamine anesthesia the right testis was brought out through a scrotal midline incision and fixed to the dartos fascia of the scrotum via the tunica albuginea with 4/0 silk sutures for 4 h, without applying torsion.

Group 2: Ischemia/reperfusion (I/R) group (n = 8). Under

ketamine anesthesia the right testis was brought out through a scrotal midline incision, torsed 720° clockwise, and fixed to the dartos fascia of the scrotum via the tunica albuginea with 4/0 silk sutures for 4 h. In this group 1 ml of serum physiologic was injected intraperitoneally immediately after detorsion and again 8 h later.

Group 3: Ischemia/reperfusion + L-Carnitine (I/R + L-Carnitine) group (n = 8): The procedures in this group were exactly the same as used for group 2, the only difference being that 500 mg/kg of L-Carnitine was injected intraperitoneally instead of serum physiologic, which was repeated 8 h later.

Then, 20 h after detorsion and the reperfusion period all rats were sacrificed with high-dose ketamine (120 mg kg^{-1} , intramuscularly). Each testis was orchietomized and placed in Bouin's solution for histologic examination.

Histologic Method

Following orchietomy, testicular specimens were individually immersed in Bouin's solution for fixation. They were dehydrated in alcohol and embedded in paraffin. Then, 5- μm sections were obtained, deparaffinized, and stained with hematoxylin and eosin, and Van Gieson. Two slides were prepared from each testicular sample. Specimens were evaluated in random order with standard light microscopy by an observer blinded to which group the samples belonged.

RESULTS

Histologic Results of the Left (contralateral) Testes

Left testes in group 1 were normal. Seminiferous tubules appeared normal and there were Leydig cells in the interstitial tissue. Sections of the left testes in group 2 showed irregularities in germinal epithelial cell configuration and degeneration in several cells, while congestion and edema were observed in interstitial tissue (Figure 1). Tissue samples of the left testes in group 3 were normal, exception for detachment of germinal epithelial cells (in patches) from the basal lamina (Figure 2).

Histologic Results of the Right (ipsilateral) Testes

Sections of the right testes in group 1 had normal seminiferous tubule structure and Leydig cell configuration.

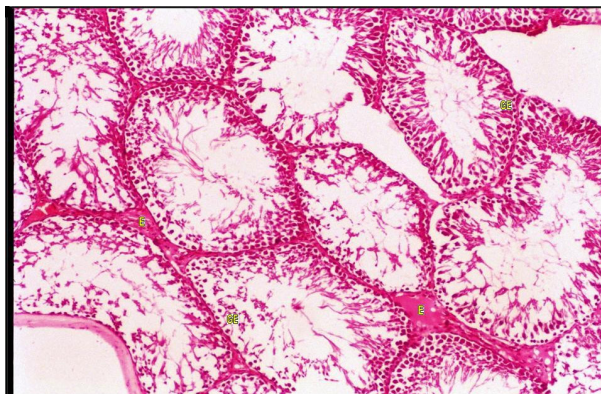


Figure-1. In group II, sections of the left (contralateral) testes showed irregularities in germinal epithelial cell configuration and degeneration in several cells while congestion and edema were found in the interstitial tissue. GE: Germinal epithelium, E: Edema (H&E, original magnification X41).

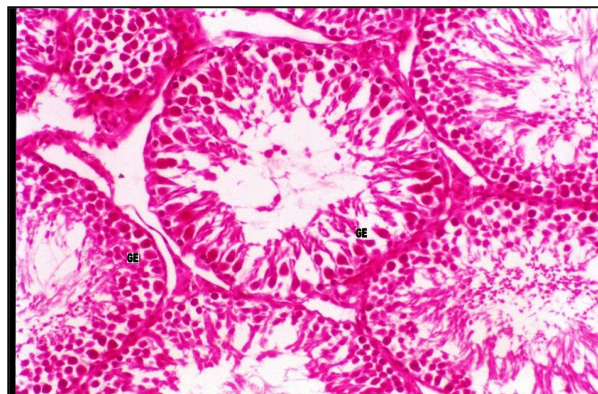


Figure-2. In Group III, samples of the left testes demonstrated a testicular tissue close to normal with the exception of the detachment of germinal epithelial cells from basal lamina in patches. GE: Germinal epithelium (H&E, original magnification X82).

In group 2 some seminiferous tubules were detached from the basal lamina and there were seminiferous tubules with no germinal epithelium, only basal lamina. In general, all the samples of the testes in group 2 had arrested sperm maturation and primary spermatocytes had a dusky appearance in some tubules. Moreover, coagulative necrosis and Leydig cell aplasia were observed in some testicular samples in group 2 (Figure 3). Sections of the right testes in group 3 appeared almost normal, with the exception of interstitial edema (Figure 4).

DISCUSSION

Testicular torsion is an acute condition that is seen most frequently in pubescent males (14,15). The principal pathology in testicular torsion is damage that occurs to the ipsilateral testis as a result of ischemia due to twisting of the testis; additional damage occurs in the ipsilateral and contralateral testes during the reperfusion period that follows detorsion (7). Elevated ROS levels during ischemia and reperfusion (I/R) lead to cellular DNA damage and cause lipid peroxidation between tissues and cell membranes (7,16). The mechanism responsible for tissue destruction in the ipsilateral and contralateral testes remains unknown. As the cells most sensitive to this tissue damage are all germ cell series, the resultant destruction may lead to infertility. Preventing such damage from occurring during the reperfusion period is as important as immediate detorsion of the tes-

tis. The literature contains many reports of the effects of ROS on I/R damage following torsion in rat testes. Moreover, there are studies that report the protective effect of antioxidants against I/R-induced damage (17). Several studies have reported that reperfusion damage was prevented with the use of certain agents during the reperfusion stage, such as L-carnitine, pentoxifylin, and allopurinol (8,18). Zhai et al. (19) reported that adding L-carnitine to the diet could improve semen production. Vicari et al. (20) reported that L-carnitine was the only effective treatment agent for infertility patients with prostatovesiculourethritis. Those results and L-carnitine's known antiradical and antioxidant activity encouraged us to use this compound in the present study.

The present study aimed to histologically determine the efficacy of L-carnitine in the prevention of testicular damage associated with ischemia/reperfusion. In the present study testes were torsioned for 4 h. Duru et al. (21) reported that detorsion significantly increased malondialdehyde (MDA) levels only if the duration of the initial torsion was less than 3 h; they do not advise increasing the duration of torsion in order to evaluate the reperfusion effect. Even though Pavabash et al. (22) preferred 1-h torsion in their study that examined the effect of N-acetylcysteine on torsioned testes, we did not consider using a torsion time less than 4 h. We chose to use 4-h

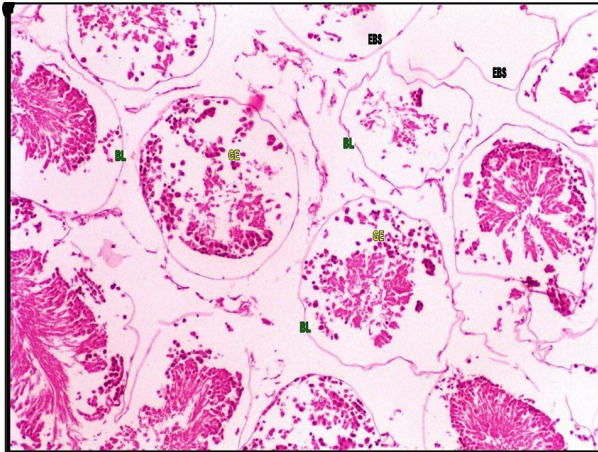


Figure-3. In Group II, some seminiferous tubules of right torted testes were observed to be detached from the basal lamina, whereas there were seminiferous tubules which had no germinal epithelium but only basal lamina. In general, all the samples of the testes in this group showed an arrest in sperm maturation and primary spermatocytes were observed to have a dusky appearance in some tubules. Moreover, coagulative necrosis and Leydig cell aplasia were determined in some testicular samples of this group. GE: Germinal epithelium EBS: Emptied seminiferous tubule BL: Basal lamina (H&E, original magnification X41).



Figure-4. In group three sections of the right torted testes manifested an appearance close to normal with the exception of interstitial edema. E: Edema, L: Leydig Cells, ST: Seminiferous tubule (H&E, original magnification X41).

torsion because clinically it is the shortest acceptable time period for this type of pathology. Histopathological results obtained in the present study show that sperm maturation in all the sections of the torted right testes was arrested, and that the primary spermatocytes in several tubules had a dusky appearance. Moreover, coagulative necrosis and Leydig cell aplasia were observed in those sections. Furthermore, several seminiferous tubules exhibited detachment of germinal epithelium from the basal lamina and absence of germinal epithelium. Torted right testes in rats that were administered L-carnitine (group 3) had a histological appearance that was almost normal, with the exception of congestion and interstitial edema in stromal tissue. L-carnitine protected the ipsilateral testis from the deleterious effects of torsion. Dökmeci et al. (23), however, did not observe a protective effect of L-carnitine in ipsilateral testis; even though the dosage of the L-carnitine was the same and the torsion time was similar to those used in the present study, their application method was quite

different. They administered L-carnitine only 30 min before the reperfusion period, whereas we administered L-carnitine immediately after the reperfusion period and then 8 h later, which we think is ideal for protecting the ipsilateral and contralateral testis from damage due to torsion.

The effect of reperfusion injury to the contralateral testis is a contentious subject. Dökmeci et al. (23) histopathologically observed reperfusion injury in contralateral testes during unilateral testicular torsion, whereas Gurdal et al. (16) did not. Gurdal et al. (16) applied torsion for only 1 h and concluded that they did not observe any pathological change in the contralateral testis. Histological examination of the sections of the contralateral left testes in the present study showed marked alterations, including irregular germinal epithelial cell configuration along with degeneration in some cells, congestion and edema in interstitial tissue in group 2. As did the present study, Dökmeci et al. observed marked alterations in contralateral testes. These alterations in contralateral testes were prevented by L-carnitine in the present study, as in Dökmeci et al.'s study.

The torsion time in the present study (4 h) and Dökmeci's

study (5 h) was longer than that used by Gurdal et al (1h). Most probably, 1-h torsion was insufficient for observing marked alterations in contralateral testes. In conclusion, it was observed that L-carnitine histopathologically reduce the reperfusion damage that occurs following detorsion of testicular torsion. We recommend the use of L-carnitine in patients with torsed testes immediately following detorsion and again 8 h later in order to preserve the ipsilateral and contralateral testes. Nonetheless, we think that there is a need for additional research in order to learn more about L-carnitine therapy, such as the optimal dose and duration of therapy, and its effect in cases with long-term (>4 h) testicular torsion.

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