DEGENERATION AND RESTORATION OF SPERMATOGENESIS IN RELATION TO THE CHANGES IN LEYDIG CELL POPULATION FOLLOWING ETHANE DIMETHANESULFONATE TREATMENT IN ADULT RATS

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Objective. To investigate degeneration and restoration patterns of spermatogenesis in relation to the changes in Leydig cells (LCs) after treatment with ethane dimethanesulfonate (EDS).

Materials and methods. Adult Wistar male rats were treated with EDS at a dose 75 mg/kg body weight and the testes were sampled at 7, 14, 21, 35 and 49 days after treatment for histological and ultrastructural studies.

Results. 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 restoration of seminiferous epithelium followed also in stage specific manner and in relation to development of a new LC population (third week). The development of new LCs after EDS treatment repeats the normal dynamics of postnatal LC development within a similar time range.

Conclusion. EDS treatment of rats causes a temporary germ cell degeneration in the testis. The kinetics of disappearance of germ cells and their regeneration broadly follows the changes in LC population.

Key words: Spermatogenesis - Germ cells - Leydig cells – Testis – Rats – Ethane dimethanesulfonate

Two major events of testis development that occurr during the sexual maturation are the establishment of spermatogenesis and the development of adult Leydig cell population. Both events are driven by gonadotropic pituitary hormones. FSH is clearly the major factor in initiating of spermatogenesis and it also plays an important role in regulating the development of appropriate number of Leydig cell population in order to high levels of testosterone can be supplied for pubertal expansion of spermatogenesis (SHARPE 1994). LH is known to be required for both proliferation and differentiation of precursor Leydig cells whereas FSH rather stimulates their differentiation (TEERDS et al. 1988; Vihko et al. 1991). LH also plays a predominant role in stimulating testosterone production by Leydig cells (EWING and KEENEY 1993; PAYNE and YOUNGBLOOD 1995).

There is general agreement that testosterone is an essential factor for maintenance of normal spermatogenesis and fertility in adult males. The evidence available in rats suggests that testosterone exerts its effect on spermatogenesis at a specific stage of the spermatogenic cycle (BREMNER et al. 1994; SHARPE et al. 1992). Additional insights into how testosterone controls spermatogenesis might be gained by analysing how the process fails following testoster-

one withdrawal. Under that experimental conditions degeneration and loss of germ cells occurred and became progressively more pronounced with time. (Bartlett et al. 1986; Ghosh et al. 1992).

EDS selectively and temporary destroys Leydig cell population thus reduces testicular and serum testosterone levels that in turn elevates pituitary secretion of FSH and LH (Kerr et al. 1986; Teerds 1996). Therefore EDS experimental model is an useful tool to investigate not only the "fate" of Leydig cell population, but also the response of seminiferous epithelium to androgen withdrawal. The effect of EDS on spermatogenesis was examined in details within the first week after treatment and a lot of data indicated stage-dependent degeneration of germ cells within the seminiferous epithelium (Sharpe et al. 1992; Kerr et al. 1993). The long lasting action of EDS is still poorly investigated and the interest arouses not only to follow degenerative changes but rather to study the pattern of restoration of spermatogenesis that occurs with time after EDS exposure. In this respect the aim of present study was to examine the dynamics of degeneration and restoration of spermatogenic cycle in relation to renewal of Leydig cell population after EDS treatment.

Materials and Methods

Mature Wistar rats were housed under conditions of 12 hour light and dark cycles. Food and water were provided ad libitum. Rats received a single intraperitoneal injection of EDS (75 mg/kg body weight) dissolved in dimethyl sulfoxide (DMSO) and water 1:3 or of vehicle alone. Animals were sacrificed at day 7, 14, 21, 35 and 49 after EDS treatment. Testes were fixed in Bouin's fluid and embedded in paraffin. Histological evaluation was performed in 6 mm paraffin sections stained with Meyer's hematoxylin-eosin. For electron microscopy (EM) testicular fragments were fixed in 2.5 % glutaraldehyde and 1 % osmium tetraoxide and embedded in Durcupan. Electron micrographs were made with the use of Opton EM 109.

Results

In histological sections the first degenerative signs in seminiferous epithelium after EDS was seen by 7 days after treatment when Leydig cells completely disappeared from the interstitial space. There was an evident loss of elongating (step 9-13) spermatids in the seminiferous tubules at the late stages (e.g. IX-XIII), whereas the early stages remained still unaffected (Fig. 1) like in controls (Fig. 2).

Two weeks after EDS single newly formed Leydig cell appeared around seminiferous tubules and blood vessels. Electron microscope evaluation revealed the presence of mesenchymal-like cells with elongated spindle-shape and a thin wall of cytoplasm. These features are characteristic for progenitor type of Leydig cells (Fig. 3). The seminiferous epithelium revealed more pronounced destructive changes and degenerating germ cells are frequently found by light microscopy in the seminiferous tubules or were sloughed out into the lumen (Fig. 4). A massive loss of elongated spermatids was found in all stages of the spematogenic cycle. During the early stages, in some seminiferous tubules a disappearance of round spermatids and pachytene spermatocytes was observed.

Three weeks after EDS an expansion of new population Leydig cells was evident. Some of progenitor cells were transformed into immature type acquiring round shape and numerous lipid inclusions and lysosomes as evident by electron microscopy (Fig. 5). Despite the seminiferous tubules were severely depleted in germ cells, in histological sections some tubules revealed first signs of recovering of spermatogenesis. Elongated spermatids appeared in late stages (e.g. 9-13) whereas they were still absent in early stages (Fig. 6).

Five weeks after EDS treatment the morphology of new Leydig cell population was similar to the control and the adult type dominated in the interstitial space. By electron microscope, they were found to have a round nucleus with a prominent nucleolus, abundant smooth endoplasmic reticulum and mitochondria with tubular crists (Fig. 7). In histological sections the spermatogenesis in late stages looked almost recovered containing full complement of germ cells whereas elongated spermatids are still absent in early stages. Seminiferous tubules with different degrees of germ cell depletion were localized in a "spotty" manner. Some of them were of SCO type, containing only Sertoli cells and few spermatogonia (Fig.8).

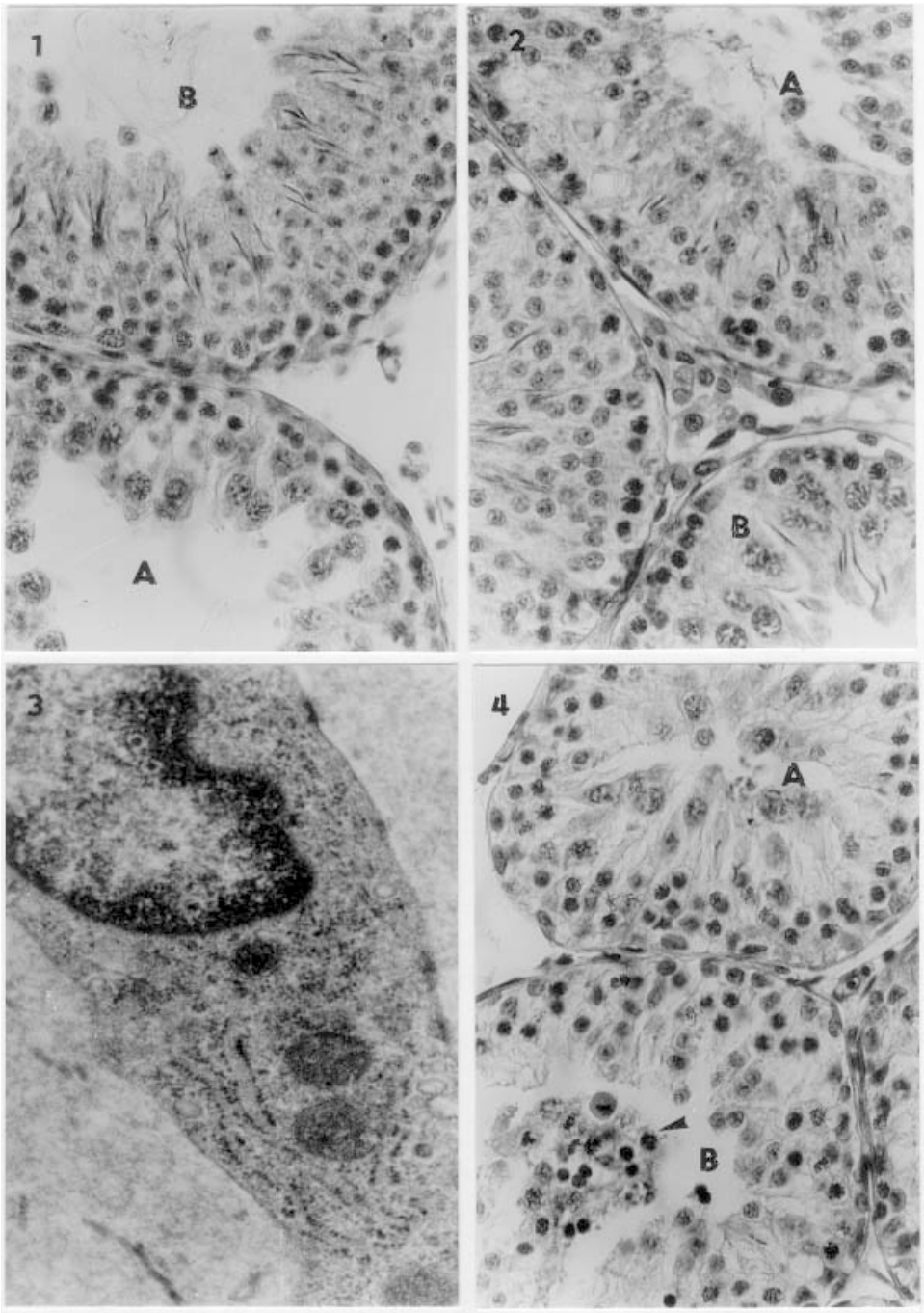

Fig. 1 Paraffin section of adult testes stained with Mayer's hematoxylin-eosin. Seven days after EDS treatment. Disappearance of Leydig cells from the interstitial space. In the seminiferous tubule at late stage (A) a massive loss of elongated spermatids was observed, whereas early stage (B) was intact. x 400

Fig. 2 Seminiferous tubules of control rat testis containing full complement of germ cells in early (A) and late (B) stages of spermatogenic cycle. x 400

Fig.3 Electron micrograph of Leydig cells at different stages of their development after EDS application. Progenitor Leydig cell - two weeks post-EDS. x 10 000

Fig. 4. Paraffin section of adult testes stained with Mayer's hematoxylin-eosin. Two weeks after treatment. Seminiferous tubules in late (A) and early (B) stages were devoid of elongated spermatids. Degenerating germ cells sloughed out into the lumen were also visible (arrow). x 400

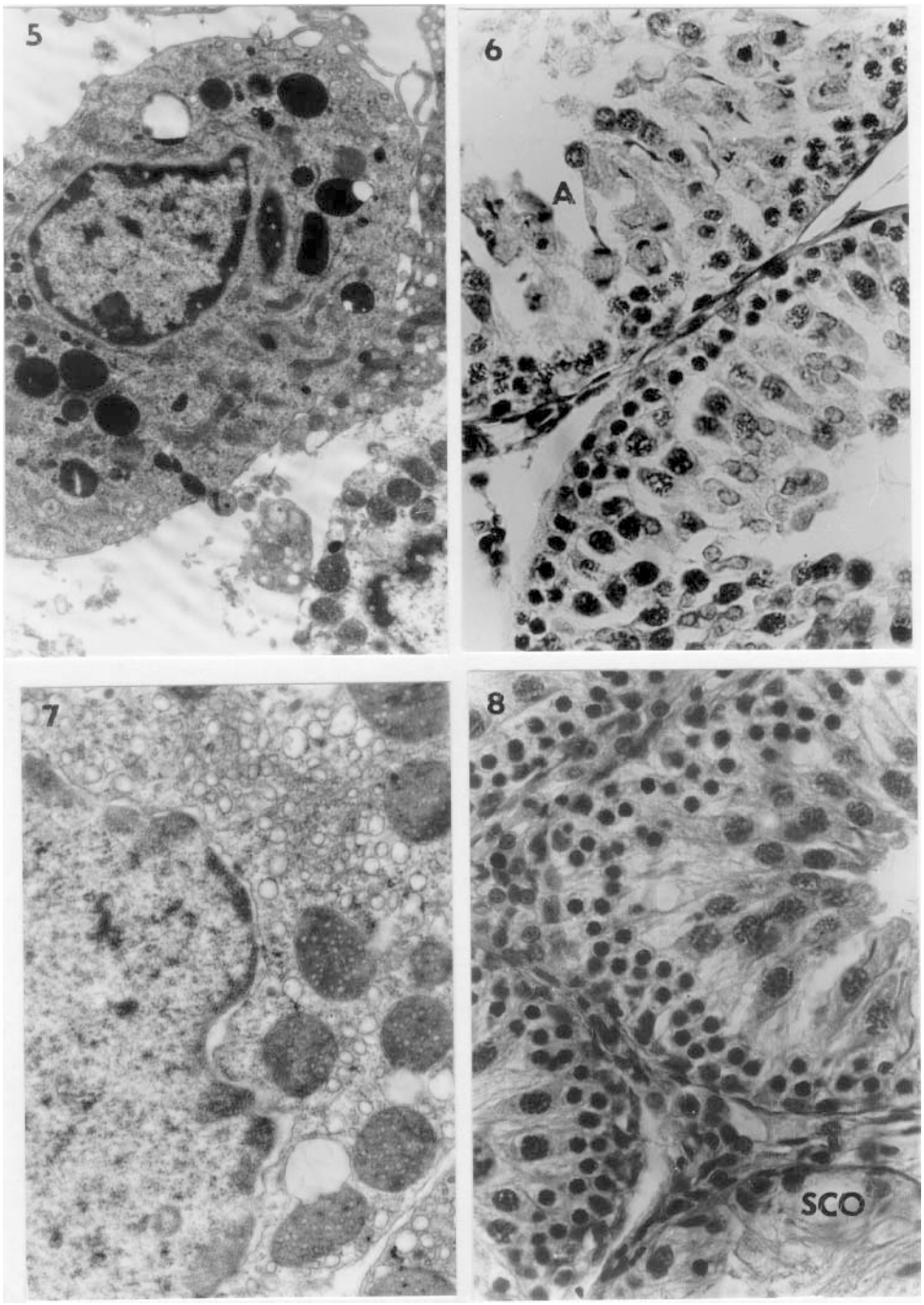

Fig. 5 Electron micrograph of Leydig cells at different stages of their development after EDS application. Immature Leydig cell - three weeks post-EDS. x 7 200

Fig. 6 Paraffin section of adult testes stained with Mayer's hematoxylin-eosin. Three weeks after EDS elongated spermatids appeared in late (A) stages of the cycle. x 400

Fig. 7 Electron micrograph of Leydig cells at different stages of their development after EDS application. Mature Leydig cell - five weeks post-EDS. x 12 000

Fig. 8 Paraffin section of adult testes stained with Mayer's hematoxylin-eosin. Five weeks after treatment seminiferous tubules in late stages were almost recovered but some germ cell depleted tubules (SCO) can be found. x 400

Seven weeks after EDS the new LC population showed normal morphological appearance. The spermatogenic cycle seems to be almost recovered. However, some seminiferous tubules of SCO type still were present in the testis, but they were more rarely found as compared to the previous treatment time point.

Discussion

The present study provides a detailed description of early and late changes in spermatogenesis of adult rat after EDS treatment. Our results show that at appropriate time intervals after EDS treatment elongated spermatids, round spermatids and pachytene spermatocytes disappeared in stage dependent manner causing a profound disruption of spermatogenic cycle. However, these changes were temporary and after three weeks following a single i.p. EDS application the spermatogenesis became activated and germ cells appeared again. Moreover, we established a close relationship between the changes of germ cell complement and the time schedule of changes of Leydig cells after EDS treatment.

In particular, our results demonstrate that germ cells began to disappear from seminiferous epithelium by the day 7 after EDS treatment leading to a massive loss of elongating (step 9-13) spermatids. Over 80 % of the elongated (step 10-12) spermatids are either absent or degenerating by day 7 post EDS and the number of degenerating germ cells at stages 10-11 far exceeds that at stage 7 (Sharpe 1994). According to him the spermatogenesis fails because of an increasing degeneration of germ cells after the stages 7-8 rather than because of a progressive maturation depletion of germ cells due to an inability of germ cells to pass successfully through these stages. This suggestion fits also with our data found on the day 14 after EDS when we observed a total loss of elongated spermatids from all stages of the cycle accompanied by the disappearance of round spermatids and pachytene spermatocytes.

The changes in Leydig cells and germ cell populations after EDS treatment are temporary and reversible which has been reported by some authors (Kerr et al. 1986; Teerds 1996; Taylor et al. 1998) and in the current study as well. Single newly formed Leydig cells appeared by 14 day post-EDS and therefore stage dependent germ loss of germ cells coincides with lack of appropriate LC population. At 21 day after EDS, when the new Leydig cell population was relatively well presented we observed the first signs of seminiferous epithelium regeneration with appearance of elongating spermatids. Together with development of new Leydig cell population, a restoration of spermatogenesis occurs also in stage specific manner following the normal dynamics of spermatogenic process. Therefore the kinetics of disappearance and reappearance of germ cells broadly follow the changes in Leydig cell population after EDS treatment.

The establishment of new LC population after EDS exposure was suggested to begin from progenitor LCs (Ge et al. 1996; Teerds 1996). The majority of postnatal Leydig cell population consisted of progenitor Leydig cells that during early sexual maturation undergo active cell division giving rise immature Leydig cells that in turn differentiate into adult mature Leydig cells (Benton et al. 1995; Hardy et al. 1991). In the present study we found progenitor LCs two weeks after EDS application. Moreover, the differentiation of new LCs passed through the same intermediate stages normally occurred during postnatal development. In our study the progenitor LCs differentiate into immature LCs within a week and two weeks later they are transformed into mature LCs. Therefore, the restoration of new LC population after EDS repeats the normal dynamics of LC development within a similar time range.

There is a strong evidence that loss of Leydig cells following EDS treatment causes a drop in the serum concentration of testosterone as well as changes in the gonadotrophic hormone levels (Teerds 1996). Furthermore, testosterone withdrawal is known to disrupt spermatogenesis causing degeneration and loss of germ cells that become progressively more pronounced with time (Bartlett et al. 1986; Kerr et al. 1993). Recent data indicate that the decrease of testosterone levels induced by EDS results in apoptosis of germ cells especially among haploid cells (Henriksen et al. 1994; Woolveridge et al. 1999). Therefore, it seems very likely that testosterone withdrawal after LC degeneration following EDS teratment is responsible for the temporary arrest of spermatogenesis in the experimental animals of the present study. Although at the days 35 and 45 the

Leydig cell population is abundant, we found that the spermatogenic cycle was not completely recovered. Even when the testosterone levels have reached the normal values. the complete regeneration of spermatogenesis probably needs appropriate stimulation of other nonandrogenic factors (Saunders et al. 1996). However, recent studies provide evidence that LCs produce numerous biological active substances, some of which play essential role in regulation of testosterone production and spermatogenesis (Angelova et al. 1996; Saez and Lejeune 1996; LeRoy et al. 1999). Thus, it is a matter of further studies to establish the exact mechanisms leading to the profound changes of germ cells observed in our study. In this relation also the significance of the other somatic cells, namely Sertoli cells must be taken into consideration, because of close functional relationships of this cell type with Leydig cells and germ cells in regulating spermatogenesis.

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