



Protective effect of tunica albuginea incision with tunica vaginalis flap coverage on tissue damage and oxidative stress following testicular torsion: Role of duration of ischemia

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Summary

Objective

This experimental study used a rat model to investigate the effect of a tunica albuginea incision with tunica vaginalis flap coverage on tissue damage and oxidative stress caused by testicular torsion and its relationship with the duration of ischemia.

Materials and methods

The test animals were divided into the following groups: G1, sham procedure; G2, testicular torsion for 1, 5, or 9 h followed by detorsion; G3, testicular torsion for 1, 5, or 9 h followed by detorsion using flap technique. Testicular torsion was induced by 720° counterclockwise rotation of the left testis. After the period of torsion, the flap technique was employed for detorsion. The oxidative stress and testosterone levels were measured at 24 h post procedure. Further assessment was carried out by histomorphometry at 30 days post procedure. The histological parameters included the Johnsen score,

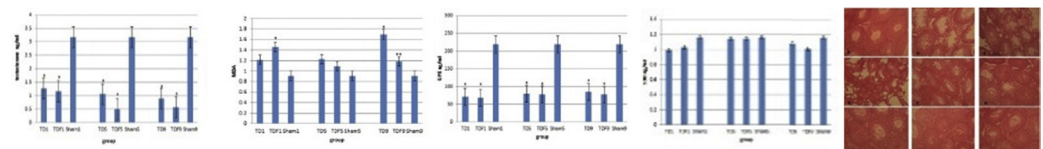
diameter of the seminiferous tubules, and thickness of seminiferous tubule epithelium.

Results

The histological parameters in the G2 group showed a significant change in relationship with the duration of ischemia. In the G3 group, flap coverage improved the histological parameters only for the 9-hour torsion subjects. The levels of testosterone, glutathione peroxidase (GPX), and superoxide dismutase significantly decreased in all subgroups of G2 and G3, and the malondialdehyde level increased as the duration of ischemia increased. Flap coverage decreased the malondialdehyde level only in the 9-hour torsion subjects.

Conclusions

Flap coverage reduced tissue damage as the duration of ischemia increased. The findings of the rat model suggested that a tunica albuginea incision with tunica vaginalis flap might have provided a protective effect in long-term ischemia.



Figure

Introduction

Scrotal pain is a common complaint, and testicular torsion accounts for 26% of cases of acute scrotum. This complaint must be differentiated from other complaints of testicular pain because a delay in diagnosis and management can lead to loss of the testicle [1,2]. Testicular torsion occurs when the spermatic cord twists around the testicles, cuts off the blood supply, and causes testicular atrophy [3]. The prevalence of testicular torsion is 1 per 4000 males aged <25 years of age [4]. Two important predictors of testicular damage are the degree and duration of torsion [5,6], as the testes can be saved in 90% of cases if detorsion occurs within 6 h of torsion occurring; this decreases to 50% after 12 h and 10% after 24 h. Accordingly, it is necessary to diagnose this condition and immediately perform the required intervention to save the testicular tissue and prevent orchiectomy [7].

The major pathophysiology in testicular torsion is damage caused by ischemia/reperfusion, followed by the production of reactive oxygen species (ROS). The increased production of ROS results in damage to the DNA, endothelial destruction, and testicular germ cell apoptosis [8]. In damage such as that caused by testicular torsion, the pressure in the spaces surrounded by non-elastic tissues, such as the tunica albuginea, increases and is a manifestation of acute compartment syndrome [9,10]. The increase in pressure in a fixed-volume space endangers normal blood circulation and tissue functioning; this often occurs in fractures, vascular damage, and ischemia/reperfusion [11].

Testicular decompression and the tunica vaginalis flap application technique were introduced in 2008 by Kutikov et al. [9]. Given that intratesticular pressure increases as the length of ischemia increases, recent studies have suggested the use of a tunica albuginea incision with tunica vaginalis flap coverage to decrease pressure on the testicular tissue after ischemia/reperfusion, although it has produced varied effects on histological parameters [12–14]. The present study examined the effect of the tunica albuginea incision with tunica vaginalis flap coverage on tissue damage and oxidative stress caused by testicular torsion and its relationship to the duration of ischemia.

Materials and methods

This experimental study was performed on 63 adult rats weighing 250–300 g. All rats were housed under a 12/12-hour light/dark cycle. The room temperature was maintained at 23 ± 2 °C and humidity at 60–70%. The rats in all groups had free access to food and water. The ethical considerations were based on the guidelines for laboratory animals from the Research and Technology Deputy of Gonabad University of Medical Sciences.

The animals were randomly divided into three groups:

Group 1: The sham group underwent a sham procedure, in which the testis was exposed without application of torsion. These were divided into three subgroups

according to length of procedure. After 1 h, 5 h, or 9 h, the left testis was exposed and replaced in the scrotum by orchidopexy. The subgroups were S1 ($n = 7$) for 1 h, S5 ($n = 7$) for 5 h, and S9 ($n = 7$) for 9 h.

Group 2: The testicular torsion detorsion (TD) group was divided into three subgroups according to length of time to detorsion: TD1 ($n = 7$) for 1 h, TD5 ($n = 7$) for 5 h, and TD9 ($n = 7$) for 9 h.

Group 3: The testicular torsion detorsion with tunica vaginalis flap coverage (TDF) group was divided into three subgroups according to length of torsion: TDF 1 ($n = 7$) for 1 h, TDF5 ($n = 7$) for 5 h, and TDF9 ($n = 7$) for 9 h.

Surgical procedure

The rats were anesthetized with ketamine (50 mg/kg), and xylazine (10 mg/kg). The left testis was exposed through a longitudinal scrotal incision and dissected. Torsion was created by 720° counterclockwise rotation of the left testis. Then the testis was carefully returned to the scrotum and pexed in position using three 6/0 silk sutures to approximate the tunica albuginea of the lower pole and the two lateral poles of the testis to the dartos. The scrotum was closed in a single plane with 5/0 silk sutures.

In the TD group, testicular torsion was maintained for 1, 5, or 9 h, followed by detorsion in all rats. After detorsion, the testis was replaced into the scrotum and fixed with 6/0 silk suture. The incision was closed in a single plane with 5/0 silk sutures and maintained for 30 days. In the TDF group, testicular torsion was maintained for 1, 5, or 9 h, followed by detorsion with tunica vaginalis flap coverage. This was performed by a longitudinal incision of the tunica albuginea from the upper pole to the lower pole of the testis, after which a tunica vaginalis flap with an intact vascularized pedicle was harvested. The flap and the testis fixation were sutured with 6/0 silk sutures and replaced in the scrotum. The incision was closed in a single plane with 5/0 silk sutures and maintained for 30 days [12].

The rats were anesthetized using ketamine-xylazine at 24 h post procedure and 1 cc of blood was drawn from an ophthalmic vein to measure the levels of testosterone and antioxidant enzymes. Blood samples were kept at room temperature and then centrifuged at 3000 rpm for 10 min. Once the serum was removed from the blood cells, serum samples were kept at -70 °C until testing. The rats underwent orchiectomy 30 days post procedure to examine the left testicular tissue. Finally, the rats were anesthetized and euthanized by guillotine.

Tissue fixation and preparation of specimens

After the orchiectomy, the testicles were placed in Bouin solution for 48 h. Once the tissue was fixed, tissue passage was performed using incremental increases in ethanol, xylene, and liquid paraffin. The testicles were molded in the paraffin and slides of tissue 5 μ thick were prepared from each specimen, stained with hematoxylin eosinophil, and studied under an optical microscope at 400x magnification.

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