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Intravital microscopic evaluation of cremasteric microcirculation in experimental testicular torsion

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Abstract *Aim:* Although absent cremasteric reflex is a significant clinical finding for testicular torsion (TT), there is limited information about microcirculation of the cremasteric muscle (CM) after TT. This experimental study was performed to evaluate CM microcirculation by intravital microscopy after TT.
Materials and methods: Twelve Wistar rats were allocated into two equal groups: control (CG) and torsion (TG). After anesthetization of the CG rats, the CM flap was dissected through a left ventral inguinal incision with its vascular pedicle. In TG rats, TT was performed by rotating left testicles 720° in clockwise direction for 1 h. Then, the CM flap was dissected as in CG, and was placed under an intravital microscope. Vessel diameters, functional capillary perfusion and leukocyte activation in post-capillary venules were measured and evaluated statistically.
Results: There was a significant decrease in vessel diameter in TG compared to CG ($p < 0.05$). The median of perfused capillaries in CG and TG was 13 (11.75—14.30) and 5.5 (4.75—7.25), respectively ($p < 0.05$). Number of granulocytes (rolling, sticking, transmigrated) was greater in TG than CG ($p < 0.05$).
Conclusion: Intravital microscopic evaluation of CM after TT showed decrease in vessel diameter and number of perfused capillaries, and increase in granulocyte activation. Clinical, electrophysiological alterations in CM after TT can be explained by deterioration of microcirculation of CM.

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Introduction

Testicular torsion (TT) is a common serious urological emergency usually involving infants and adolescents. Early diagnosis and surgical intervention are required to prevent testicular loss. Important signs of TT include a firm testicle high-riding in the scrotum, an abnormal transverse orientation of the testis, and the absence of cremasteric reflex [1]. Although ultrasound and nuclear scintigraphy have been valuable tools in diagnosis, history and physical examination remain the cornerstones of the diagnosis of TT [2,3]. The cremasteric reflex has been reported to be a useful sign in diagnosis of TT [4,5]. Although absent cremasteric reflex is a significant clinical finding for TT, there is limited information about the microcirculation of cremasteric muscle (CM) after TT.

Intravital microscopy is a qualitative and quantitative technique used for observing and quantifying microcirculatory dynamics in vivo [6]. In 1973, Baez described the direct observation of cremasteric muscle microcirculation under microscope [7]. The isolated CM with its vascular pedicle is a suitable model for investigating microcirculatory hemodynamics [8,9]. An experimental study was performed to evaluate the cremasteric microcirculation using intravital microscopy after TT in vivo.

Materials and methods

Twelve Wistar rats, weighing 150—200 g were allocated into two equal groups: control (CG) and torsion (TG) groups. Animals were anesthetized with intraperitoneal injections of thiopental sodium (40 mg/kg, Pental Sodium, İ.E Ulagay, Istanbul). Intravital microcirculatory hemodynamics were observed in the rat CM flap. After anesthetization of the CG rats, the cremaster flap was dissected through a left ventral inguinal incision with its vascular pedicle (Fig. 1A). In TG, testicular torsion was performed by rotating left testicles 720° in a clockwise direction as described by Turner [10]. After 1 h torsion time, the cremaster flap was dissected as in the CG (Fig. 1B). The CM flap was placed under an intravital microscope, and all microcirculatory images were recorded. Vessel diameters, functional capillary perfusion and leukocyte activation in post-capillary venules were measured and evaluated statistically. After microcirculatory observations were completed, all rats were sacrificed with cardiac blood withdrawal.

The experiments were performed in adherence to the Declaration of Helsinki and by approval of the Ethics Committee of Kırıkkale University (2012). The data obtained from the experiments were analyzed using the Kruskal Wallis test (SPSS 15.0). The *p* values lower than 0.05 were considered as significant.

The cremaster muscle flap model

After anesthetization, the cremaster flap was dissected through a left ventral inguinal incision, as described previously [6,8,9,11]. A slight pressure was used to push the testicle down into the scrotum from inside the body cavity. A left ventral incision was made above the scrotum, and then extended down to the distal end of the scrotum. The underlying connective tissue and fascia were then carefully separated from the cremaster sack. The testis and spermatic cord were extracted, and CM was isolated with its supplying pudic-epigastric pedicle. The flap was opened along its anterior wall. A round island flap was created with an axial pattern of main feeding vessels. The animal was secured onto a custom-designed Plexiglas chamber; the CM was stretched and secured with 6-0 silk sutures (Silk, 6/0, Hünnigen, Belgium) (Fig. 2A). The muscle flap was kept moist with Ringer's solution and sealed in a standard fashion with a vinylidene-chloride sheet semi permeable to oxygen (Saran wrap, US), and left for 30 min before examination.

Direct in vivo microcirculatory monitoring

The prepared flap on the Plexiglas chamber was transilluminated from below using a fiber optic tungsten lamp

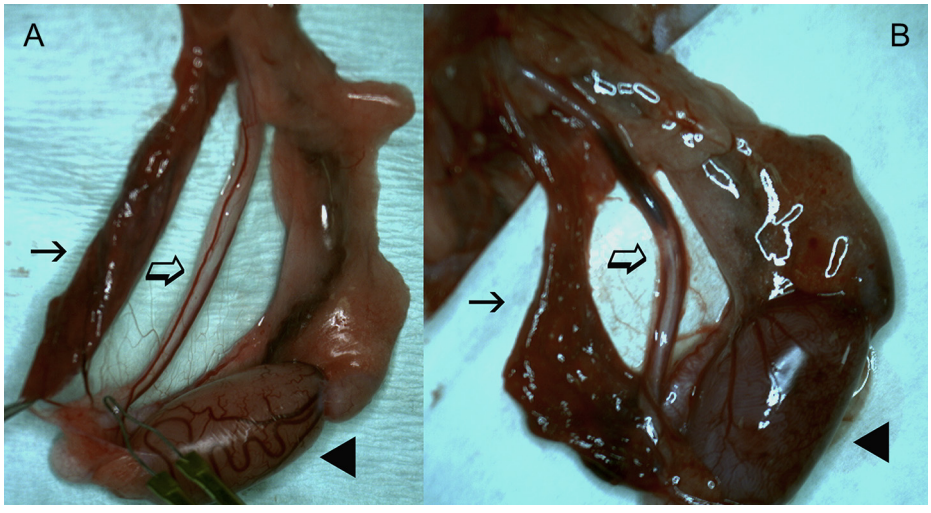

Figure 1 Dissection of the cremasteric muscle flap. (A): Cremaster flap dissected with its vascular pedicle in control group. (➔: Cremaster muscle, ⇨: spermatic cord, △: testicle). (B): Cremaster flap dissected with its vascular pedicle in torsion group. (➔: Cremaster muscle with hematoma, ⇨: spermatic cord with hematoma, △: testicle with microvascular hemorrhage).