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Effects of Experimental Torsion of the Spermatic Cord on Leydig Cell Function in the Guinea Pig Testis: An Ultrastructural Stereological Analysis

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Summary. An ultrastructural stereological analysis of Leydig cells of the guinea pig testis was carried out following surgically induced testicular torsion. Morphometric analyses of the Leydig cells of the experimental group of animals revealed an increase in the nucelar and mitochondrial volume and a decrease in the lipid volume, in comparison to those in the Leydig cells of the control group of animals. We believe that these changes in the Leydig cells of the experimental group of animals are indicative of cellular hypertrophy. The possible mechanisms of the Leydig cell hypertrophy in the guinea pig testis following the induction of spermatic cord torsion are discussed.

Key words: Guinea pig, Spermatic cord torsion, Stereology, Electron microscopy, Leydig cell.

Torsion of the spermatic cord with acute scrotal pain and swelling is a fairly common urological emergency. We have previously reported that unilateral torsion of the spermatic cord not only affects the experimental testis but also induces germ cell degeneration in the contralateral, so-called unaffected testis [4–7, 14]. These findings have been substantiated by other inverstigators [3, 19, 23]. During our studies on the effect of unilateral torsion of the spermatic cord on the contralateral testis of guinea pig, we found Leydig cell hypertrophy associated with spermatogenic disruption [6]. This type of Leydig cell hypertrophy in association with seminiferous tubular involution has been previously reported in the rat after experimentally induced cryptorchidism or after administration of antifertility drugs [10].

During the present investigation we employed an ultrastructural stereological procedure to quantitatively assess the effect of torsion of the spermatic cord on Leydig cells in the guinea pig testis.

Materials and Methods

Twelve adult Hartley strain guinea pigs weighing 600–800 g were used for the present study. The animals were divided into two equal groups. In the first group. of 6 animals, a 540^0 unilateral torsion of the spermatic cord was surgically induced under pentobarbital anesthesia [4, 5]. This group was designated as the torsion-maintained group. The rest of the 6 animals were given a single injection of pentobarbital and severd as control. All animals were sacrificed four months after the induction of torsion of the spermatic cord. Specimens were obtained from different areas of each testis for light and electron microscopic studies.

Tissues collected from the animals were immediately fixed in 2.5% buffered glutaraldehyde, post-fixed in 1% O_SO_4 and processed for routine epon embedding. Semi-thin (1 μm) sections were cut from the epon embedded blocks and stained with 1% toluidine blue for light microscopic examination. Thin sections (90–100 nm) were cut from the well preserved regions of the block, stained with lead citrate and were examined under a Philips EM 300 electron microscope.

The morphometric analyses of the Leydig cells were based on standard stereological principles [21, 22]. Both the cell and nuclear volumes of the Leydig cells from control and affected testes were determined at the light microscope level [6, 9, 17]. The same sampling procedure described in our previous report [6] was employed for morphometric analyses. Briefly, 10 sections (1 μm) from each testis (one section/block) were examined at 800x with a Nikon-Optiphot light microscope. The nucleus-cell ratio was obtained from the sums of the number of points failling on the nuclei (Pn) and on whole cells (Pc). Point-counting was performed with a square lattice grid containing 100 test points, fitted to the eye piece (6, 7). The total number of grid fields counted for each animal varied from 60–80. The nuclear volume (Vn) was estimated from the mean diameter ($\bar{d}$) of the nucleus using the standard formula ($Vn = 1/6\ \pi\bar{d}^3$). The mean diameter ($\bar{d}$) of the nucleus was obtained by direct measurements of the long and short axes of the nuclear profiles with a Nikon-Optiphot light microscope at 800x using an ocular micrometer fitted to the eye piece. Only those nuclei having the largest profile diameter in sections were considered. For each animal in each group, at least 50 nuclear profiles were measured.

The Leydig cell volume (Vc) was calculated from the nucleus-cell ratio (Pc/Pn) multiplied by the volume of the nucleus (Vn). The cytoplasmic volume of the Leydig cell (Vcy) was also determined by subtracting the nuclear volume (Vn) from the cell volume (Vc). Correction for shrinkage during tissue preparation or for section thickness was not performed since semi-thin epon sections were used for quantitation [11, 13].

Only severely damaged testes were used for ultrasturctural morphometric analysis. Seminiferous tubules of these severely damaged testes were depleted of differentiating germ cells, containing ony a

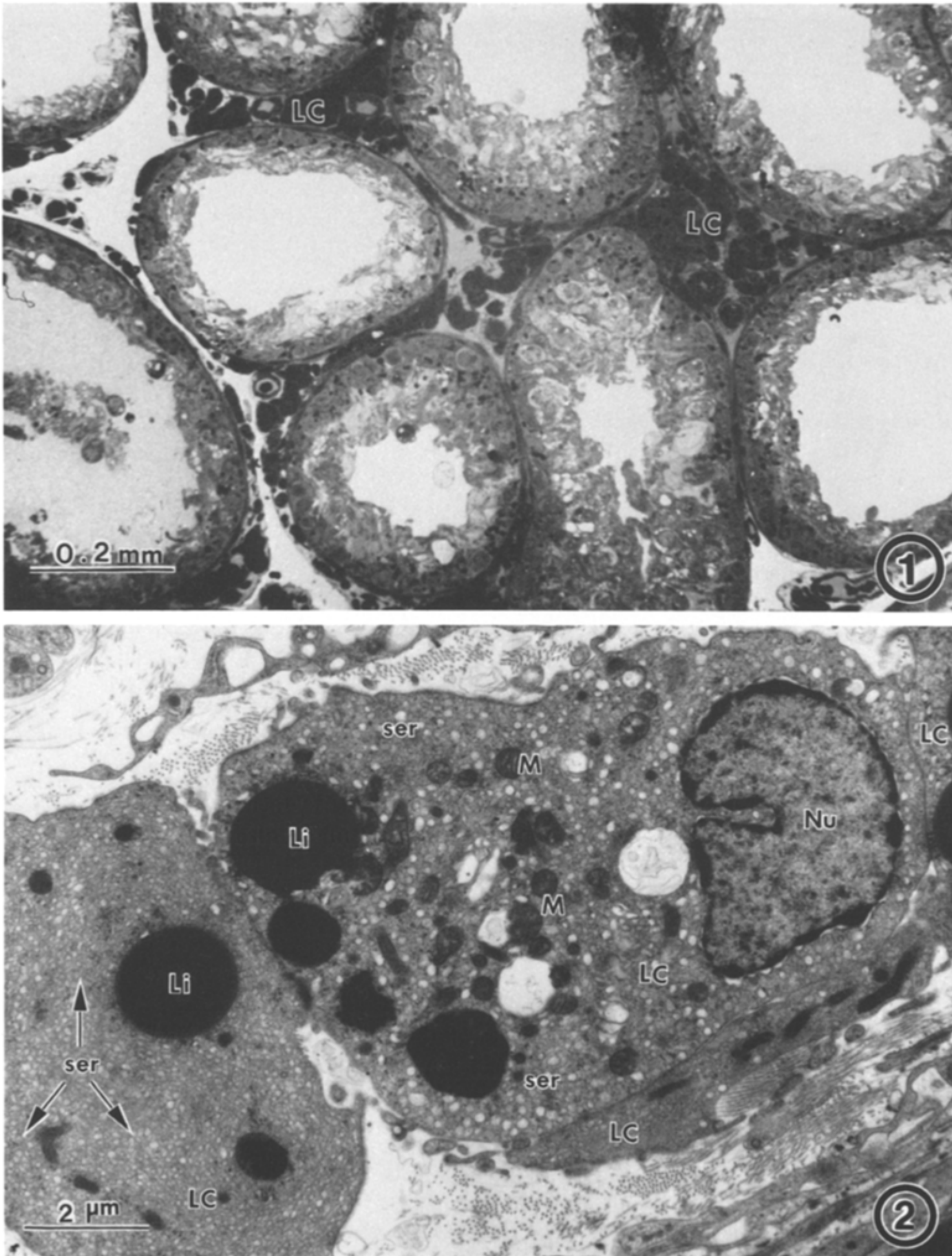

Fig. 1. Light micrograph of a section of severely damaged guinea pig testis from an animal, with spermatic cord torsion for four months. This is a representative figure showing the overall histological appearance of seminiferous tubules and interstitium. Seminiferous tubules are lined by a layer of Sertoli cell with a few spermatogonia. Clusters of Leydig cells (*LC*) are readily observed in the interstitium. x97

Fig. 2. Electronmicrograph of a portion of same testis as in Fig. 1, showing the typical appearance of normal Leydig cells (*LC*), containing abundant smooth endoplasmic reticulum (*ser*), nucleus (*Nu*) with patches of heterochromatin, mitochondria (*M*) and lipid droplets (*Li*). x8,550

layer of Sertoli cells with occasional spermatogonia. The percentage of cell volume occupied by mitochondria, lipid and membrane space (space occupied by the smooth and rough endoplasmic reticulum and Golgi complex) was estimated from electron micrographs by the differential point-counting method [21, 22]. Twenty micrographs at final magnifications of x25,500 for each animal from each gorup were selected randomly and used for quantitative analyses. The volume fraction obtained at the ultrastructural level was then expressed as the absolute volume (μm^3) occupied by the organelles in the cytoplasm of Leydig cells from each group of animals. Results are finally expressed as the mean ± SE. Statistical comparison between control and experimental groups was performed by student's "t" test. *P* values less than 0.05 were consiedered to indicate significant differences.

Results

Cytological Observation

Out of 6 animals in the experimental group with spermatic cord torsion, two animals had no recognizable testicular

Table 1. Morphometric data on Leydig cells in control and torsion-maintained guinea pigs

Components volume (μm^3)	Experimental group		Significance level
	Control (6)	Torsion-Maintained (4)	
Cell	2,195.2 ± 130.3[a]	2,224.5 ± 125.6	N.S.
Nucleus	236.3 ± 4.38	276.7 ± 10.6	$P < 0.01$
Cytoplasm	1,958.9 ± 132.8	1,947.8 ± 127.8	N.S.

[a] Mean ± S.E.M. The number of animals in each group is indicated in parentheses. N.S. – Not significant

tissue; testicular tissue of two animals had severe germ cell degeneration characterized by the presence of a layer of Sertoli cells with a few basal spermatogonia (Fig. 1); while the remaining two animals had moderately damaged testes containing some differentiating germ cells.

The ultrastructural features of Leydig cells in control guinea pigs were normal [8]. Briefly, these were polyhedral cells located in the interstitial tissue in small compact clusters. The nucleus was usually ovoid with clumps of heterochromatin located mainly adjacent to the nuclear envelope. The cytoplasm contained an exceptionally abundant and highly developed smooth endoplasmic reticulum (SER). Small patches of rough endoplasmic reticulum were scattered in the cytoplasm. Other cellular organelles included moderately abundant mitochondria, many lipid droplets, few lipofuchsin pigment granules and dispersed golgi elements. No appreciable change was observed in the Leydig cell ultrastructure of the experimental animals (Fig. 2) in comparison to that of the control group.

Morphometric Analysis

Results of the morphometric analysis of Leydig cells of control and experimental guinea pigs are summarized in Tables 1 and 2. The Leydig cell volume of control and experimental groups of animals remained unchanged. A significant increase in the nuclear volume of these cells was noted in the affected testes in comparison to those of controls (Table 1). An increase in the mitochondrial volume was also apparent, although it was not statistically significant (Table 2). However, there was a significant decrease in lipid droplets in the Leydig cells of the experimental group of animals as compared to those of controls (Table 2). The volume of other cytoplasmic organelles, smooth and rough endoplasmic reticulum, golgi apparatus, and lysosomes remained unchanged in the affected testis in comparison to the control specimens (Table 2).

Discussion

Ultrastructural stereological methods were employed during the present investigation to assess the Leydig cell function in guinea pig testis after experimental induction of spermatic cord torsion.

The basic stereological data on Leydig cells in normal guinea pig testis are available [18, 24]. Data obtained during the present investigations on Leydig cells from the control group of guinea pigs are in agreement with the previously reported values. For example, the volume fraction of mitochondria (11.4% of cytoplasmic volume) and lysosomes (0.8% of cytoplasmic volume) obtained in the present investigation were similar to the values (9.3% and 1.17% respectively, of guinea pig, Leydig cell cytoplasm) reported by Mori [18]. The present value for volume fraction of the membrane space (72.6% of the cytoplasmic volume) is also similar to the value of SER (60.9%) obtained by Zirkin [24] but considerably higher than the value (13.8% of the cytoplasmic volume) obtained by Mori [14]. However, there are disagreements in the data on lipid fraction between present and earlier studies. The volume fraction of lipid (15.15% of cytoplasmic volume), obtained in our study, is higher than that of the ones measured by Mori [18] (6.22%) and Zirkin [24] (7.9%). Similarly, our estimate of the average Leydig cell volume (2,195.2 μm^3) and nuclear volume (236.3 μm^3) are also comparatively higher than

Table 2. Morphometric data on components of Leydig cells of control and torsion-maintained guinea pigs

Cytoplasmic organelles	Experimental group			
	Control (2)		Torsion Maintained (2)	
	Volume%	Absolute volume (μm^3)	Volume%	Absolute volume (μm^3)
Mitochondria	11.4 ± 1.2[a]	223.1 ± 24.3	16.3 ± 1.5	318.4 ± 30.2
Lipid	15.1 ± 0.5	295.7 ± 9.8	5.2 ± 0.4[b]	101.3 ± 7.8[b]
Membrane space	72.6 ± 1.1	1,422.7 ± 22.9	77.2 ± 2.4	1,503.7 ± 46.7
Lysosome	0.8 ± 0.5	15.5 ± 10.0	1.2 ± 0.4	23.0 ± 7.4

[a] Mean ± S.E.M. The number of animals in each group is indicated in parentheses
[b] $P < 0.01$

the values (1,443 μm^3 and 188 μm^3) reported by Mori [18]. It should be mentioned that a wide range of variation can be noted in the average volume of the rat Leydig cells estimated by previous investigators. The average Leydig cell volume ranges from 367 μm^3 [15], 712.3 μm^3 [16], 1,209 μm^3 [17], and 2,199 μm^3 [9]. These discrepancies are probably due to differences in the preparation of the specimens, or due to the application of different morphometric procedures. Since we used the same techniques during the present investigation for all control as well as experimental animals, the relative differences in the data obtained during morphometric analysis between the Leydig cells of control and experimental groups of animals, reported in this study, are valid.

The present study indicated a significant increase in the nuclear volume, an apparent increase in the mitochondrial volume and a decrease in the lipid droplets in the Leydig cells of severely affected testis due to the torsion of the spermatic cords compared with measurements from control testes. These findings were indicative of Leydig cell hypertrophy in the damaged testis. Alteration in Leydig cell morphology accompanying spermatogenic disruption under diverse experimental condition is well known [1, 6, 10, 15,20]. Previous studies suggested that cellular hypertrophy was a consequence of increased gonadotrophin which in turn stimulated the Leydig cell [10, 15, 20]. Aoki and Fawcett [1], however provided an alternative explanation and suggested the possibility of a local feedback from the tubules modulating the Leydig cell function. Bartsch et al. [2] reported a significant elevation of FSH and LH levels in patients with abnomral sperm analyses following unilateral spermatic cord torsion for more than 24 h. By contrast, Goldwasser et al. [12] was unable to detect any appreciable alterations in FSH, LH and testosterone levels in patients after 4 months to 5 years following unilateral torsion. Although comparable hormonal analyses are not available, the Leydig cell hypertrophy in the affected testis of the guinea pig could be the result of local stimulation from damaged seminiferous tubules following spermatic cord torsion leading to cellular hypertrophy [1].

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