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Experimental Model for Streptozotocin-Induced Diabetes Mellitus Neonatally or in Adulthood – Comparative Study on Male Reproduction in Condition of Hyperglycaemia

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Reproductive dysfunction is a consequence of diabetes mellitus (DM), but the underlying mechanisms are poorly understood. In this respect the aim of the present study is comparative evaluation of spermatogenesis in conditions of experimentally induced DM neonatally (NDM) or prepubertaly (PDM) or in adulthood (ADM) in relation to diabetes status. DM was induced by single i.p. injection of streptozotocin (65 mg/kg) Germ cell development/spermatogenesis was assessed by immunohistochemistry for tACE. Gross morphology of the testis in ADM rats is relatively normal whereas the histology of the testes from 50 day-old NDM rats is more altered than in ADM. Testicular morphology was most affected in 50 day-old PDM. Spermatogenesis is not completed and different degree of delay in spermatid development was observed. In conclusion the long-term diabetes with sustained hyperglycemia leads to significant testicular dysfunction that could be considered as a risk factor for male fertility.

Key words: ACE, diabetes, spermatogenesis, germ cells, testis

Introduction

Angiotensin I converting enzyme (ACE) is well-known component of rennin-angiotensin system (RAS) that plays an essential role in male reproduction [6]. This enzyme exists in two isoforms – somatic (sACE) and testis (tACE) specific and they are differently distributed in the male reproductive system [5]. The tACE is expressed in germ cells during spermiogenesis, particularly in elongating spermatids in a stage specific manner. Therefore tACE can be used as a good marker for spermatid differentiation [10, 1]. Our data from previous studies [1] indicated that tACE is a marker for developmental stage of germ cell differentiation in the course of the first spermatogenic wave. Our data from experimental models that affect spermatogenesis characterize tACE as a marker for germ cell depletion in pathological conditions [2, 3].

Reproductive dysfunction is a consequence of diabetes mellitus (DM), but the underlying mechanisms are poorly understood. Abnormal sperm production and failure of reproduction is a long time recognised consequence of DM, and it is accepted that infertility is a common complication in diabetic men [11]. In rats, DM induces decreased testicular weight, sperm number and motility, testosterone levels that are an expression of abnormal spermatogenesis [9]. Data from experimental model in rat revealed that spermatogenesis is more severely altered by DM induced prepubertally than DM induced in adulthood [8]. Nevertheless, the histological alteration of testes has never been deeply studied, and the molecular mechanisms underlying this dysfunction are poorly understood. In this respect the aim of the present study is comparative evaluation of spermatogenesis in conditions of experimentally induced DM neonatally or prepubertally or in adulthood in relation to diabetes status.

Materials and methods

DM was induced by single i.p. injection of streptozotocin (65 mg/kg) in adulthood (10 week-old rats) or neonatally on d1 p.p or prepubertally on d10 DM status confirmed by blood glucose > 15 mmol/l 2-3 days after injection. Testes were sampled at d50 (neonatal and prepubertal treatments); or at 1 and 2 months after injection (adult treatment) and they are fixed in Bouin's fluid and embedded in paraffin. Germ cell development/spermatogenesis was assessed by immunohistochemistry (ABC-HRP technique) for tACE using rabbit polyclonal antibody at dilution 1:500 (Santa Cruz) [2].

Results

Our data from experimental model for DM induced in adulthood (ADM) revealed significant reduction of body weight by 20-30% whereas the 10% decrease in testis weight was not significant. The similar tendency for testis weight was found in animals with induced DM neonatally (NDM) or prepubertally (PDM) (data not shown). Blood glucose levels were strongly elevated up to 4 times in ADM and they were higher by 20-30% in NDM and PDM compared to the controls. Plasma testosterone levels were not significantly lower than controls in all the experimental groups.

Histology and immunohistochemistry for tACE showed that on d50 p.p.in all the experimental group spermatogenesis is completed as indicated by full germ cell complement in the seminiferous tubules (ST) of the testis. The stage specific pattern of expression of tACE is intact starting in step 8 round spermatid at stage VIII of the spermatogenic cycle and reaching maximum in step 19 mature elongated spermatids at the same stage. Gross morphology of the testis in ADM rats is relatively normal and at some places shrinkage of seminiferous tubules is found accompanied by thickening of blood vessel wall. Histology of the testes from 50 day-old NDM rats is more altered than in ADM rats. Differential response to the hyperglycaemia was found as in some animals ST with enlarged lumen were seen and in others rats shrinkage of ST in stage VIII was found. Comparative analysis revealed that testicular morphology was most affected in 50 day-old PDM. Spermatogenesis is not completed and different degree of delay in Sd development was observed. It was indicated by lack of elongating spermatids in stages IV-VII or in early stages (I-VII) or total loss of elongating spermatids in all the fourteen stages was seen (Fig.1.).

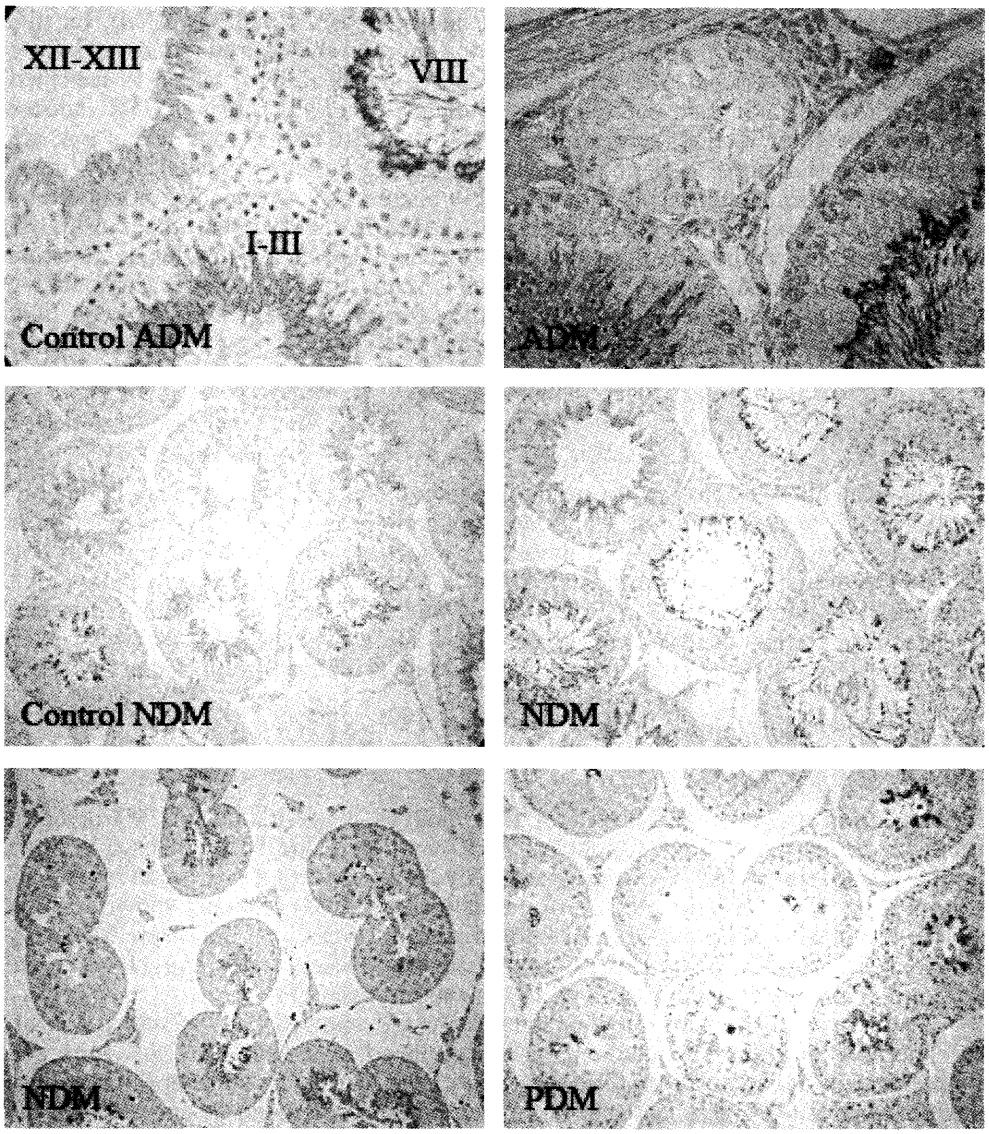

Fig.1. Immunohistochemical localization of tACE in the elongating spermatids of the testes from ADM, NDM and PDM. Enlarged ST lumen and shrinkage of ST is evident in NDM. Note lack of spermatids in PDM. Magnification: ADM – x400; NDM and PDM – x200.

Discussion

Our data provide evidence that diabetic state results in decreased body and testis weight and induced obvious changes on the testicular morphology, especially in neonatal and prepubertal diabetic animals. Recent study by Ricci et al [8] also reported frequent abnormal morphology of ST in which disorganized epithelium was present. As the authors found abnormal localization of occludin in Sertoli cells they conclude

that diabetes directly or indirectly impairs tight junctions and probably prevents the formation of peculiar microenvironment that in turn produce negative impact on germ cells.

It is a long time known that in diabetes, the cellular defense against toxic free radicals is reduced and induction of diabetes with streptozotocin dramatically decreased superoxide dismutase (SOD) in pancreatic islets [7] and in the testis [8]. The role of oxidative stress due to diabetic conditions in testicular function is not well understood.

It has been described that the anti-oxidant defence systems in rat Leydig cells decrease with age [4], and in the total testes the SOD activity is age-dependent [12]. Diabetes appears to mimic the effects of ageing, inducing an increase in OS in the testes. Therefore, the alteration in spermatogenesis in diabetic state is mainly due to Leydig cell functional impairment caused by oxidative stress. As a consequence decreased testosterone production is responsible for the suppression of germ cell development [8].

Our data provide new evidence for expression of t ACE in diabetic condition and demonstrated the dynamic of spermatid population, in particular the delay in development of postmeiotic stages of spermatogenesis. Previously we suggested tACE as a marker for germ cell depletion during aging and pathological conditions [2, 3]. In case of DM tACE also could be considered as a marker for assessment of developmental stage of delayed spermatogenesis. Moreover our comparative analysis provided the first evidence that neonatal and pubertal testis is more affected by hyperglycaemia that adult testis. Induction of DM on d10, when the first proliferative wave have started, affects germ cell development in a stronger extent compared to DM induced on day 1when gonocytes are still quiescent GC. Our data indicate that the spermatogenesis is more vulnerable to DM at the time of proliferative phase of spermatogonia (d4.5-d12) rather than the time of their mitotic arrest/quiescent period before d4.5 p.p.

In conclusion our results indicate that long-term diabetes with sustained hyperglycemia leads to significant testicular dysfunction associated with decreased fertility potential.

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