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Morphometric study on Leydig cells in capsulotomized testis of rats

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Abstract **Aim:** To further clarify the changes occurred in the testicular capsulotomized rats. **Methods:** In testicular capsulotomized and sham-operated rats, the cross sectional area, the nucleus diameter and the number of Leydig cells were morphologically analyzed by the Vidas Image Processing System connected to a microscope. **Results:** In the capsulotomized animals, the cross sectional area of Leydig cells was gradually increased from 30 days onwards. There was no obvious change in the nucleus diameter of Leydig cells. However, The Leydig cell number was significantly increased from day 30 onwards. **Conclusion:** In rats, testicular capsulotomy may induce hyperplasia/hypertrophy of Leydig cells in the testis. *(Asian J Androl 2002 Mar; 4: 49-53)*

1 Introduction

It was found in our previous studies that in adult male rats, a progressive degeneration of the seminiferous tubules could be induced by testicular capsulotomy with a progressive reduction in the testosterone concentration in the testicular venous blood and a gradual increase in the LH and FSH levels in the peripheral blood from approximately 30 days post-capsulotomy. At the same time, the fertility of the capsulotomizd rats was gradually depressed [1-4]. It was traditionally believed that the blood level of testosterone was principally controlled by LH released from the pituitary gland. However, in the testicular capsulotomized rats, although there was a significant increase in the blood LH level, the secretion of testosterone remained low. A progressive reduction in the endogenous LH binding to the testis was also observed[5]. In the present study, the cross sectional area, the nucleus diameter and the number of Leydig cells of the capsulotomized rats were morphometrically analyzed in an attempt to further clarify the changes occurred in the testicular capsulotomized rats.

2 Materials and methods

2.1 Animals and capsulotomy

Mature (60 days of age) Sprague-Dawley male rats were obtained from the Laboratory Animal Unit, University of Hong Kong and were randomly divided into the sham-operated and the capsulotomized groups of 30 animals each. Both groups were further divided into 6 subgroups of 5 rats each. Surgical intervention was performed in the Minimal Disease Operation Theatre of the University. The animals were anaesthetized intraperito

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neally with sodium pentobarbitone (Sigma, USA) at a priming dose of 60 mg/kg and a maintenance dose of 10 mg/kg/h. Testicular capsulotomy was carried out as previously described [1]. Briefly, with the aid of a dissecting microscope (Wild M60, Switzerland), the two outer layers of the capsule, i.e., tunica vaginalis and tunica albuginea, were carefully incised starting half-way down the rostral half of the testis along the two lateral borders down to the middle of the caudal half of the testis.

2.2 Tissue preparation

The testes were isolated, fixed and processed as previously described [6]. Briefly, the testes were fixed in Helly's fluid [6] overnight, then cut into two halves and placed again in the Helly's solution for another 32 hours. Paraffin section were cut in ribbon at a thickness of 4 μm.The Periodic Acid-Schiff (PAS) staining method [6] was used and the stained sections were observed under a light microscope (Leitz, Germany).

2.3 Leydig cell observation

The Leydig cells were morphometrically analyzed by the Vidas Image Processing System (Kontron, Germany) connected to a microscope (Leitz, Germany). For each rat, five sections from the polar and the equatorial regions of the testis were sampled and the cross sectional area and the nuclear diameter of 20 Leydig cells were measured per section. The cross-sectional area was obtained by tracing the outline of Leydig cells with a cursor, coupled to the Image Processing System and selecting the morphometric parameter of "AREA" from the measurement menu of the Videoplan Software. The average nuclei diameter was measured by tracing the outline of the nuclei of Leydig cells and selecting the morphometric parameters of "DMAX" and "DMIN" from the measurement menu. Since the nuclei were commonly somewhat oblong, both the maximal and minimal diameters were measured and the mean calculated.

The number of Leydig cells per testis was estimated by multiplying the numerical density, Nv, of the Leydig cells (the number of Leydig cells per unit volume of testis) with the testicular volume (V). The numerical density of Leydig cells was calculated using the equation: Nv = Na/D+t-2h, where Na is the number of nuclei scored in sections within a defined reference area a, D is the mean diameter of nuclei, t is section thickness and h the thickness of the smallest recognizable cap of the nuclei. The testicular volume was obtained from its weight and specific gravity. Since the specific gravity of the rat testis is nearly 1 [7], the absolute value of testicular volume in mL is similar to that of its weight in gram. For each testis, five sections from the polar and the equatorial regions were sampled and the fields to be counted were selected over the whole specimen using a systematic random scheme [8]. The number of Leydig cell nuclei within the reference area of each counting field was determined by counting the nuclei within the area and selecting the parameter of "COUNTS/POINTS" in the measurement menu. The height of the lost nuclear caps was estimated to the one tenth nuclear diameter as previously described [9].

All morphometric analyses were performed in a blind fashion without the knowledge of the groups. The accuracy of sampling by these methods was assessed by measuring the above parameters from tissue specimens taken from a single rat and measured independently on five occasions using the criteria listed above. The coefficent of variation of these five estimates were found to be 1.6% - 1.8%.

Tissue shrinkage normally occurs during the fixation, dehydration and embedding processes. The shrinkage index was measured in four testicular tissue blocks from each of the five animals used in the morphometric measurements. The index of shrinkage in one dimension was derived from the ratio of the length of the axis after embedding to the length of the same axis before fixation. The average value obtained was 0.7956±0.0113 (SEM), showing a reduction of 20.44%. This correction factor was applied to all the morphometric data in this study in order to provide values expressed in terms of the intact testis.

2.4 Statistical analysis

Results were expressed in mean±SEM. Two-group comparisons were made by Student's *t*-test and multiple group comparisons by two-way analysis of variance (ANOVA) followed by the Student-Newman Keuls test. $P<0.05$ was considered significant. Results are the means from groups of 5 rats and the value for each rat is derived from the data of 5 sections.

3 Results

3.1 Changes in interstitial tissues and Leydig cells

In the early post-operation period (day 20), no obvious changes in Leydig cells and interstitial tissue were observed (Figure 1A). With increasing duration after capsulotomy, the interstitial tissues appeared to increase in size progressively and the Leydig cells increase in number. In the late post-operation period (days 50-60) in the interstitial tissue, there were gradually increasing number of fusiform and irregularly-shaped cells as well as a marked hyperplasia of the interstitial cells (Figures 1B and 1C) .

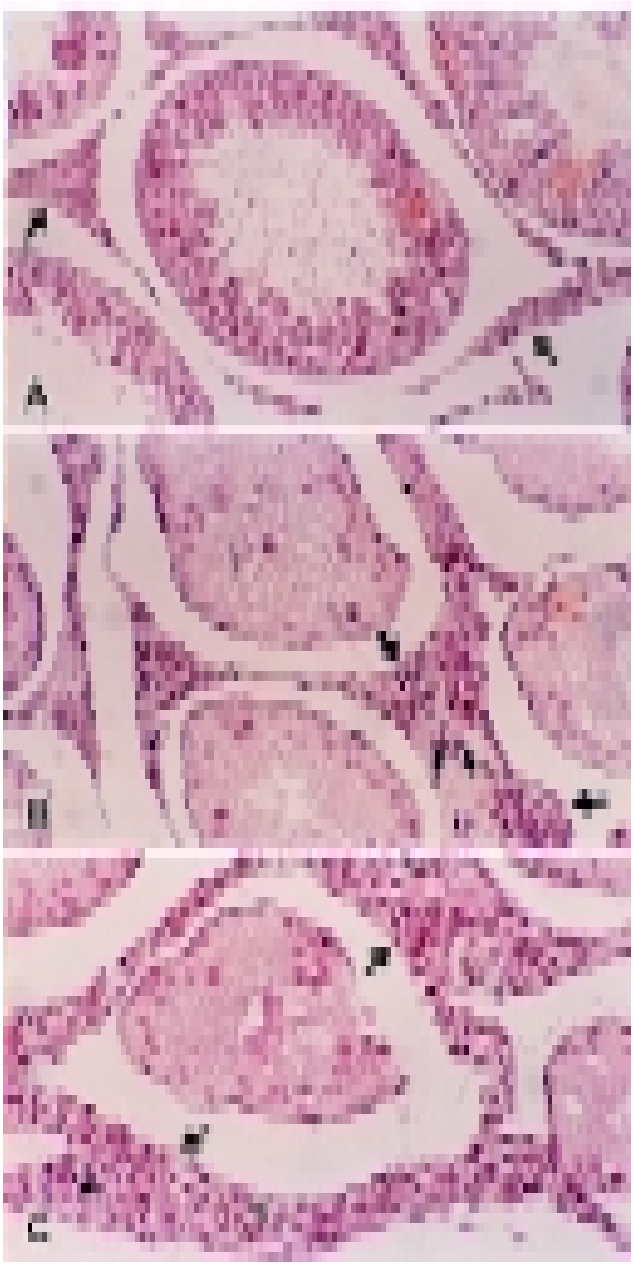

Figure 1. Light micrographs of the testis: (A) at 20 day post-capsulotomy showing normal structure of interstitial tissues (big arrows) between the seminiferous tubules; (B) at 50 day post-capsulotomy showing hyperplasia of Leydig cells (big arrows); (C) at 60 day post-capsulotomy showing marked hyperplasia of Leydig cells (big arrows), fusiform cells and irregular shaped interstitial cells (small arrows). PAS stain, × 240.

3.2 Cross-sectional area of Leydig cells

The effect of testicular capsulotomy on the cross-sectional area of Leydig cells are summarized in Table 1. It can be seen that the cross-sectional area was significantly increased from 30 days onwards, being 119±1.2 μm^2 by day 30 and 127±2.5 μm^2 by day 60. When compared with those at 10 days, the cross-sectional areas in the capsulotomized rats were significantly (P<0.05) increased by 11% at 30 days, 14% at 40 days, 21% at 50 days, and 19% at 60 days post-operation. Sham-operation did not cause any change in Leydig cell cross-sectional area throughout the post-operation period.

Table 1. Effect of testicular capsulotomy on cross-sectional area of Leydig cells in rats. *n*=5, mean±SEM, $^bP< 0.05$, compared with controls.

Post-operation (days)	Cross-sectional area (μm^2)	
	Control	Capsulotiomized
10	104±2.7	107±2.1
20	103±2.3	113±3.2
30	106±3.5	119±1.2^b
40	105±2.6	122±2.6^b
50	107±2.8	129±2.6^b
60	107±3.1	127±2.5^b

3.3 Nuclear diameter of Leydig cells

As indicated in Table 2, testicular capsulotomy does not significantly affect the nuclear diameter of Leydig cells.

Table 2. Effect of testicular capsulotomy on nuclear diameter of Leydig cells in rats. *n*=5, mean±SEM, $^bP< 0.05$, compared with controls.

Post-operation (days)	Nuclear diameter (μm)	
	Control	Capsulotomized
10	6.37±0.05	6.33±0.02
20	6.35±0.04	6.36±0.03
30	6.37±0.04	6.35±0.03
40	6.36±0.03	6.36±0.04
50	6.38±0.02	6.39±0.04
60	6.35±0.03	6.38±0.02

3.4 Number of Leydig cells

It is indicated in Table 3 that in capsulotomized rats, the number of Leydig cells per testis was not significantly different from those of the corresponding controls at days 10 and 20 post-operation. However, it was significantly increased from day 30 onwards. Sham-operation did not cause any change in the number of Leydig cells throughout the post-operation period.

4 Discussion

After testicular capsulotomy, there was a progressive increase in the cross-sectional area of the interstitial

Table 3. Effect of capsulotomy on number of Leydig cells per testis in rats. n=5, mean±SEM, $^bP< 0.05$, compared with controls.

Post-operation (days)	Number of Leydig cells per testis ($\times 10^6$)	
	Control	Capsulotomized
10	24.6±0.5	24.1±0.6
20	24.2±0.4	25.0±0.5
30	23.9±0.5	28.8±0.6^b
40	26.7±0.3	32.1±0.9^b
50	25.2±0.7	37.0±1.5^b
60	23.2±0.7	38.1±1.8^b

cells and the number of Leydig cells, indicating the presence of hypertrophy and hyperplasia of the cells. However, there was no obvious change in the diameter of their nuclei, suggesting that the activity of Leydig cells was not enhanced. With increasing duration after capsulotomy, in the interstitial tissue there were more and more fusiform and irregularly shaped cells which might be the precursors of the newly-formed Leydig cells.

Experimental disruption of spermatogenesis induced by various treatments such as anti-androgen, vitamin A deficiency, X-irradiation, crytorchidism, efferent duct ligation and heat treatment is always associated with hyperplasia and hypertrophy of the Leydig cells [10-14]. Although the gonadotrophin levels in these situations are usually elevated [15], the response of the Leydig cells is believed to be unrelated to the hormonal changes as if the spermatogenic disruption is unilateral, the changes only occur at the ipsilateral testis. In the capsulotomized rats, we did find a progressive increase in the levels of gonadotrophins starting from day 20 post-operation [3]. The possibility that Leydig cell hyperplasia is caused, at least in part, by luteinizing hormone elevation can not be ruled out.

The paracrine control of Leydig cells by factors released from the seminiferous tubules has been the subject of many investigations in recent years [16]. Both inhibitory (activin and transforming growth factor-β) and stimulatory (inhibin and insulin-like growth factor-1) factors have been identified in *in vitro* preparations [17-20]. Recently, Wu and Murono [21] have found a yet unidentified testicular growth factor(s) which stimulates proliferation but inhibits steroidogenesis of the rat Leydig cells. It may be postulated that in physiological conditions, some locally produced factors from the seminiferous tubules could regulate not only the function but also the proliferation of the adjacent Leydig cells, and that damage to the seminiferous tubules could interfere with the production of these regulatory factors. Whether the Leydig cell hyperplasia is the consequence of the secretion of newly-formed stimulatory factor(s) or an alleviation of some local inhibitory mechanism(s) occurring after testicular capsulotomy awaits further investigation. Although there were a marked hyperplasia and hypertrophy of the Leydig cells and a significant increase in LH levels after testicular capsulotomy, the secretion of testosterone remained low [3]. It has been shown that under the influence of FSH the Sertoli cells metabolize testosterone to estradial. Hence it is also possible that in the capsulotomized testis, the metabolic status of the Sertoli cells is changed under a high FSH influence, favoring the conversion of testosterone to estradiol with a resultant decline in the testosterone level.

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