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Study of Level of Inhibin B and Ultra structure of Sertoli Cells in Contra-Lateral Testis after Unilateral Blunt Testis Trauma in Rat

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Abstract: This study was designed to evaluate the ultrastructure of contra lateral testis tissue and measurement of Serum inhibin B following unilateral blunt testis trauma. Twenty pre-pubertal male wistar albino rats aged 3 weeks were divided into 4 equal groups that each containing five rats. Group I was the control group. Group II was used as a Sham group. Group III had right orchiectomy initially. Group IV was the trauma group in which the right testis was placed on a firm sterile surface and the metal rod weighting 100 g was drooped on to the testis from a height of 5.5 cm. Seven weeks after initial operation 3 mL blood samples were obtained from each rat to determine inhibin B levels and contra lateral orchiectomies were performed in all groups to microscopically investigate electron. Inhibin B levels decreased in groups 3 and 4. The difference between group 3 with groups 1 and 2 was significant ($p = 0.003$ and 0.02). Also the difference between group 4 with groups 1 and 2 was significant ($p = 0.006$ and 0.002) but the difference between group 3 and 4 was not significant ($p = 0.08$). In group III (orchiectomy) TEM showed a normal sperm morphology and normal disruption of different stages of the spermatogonial maturation. Debris and vacuolar changes were seen in sertoli cells. Morphology of leydig cells slightly modified and the dilated cisternae of the Smooth Endoplasmic Reticulum (SER) were observed in group IV(trauma) mitochondria with degenerated cristae and enlarged vacuole were observed.

Key words: Testis, electron microscope, apoptosos, sertoli cell, rat

INTRODUCTION

Testicular trauma is defined as any injuries sustained by the testicle. Types of injuries include blunt, penetrating or degloving. Blunt Trauma refers to injuries sustained from objects applied with any significant force to the scrotum and testicle. This can occur with various types of activity. Blunt trauma accounts for approximately 85% of cases (Saleh *et al*., 2009). The most common cause of blunt testicular trauma is sport injuries. Most blunt testicular injuries are unilateral and isolated (i.e., without other associated injuries) (Luchey *et al*., 2009). Unilateral testicular injury is always considered with contralateral testicular damages. Any kind of testicular injury may result in unilateral and contralateral testicular atrophy which will impair fertility (Ozkan *et al*., 2003). There is evidence that unilateral blunt testicular trauma affects contralateral testicular germ cell maturation through an autoimmune response (Shaul *et al*., 1997). The testicular maturation is not complete until puberty and any injury to the pre-pubertal testis is likely to affect the spermatogenesis maturation in later periods (Srinivas *et al*., 1999a). Inhibin B is the relevant circulating inhibin form in the human male. Inhibin B is a dimmer of α- and β_B-subunits. It is produced exclusively by the testis. The Sertoli cell is considered the predominant source of inhibin B (Srinivas *et al*., 2003; Meachem *et al*., 2001). This

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notion is currently challenged and confounded by a mounting number of sometimes contradictory studies, based on immunolocalisation of inhibin subunits and on the *in vitro* and/or *in vivo* production of inhibin B in various experimental settings. These studies indicate that germ cells and possibly even Leydig cells would produce inhibin as well (Grootenhuis *et al*., 1990; Handelsman *et al*., 1990). The human male inhibin subunits seem to be differentially localized and secreted depending on age and cell type. For example, in the fetal testis and bB subunits, but not bA were immunolocalised in the sertoli and leydig cells (Risbridger *et al*., 1998). Ozkan *et al*. (2003) showed that Unilateral Testis Trauma (UTT) has an adverse on the contralateral testis and decreased levels of inhibin B following unilateral testis trauma may reflect this contralateral testis damage. This may be consequence of sertoli cell degeneration or less in the contralateral testis because the serum inhibin B level in orchioctomy group was normal. Srinivas showed that grade I unilateral blunt testicular trauma in prepubertal rats significantly affected germ cell maturation in both ipsilateral and contralateral testis and altered the sex hormone profile (Andersson *et al*., 1998; Majdic *et al*., 1997). This study was designed to evaluate the ultrastructure of contra lateral testis tissue following unilateral testis trauma and measurement of Serum inhibin B.

MATERIALS AND METHODS

This experimental study was performed in the Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences (AJUMS) from March 2008 to August 2009. Twenty male wistar rats aged 3 weeks were divided into 4 equal groups each containing 5 rats. All the rats were provided with equal amount of commercial pellet feed once a day at a scheduled time and water was made available and libitum. They were housed in cages under standard elaborating conditions with 12 h light and dark cycles. Rats were anesthetized by intraperitoneal ketamine (1 mg 100 g^{-1} b.wt.). All instruments used for the procedure were sterilized. Abdomens of group II (sham) were sham operated without disturbing either testis. In group III right orchiectomy performed initially. In group IV a transverse lower abdominal incision was made and the right testis was placed on a sterile firm surface and 100 g sterile weight was dropped on to the testis from a height of 5.5 cm. The tunica albuginea was open thereby exposing seminiferous tubules and the tunica vaginalis was then closed with fine absorbable suture. The abdominal incision was closed in layers with polyglactin suture and the wound was cleaned with povidone-iodine. Seven weeks after initial operation 3 mL blood samples were obtained from each rat to determine inhibin B levels and contra lateral orchiectomies were performed in all groups to microscopically investigate electron.

Electron Microscopy

For electron microscopy, the testicular specimens were fixed with 2.5% glutaraldehyde in 0.1 M sodium buffer phosphate (pH 7.2) for 3 h at 4°C, washed in the same buffer for 1 h at 4°C and post-fixed with 1% osmium tetroxide in sodium phosphate buffer for 1 h at 4°C. The tissues were then dehydrated in graded series of ethanol, starting at 50% each step for 10 min, after two changes in propylene oxide. The tissue ecimens were embedded in araldite. Ultrathin sections were prepared with Mg-uranyl acetate and lead citrate for the electron microscopic evaluation (Philips CM 10 TEM).

Measurement of Inhibin B Levels

Serum inhibin B was measured using specific two-site ELISAs.

Statistical Analysis

Level of serum inhibin B was reported as the Means±SD. The statistical significance of difference between the control and experimental groups was determined by the unpaired t-test. Differences between the means were considered to be significant when $p<0.05$ was achieved.

RESULTS

Electron Microscopic Findings

The EM sections were studied under the following categories: (1) Seminiferous tubules: Basement Membrane (BM); spermatogenesis series: various stage of maturation and sperm morphology and sertoli cells: nuclear membrane and organelles. (2) Extra Cellular Matrix (ECM) and leydig cells. Features of degeneration were fragmentation of the cell membrane, disorganization of organelles. Disruptions of the Endoplasmic Reticulum (ER) and rarefaction of mitochondria. In group I (control) and group II(sham), TEM showed a normal BM, normal sperm morphology and normal disruption of different stages of the spermatogonial maturation, normal leydig cells, sertoli cells, normal ECM and interstitial tissue. In group III(orchiectomy) TEM showed a normal sperm morphology and normal disruption of different stages of the spermatogonial maturation. Debris and vacuolar changes were seen in sertoli cells. Morphology of leydig cells slightly modified and the dilated cisternae of the Smooth Endoplasmic Reticulum (SER) were observed(Fig. 1a). In group IV (trauma) mitochondria with

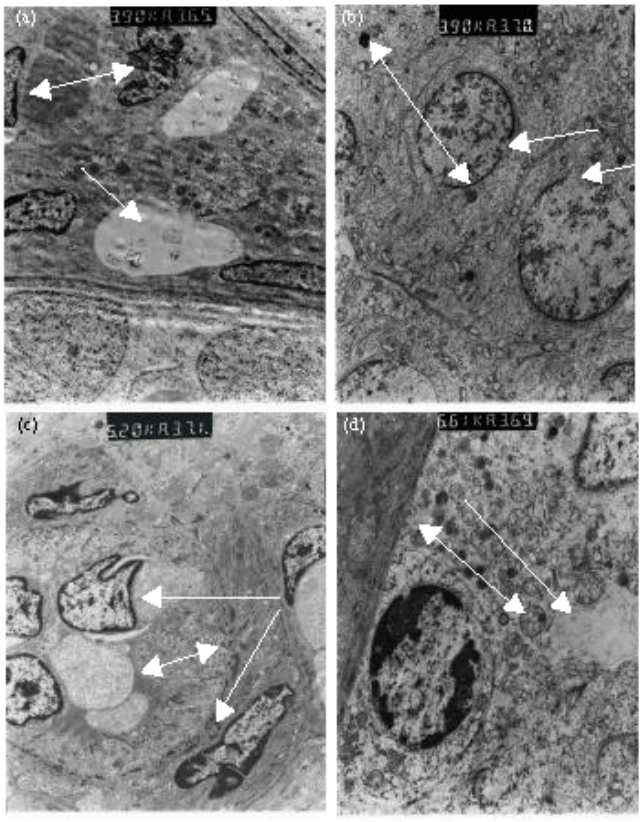

Fig. 1: (a) Electron microscopy of testicular tissue in orchiectomy group. vacuolar changes → of interstitial tissue and slightly morphological changes ↔ of leydig cells were observed, (b) electron microscopy of testicular tissue in trauma group. Nuclear membrane degeneration → of primary and secondary spermatocytes was observed in this group. Dens apoptotic body ↔ increase in cells, (c) electron microscopy of testicular tissue in trauma group. Morphological changes of interstitial cells specially leydig cells → and vacuolar changes of interstitial tissue ↔ and (d) Electron microscopy of testicular tissue in trauma group. Mitochondria with degenerated cristae ↔ and enlarged vacuole → were observed

Table 1: Inhibin B level in all groups

Group	Number	Inhibin B level (pg mL^{-1})
I (Control)	5	410.3±15.9
II (Sham)	5	435.5±20.3
III (Orchiectomy)	5	290.5±28.5
IV (Trauma)	5	270.8±30.8

degenerated cristae and enlarged vacuole were observed. Markedly morphological changes of interstitial cells specially leydig cells and nuclear membrane degeneration of primary and secondary spermatocytes were observed in this group. Dens apoptotic body increase in cells (Fig. 1b-d).

Inhibin B Levels

The level of inhibin B was 410.3±15.9, 435.5±20.3, 290.5±28.5, 270.8±30.8 pg mL^{-1} in control (I), sham (II), orchiectomy (III) and trauma (IV) groups, respectively (Table 1). Statistical analysis show that the difference between group 3 with groups I and II was significant ($p = 0.003$ and 0.02). Also, the difference between group IV with groups I and II was significant ($p = 0.006$ and 0.002) but the difference between group III and IV was not significant ($p = 0.08$).

DISCUSSION

In present study we observed that the level of inhibin B in two experimental groups (groups III and IV) significantly decreased in compared to others two groups. This can consequence of sertoli and leydig cells degeneration in contralateral testis. Because in these groups TEM showed vacuolar changes in ECM, morphological changes of leydig cells, dilated cisternae of SER, mitochondria with degenerated cristae in sertoli cells and degeneration of spermatocyte cells. Earliely Ozkan *et al.* (2003) declare that in peripubertal rats the serum inhibin B levels in trauma groups in agreement with our result decreased but in contrast with present study the serum inhibin B level of the orchiectomy group was not different from the control group. Ozkan *et al.* (2003) said that the inhibin B levels of orchiectomy group might have been decreased but after a 6 week period the remaining testis might have improved its function by compensating for impaired fertility (II). Present results reinforced the Ozkan *et al.* (2003) study. In other study that performed by Shaul *et al.* (1997) in postpubertal rats, the group undergoing orchiectomy following UTT had a similarly high fertility rate compared to the control group (III). This finding is confirm by this results. Wolfe *et al.* (1985) studied the serum quality of contralateral testicle of bulls after unilateral orchiectomy. They observed a decreased percentage of normal spermatozoa only on postoperative day 6 but at the end of the 8 weeks there was no difference in sperm mobility scores and percentage of normal spermatozoa (Wolfe *et al.*, 1985). Srinivas *et al.* (1999b) reported that UBTT affects the contralateral testis and fertility. Anti Sperm Antibody (ASA) mediate this damage and orchiectomy performed around 6 h after trauma or short-term cyclosporine therapy prevents the damage. Experimental models were chosen to study the effect of UBTT, in which reduced fertility (Slavis *et al.*, 1990; Srinivas *et al.*, 1999b), antibody mediated (Srinivas *et al.*, 1999a) and cell-mediated (Sakamoto *et al.*, 1995, 1998; Sharma *et al.*, 1999) immune response have been demonstrated. In these studies, the technique of inflicting injury and documentation of the injury was not standardized. In this study serum inhibin B levels in orchiectomy and trauma groups were less than control groups. The difference between orchiectomy and trauma group was not significant. Also, the electron microscopic findings in these two group almost similar. Present results indicated that the injury in prepubertal period was sensitively and repair might need a long period. Additionally the grade of injury is importance. In the present study, the ultrastructure details of the contralateral testis were studied using Transmission Electron Microscopy (TEM). According to our best knowledge out report represents the first study designed to evaluate the

ultrastructure of contra lateral testis tissue following unilateral testis trauma. In agreement with our present study, earlier, Nambirajan *et al.* (2002) reported the ultrastructure changes observed in the undescended testis (UDT) were also reflected in the Contralateral Descended Testis (CDT). These changes in the CDT are significant. In this study electron photomicrographies of contralateral descended testis showed a irregularly thickened basement membrane, vacuolation, focal degeneration of spermatocyte and dens apoptotic body. Also, the size of ER and number of sertoli cells increased (Nambirajan *et al*., 2002). Dokmeci *et al*. (2007) considered the protective effects of ibuprofen on testicular torsion/detorsion-induced Ischemia/Reperfusion (I/R) injury in rats. After I/R, slightly dilated cisternae of SER and markedly swollen mitochondria with degenerated cristae were observed in sertoli cells in the ipsilateral testis of the late orchiectomy model. After I/R markedly degenerated cristae of mitochondria and slightly dilated cisternae of SER were observed in the spermatid cells contralateral testes of the late orchiectomy group.

Mogilner *et al.* (2006) concluded that testicular ischemia in rats led to histological damage in the ipsilateral testis. In the contralateral testis , minimal damage was observed and testicular ischemia additionary caused an increase in germ cells apoptosis in the contralateral testis. In present study on prepubertal rats, histological changes of contralateral testis following unilateral testis trauma were observed in orchiectomy and trauma groups. Some changes were similar to histological changes in torsion and undescended testis. In group III (orchiectomy) TEM showed a normal sperm morphology and normal disruption of different stages of the spermatogonial maturation. Debris and vacuolar changes were seen in sertoli cells. Morphology of leydig cells slightly modified and the dilated cisternae of the Smooth Endoplasmic Reticulum (SER) were observed in sertoli cells. In group IV (trauma) mitochondria with degenerated cristae and enlarged vacuole were observed. Markedly morphological changes of interstitial cells specially leydig cells and nuclear membrane degeneration of primary and secondary spermatocytes were observed in this group. Dens apoptotic body increase in cells. These changes could probably be responsible for the decrease in serum inhibin B levels and decrease in fertility seen in previous studies.

CONCLUSION

Recent study indicated that UTT affected the contralateral testis and serum inhibin B levels reflect sertoli cell function and spermatogenetic activity.

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