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Response of the Seminiferous Epithelium of the Rat Testis to Withdrawal of Testosterone: an Ultrastructural Study[1]

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The aim of the present study was to investigate the ultrastructure of different cell populations in seminiferous tubules at condition of testosterone withdrawal. Ethane dimethanesulfonate (EDS) selectively eliminates by apoptosis Leydig cells in adult rat testis that causes the temporary lack of testosterone production. During the first two weeks after treatment stage dependent loss of germ cells was found within seminiferous tubules that led to a profound disturbance of spermatogenesis. The predominant cell types undergoing degeneration were pachytene spermatocytes and round spermatids. The ultrastructural changes of Sertoli cells were also observed. The kinetics of destruction, disappearance and regeneration of cells in seminiferous tubules follow the changes in Leydig cell population.

Key words: EDS, spermatogenesis, ultrastructure, seminiferous epithelium.

Introduction

The sensitivity of the seminiferous epithelium to a wide variety of factors which disrupt the process of spermatogenesis has provided a useful approach to the study of the biology of the Sertoli and Leydig cells and their role in regulating germ cell development [15].

In studies concerned with the hormonal control of spermatogenesis in the rat testis, it has been shown the histological alterations following the partial or complete withdrawal of testosterone, achieved by surgical hypophysectomy [6] or selective elimination of the Leydig cells with the Leydig cell toxicant EDS [8, 10, 17,].

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Testosterone withdrawal is known to disrupt spermatogenesis causing degeneration and loss of germ cells that become progressively more pronounced with time [3, 8]. Recent data indicate that drop in testosterone levels induced by EDS results in apoptosis of germ cells especially among haploid cells [7, 19].

The aim of the present study was to investigate the effect of testosterone withdrawal (after EDS application) on the ultrastructure of different cell populations in seminiferous tubules.

Material and Methods

Adult male Wistar rats (250-300 g body weight) were maintained under standard conditions. The animals were given a single intraperitoneal injection of ethane dimethanesulphonate (EDS, 75 mg/kg body weight) in dimethyl sulfoxid-water (1:3 V/V), known to destroy selectively the Leydig cells within 3 days and to reduce the intratesticular and serum levels of testosterone. Rats were killed at 3, 14 and 35 days after treatment. Testis were fixed in Bouen's solution, embedded in paraffin and examined by light microscope. The stages of spermatogenic cycle were assessed using morphological criteria of R u s s e l l et al. [13]. For electron microscopy, testicular fragments were fixed in 2.5% glutaraldehyde, 1% osmium teraoxide and embedded in durcupan. Electronograms were made on an Opton EM 109.

Results

A single dose (75 mg EDS/kg bw) of EDS administrated to an adult male rat kills completely the mature Leydig cell population of the testis. Within 72 h no histologically recognizable Leydig cells can be found in the interstitium. Removal of the LCs and thus the source of testosterone was followed by degeneration of the different cell populations in seminiferous tubules.

Sertoli cells: Ultrastructural analysis revealed many vacuoles occupied the cytoplasm of Sertoli cells (Fig.1). Often the fagosomes were also observed.

Spermatocytes: A dilatation of perinuclear space, chromatin condensation, cytoplasm vacuolization and mitochondrial destruction can be observed in pachytene-spermatocytes (Fig. 2).

Spermatids: In some seminiferous tubules in early stages there was disappearance of elongated spermatids. At the ultrastructural level many of the round spermatids posed a disorganization of chromatin structure and vacuoles in caryoplasm (Fig. 3). Degenerating spermatids showed morphological features with chromatin aggregation at the nuclear periphery, which appeared to resemble those of early apoptotic cells.

Two weeks post EDS a massive loss of elongated spermatids was found in all stages in the spermatogenic cycle. Regression of the seminiferous epithelium and subsequent infertility also occur.

The selective effect of EDS on the Leydig cell population is reversible, as precursor Leydig cells begin to repopulate the interstitium during the third week after EDS administration and testosterone concentrations return to normal levels.

Seven weeks after EDS treatment when the morphology of new Leydig cells population was similar to the control, the spermatogenic cycle seems to be almost recovered. However, some seminiferous tubules with abnormal morphology are still present in the testis.

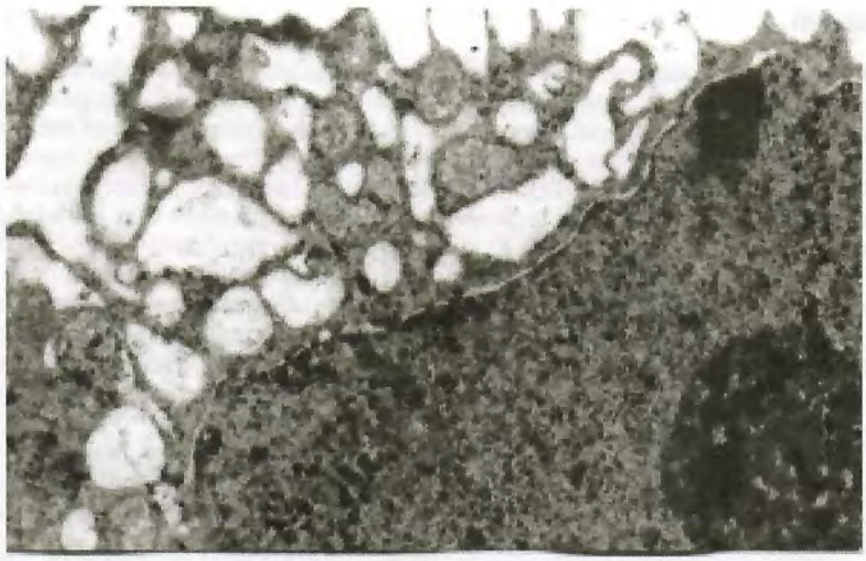

Fig. 1. Fragment of Sertoli cell — abundant vacuolization in the cytoplasm. 14 days after EDS treatment (×12 000)

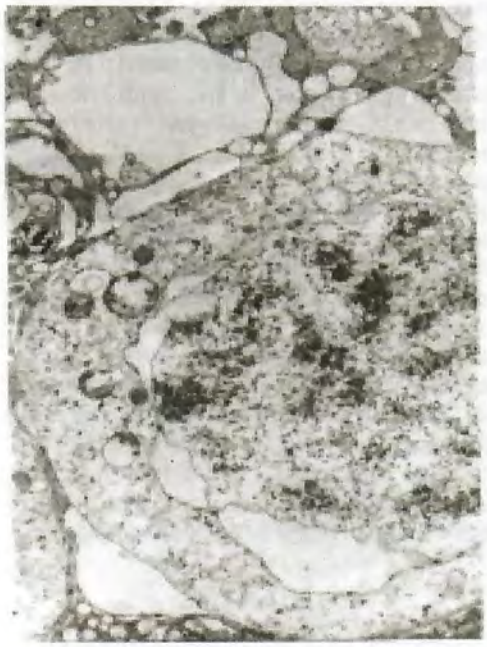

Fig. 2. Pachytene spermatocyte with marked cytoplasm vacuolization, dilation of perinuclear space and chromatin condensation. 14 days after EDS (×7 000)

Fig. 3. Degenerated round spermatid. 14 day after EDS (×12 000)

Discussion

The process of spermatogenesis is well known to be regulated by testosterone and FSH. The acute withdrawal of testosterone produces disruption of spermatogenic cycle. Then testosterone was removed over the period of a few days by the use of the Leydig cell cytotoxine EDS there was a sharp increase in the number of pyknotic nuclei and cytoplasmic vacuoles (both markers of cellular degeneration) of different cell populations in many stages of the cycle. Recent studies demonstrated that EDS leads to apoptotic death both of Leydig cells as well as germinative epithelium [7,9,10]. There was a marked increase in germ cell apoptosis 8 days after EDS administration. The predominant cell types undergoing apoptosis following androgen withdrawal were spermatocytes and round spermatids [19]. Our study confirmed these data. A role for testosterone in prevention of spermatocyte degeneration may be important particularly at stages VII-VIII where degeneration was prevented by concomitant testosterone administration [10].

The time-dependent increase in germ cell apoptosis in the seminiferous epithelium as a result of testosterone withdrawal is consistent with previous studies [4, 16, 18]. It has been well established that apoptosis occurs in the normal testis and is associated predominantly with spermatogonia [1, 7]. On the other hand, in response to androgen withdrawal as a result of hypophysectomy [13] or administration of EDS [7, 18] the cell types that have been shown to undergo apoptosis are pachytene spermatocytes and spermatids. In more extensive studies examining germ cell populations by determining the hourly production rates O'D o n n e l l et al. [11] demonstrated that the conversion of steps 1 to 7 round spermatids was maintained in the presence of lots of testosterone, but the conversion of step 7 to 8 spermatids proceeded at only 15% of its normal efficiency. Furthermore, adhesion of round spermatids at this stage appeared to be lost, resulting in their sloughing into the lumen and appearance in the epididymis. A massive loss of elongated spermatids found in all stages of spermatogenic cycle in our study might be connected with these phenomena.

The testosterone withdrawal leads to essential destruction of Sertoli cells observed by G h o s h et al. [6], R o b e r t s & G r i s w o l d [12], S h a r p e et al. [15] and also by us [2]. It seems likely that it is the Sertoli cells, rather than the germ cells, which first respond to androgen withdrawal because the Sertoli cells are exclusive site for androgen receptors within the seminiferous epithelium [14]. C a m e r o n et al. [5] described the disruption of the ectoplasmic specialization between Sertoli cells and round spermatids, responsible for normal development of spermatids.

According McLachlan et al. [10] testosterone stimulates Sertoli cell secretion of some proteins (e.g. androgen-binding protein) but inhibits the production (e.g. plasminogen activator) or gene expression of others (e.g. nerve growth factor). However, the relation of these changes to testosterone action on spermatogenesis has not been established. Future morphological and immunohistochemical studies might provide important information on how androgens support the process of spermatogenesis.

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