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Enclothelial Cell Toxicity of Cadmium: Transmission Electron Microscopy Examination

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Abstract: Cadmium induces cellular damage in reproductive system. We investigated vascular endothelial cellular injury induced by cadmium on blood vessels of the testis. Here we compared the fine structure of cellular changes in two groups of mice (SWR), experimental and control. The experimental group underwent cadmium ingestion at 1 mg.kg^{-1} daily for 4 weeks. The control group underwent ingestion of distilled water with equal dosages, using the same type of injectors, for 4-weeks. After cadmium exposure period both control and experimental groups were sacrificed and samples of the testis were processed for microscopic examination. The prepared samples were cut, stained and examined by Transmission Electron Microscope (JEOL-100 CX) at 80 KV. Ultrastructure examination revealed narrowing the lumen of the blood vessel associated with nuclear hypertrophy of the endothelial cells, irregular thickness of the endothelial cell cytoplasm. The endothelial cellular injury was associated with numerous pathological signs in germinal cell differentiation, heterogeneous chromatin distribution, irregular nuclear envelope and acrosomal dislocation. Based on the results of this investigation it can be concluded that endothelial cellular injury mediate early testicular failure secondary to microcirculatory impairment.

Key words: Blood vessel, cadmium, endothelial, testis and nuclear envelope

INTRODUCTION

Pioneering reports[12,13] indicated that cadmium caused toxic effects in the male reproductive system that resulted in an increased incident of impaired reproductive function and infertility. Epidemiological studies[3,5,11,16,18,27] revealed a relationship between paternal cadmium exposure and post-conception abnormalities. Many of these epidemiological observations have been interpreted as secondary effects to neuroendocrine malfunction - hormonal imbalances. In addition, clinical investigations had attributed these epidemiological observations to an increased incidence of interstitial cell tumors of the testis and proliferative lesions of the prostate[1,24]. Furthermore, the cellular markers of those epidemiological reports were attributed to chromatin instability and degeneration of the seminiferous epithelium[4]. More recent light microscopic studies[2,9,21-23,25] along with histological studies[7,11,17] have focused on seminiferous degeneration induced by cadmium. In spite of the broad investigations reviewed above the pathophysiology of testicular microcirculation that might be responsible for testicular failure remains unclear. From pathophysiological standpoint, early testicular injury is initiated as a result of loco microcirculation failure.

In vitro experiments revealed that the number of endothelial cells was decreased significantly in metal cadmium treated cell culture[14]. These findings indicate inhibition of cell proliferation and increased in the cell detachment and retardation of repair of the space. We hypothesized that cadmium reacts with endothelia mediators of testicular blood vessels leading to inflammation of the endothelial vessel layer and the subsequent enclothelial cellular swelling and injury. There is no previous research to provide information with regard to the effects of cadmium on the pathology of the enclothelial cell on the blood vessel of the testes. To reveal important pathological signs of cadmium effects on testicular enclothelia cells, here we report their fine structure changes induced by cadmium treatment.

MATERIALS AND METHODS

Experimental design: Twelve mature mice (SWR) matched with age, with mean body weight of 27.48 gm, was divided randomly into two groups, six controls and six experimental. Experimental group underwent cadmium ingestion of 1 mg.kg^{-1} day^{-1}, for four weeks period. Animal in the control group was ingested equal dosage of

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distilled water, using the same type of injectors for the same period. The treatment room was maintained at a constant temperature of 25°C.

Toxicant solutions preparation: A stock solution was prepared by dissolving cadmium salt -cadmium acetate $(CH_3COO)_2Cd_2H_2O$ (Reide Dehaven, Hanover - Germany) in deionized water and from each stock solution a measured amount of metal solution was taken and thoroughly mixed with distilled water to make it up to 100 microgram.ml^{-1} solution, the amount of the daily dosage in accordance with the concentration mentioned above.

Ultrastructure procedures: Animals were sacrificed and tissue samples were obtained from the left and right testes. Immediately after all samples underwent fixation, dehydration, infiltration, imbedding as described in the following sections.

Fixation: Tissue samples were immersed in 3% buffered cold (4°C) of glutaraldehyde fixative on a glass surface for 10-15 min to attain a proper hardness and then chopped into pieces approximately I mm^3 in size and transferred to viles containing 2% buffered glutaraldehyde for 2-4 h. Tissue blocks were post fixed in 1% osmium tetroxide in the buffer for 2 h at 4°C, then washed overnight in 3 changes in cacodylate buffer (pH 7.4) at 4°C.

Dehydration: Fixed tissue samples were dehydrated in graded concentrations of ethyl alcohol, (30, 50, 70, 90%) for 30 min each and finally in absolute ethanol (100%) for 40 min. Tissues were infiltrated gradually in resin and embedded in plastic capsules in fresh full strength agar 100-epoxy resin before being cured in an oven at 60-700°C for 2 days.

Transitional fluid: Dehydrated tissue samples were then placed in propylene oxide to get rid off ethanol and render the tissues penetrable for the embedding media. This step was done at room temperature for 60 min.

Infiltration: Tissue samples were transferred from propylene oxide to the mixture of epoxy resins. First, samples were placed in a mixture of propylene oxide and resins at the ratio of 1: 1 for 2 h and lastly placed in pure epoxy mixture overnight.

Embedding: Tissue samples were embedded in the epoxy mixture using polyethylene capsules. Polymerization of the resin was done at 600°C for 48 h.

Sectioning (UCT-GA): Polymerized resin blocks containing tissue samples were prepared for sectioning, first semi-thin sections (I μm) which were stained with toluidine blue for purpose of orientation. Accordingly ultra sections (70 nm) were made and double stained with uranyl acetate and lead citrate. Ultra sections were mounted on carbon-coated grids, then examined and photographed by Transmission Electron microscope (JEOL- 100 CX) at 80KV.

RESULTS

The fine structure of endothelliall cell of blood vessel in the control group: As can be seen from the Fig. 1, the wall consisted of a thin continuous layer. Approximately three enclothelial cells forming the entire enclothelial circumference. The lumen contained erythrocytes that were floating freely inside the intravascular space. The nucleus of the enclothelial cell had an oval shape with convoluted appearance. The peripheral chromatin distribution is homogenous interior to nuclear envelope. The location and the orientation of the nucleus within the cytoplasm maintained the shape that optimized nucleocytoplasmic exchanges needed for cellular differentiation and developmental stages of the spermatogenesis.

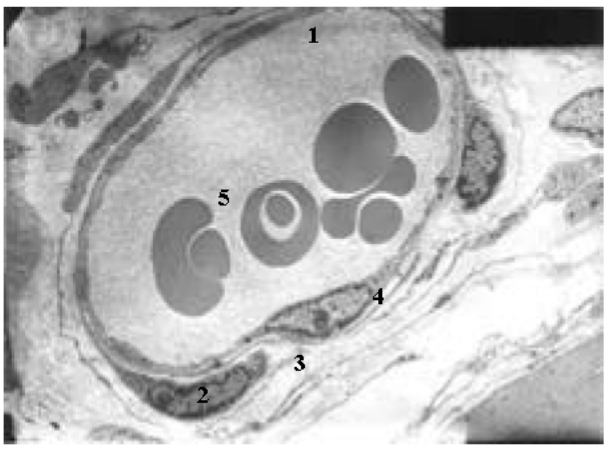

Fig. 1: Fine Structure of testicular blood capillary in the Control Group; 1) lumen, 2) endothelial nuclei, 3) vessel wall, 4) endothelial layer, 5) red blood cell x = 58, 180

Vascular endothelliall injury in cadmium treated group: Figure 2 showed various abnormalities such as hypertrophy of the nucleus of the endothelial cell, swelling of the cytoplasmic extension of the endothelial cell, indistinguishable capillary basement membrane, the basal blebs were extending from the cytoplasm and lamina thickness was not uniform. The resulting nuclear hypertrophy caused narrowing of the internal diameter of

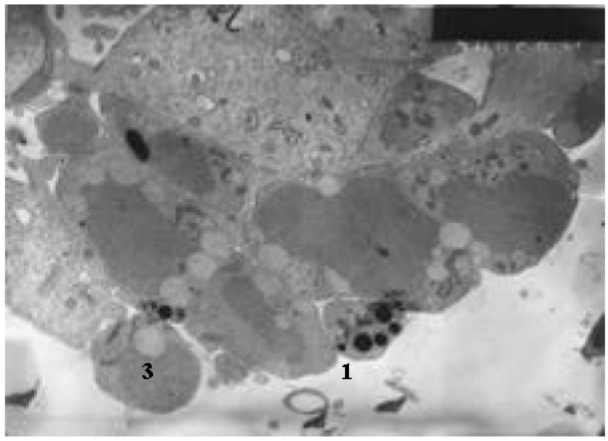

Fig. 7: Impaired germinal cell differentiation in cadmium treated group, 1) uneven chromatin allocation, 2) irregular nuclear envelope, 3) abnormal late spermatid development. x = 15, 750

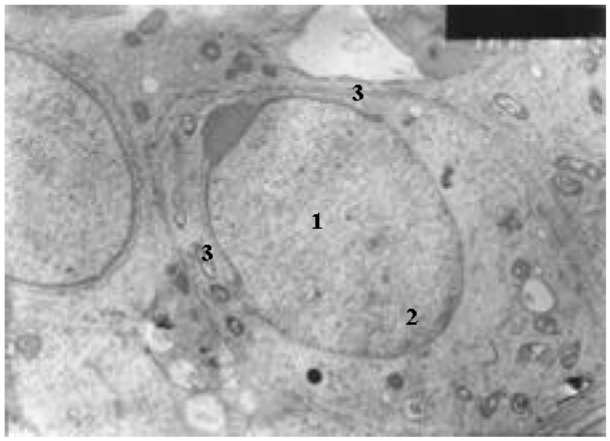

Fig. 8: Nuclear shape during spermatogenesis in controls group, 1) oval shape and slightly massive and coarse, 2) homogenous peripheral chromatin distribution and slightly condensed towards the nuclear pole, 3) symmetrical AG growth. x = 30, 000

the vessel's lumen with potential complete block and the subsequent prevention of blood supply and hence oxygen delivery -ischemic tissue. Moreover, the endothelial cells looked like undergoing cytolysis and their residuals were pointed toward the lumen (Fig. 3).

Vascular arteriole injury in cadmium treated group: Figure 4 showed damaged testicular arterioles, with detached endothelial cytoplasm masses and an overall swelling appearance. The nuclei of the endothelial cells were unfolded and enlarged associated with irregular nuclear envelope. Moreover, the smooth muscle in the middle layer was damaged as a result of reduced oxygen supply -tissue dysoxia.

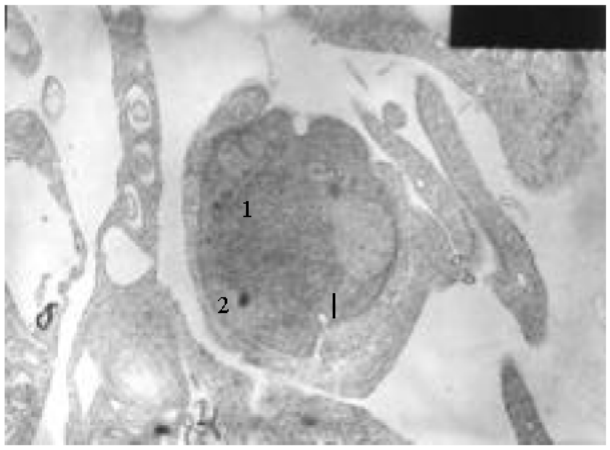

Fig. 9: Nuclear shape in the cadmium treated group, 1) deformed nuclear envelope, 2) uneven chromatin distribution. X = 30, 000

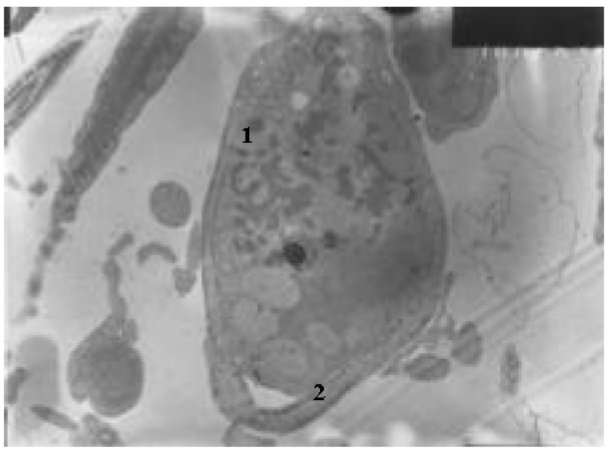

Fig. 10: Acrosomal shape in cadmium treated group, 1) lateral indention and dislocation of the AG, 2) the absence of the manchette. X = 13, 000

Spermatogenesis and sertoli, interstitial cell in the control group: The evolutions of germinal cells were homogenous and complete as evident by the formation of spermatozoa in clusters of 4-6 spermatozoa each (Fig. 5). As was noted the embeddment of the spermatozoa onto the Sertoli cell was noticeable. Further Fig. 6a showed normal attachment and embeddment of the spermatozoa in the process of Sertoli cells. Generally, normal spermatogonial development leads to normal spermatozoa development and pattern, with their attachment on the process of the Sertoli cells, with tail extended exteriorly. Furthermore, the interstitial cell differentiation followed the normal pattern of excretory cell differentiation (Fig. 6b) in which lipid droplets was observed within the cytoplasmic space. Furthermore Fig. 6b showed that phagocytosis of the lipid droplet succeeded to spread out

into the cell indicating their migration from a basal to an apical position toward Golgi apparatus for exporting. The interstitial cells of the testes produce male hormones, which are made of lipid materials. Evidently the normal and complete spermiation development that was presented in Fig. 5 appeared to be brought about by the action of gonadotropins excretion from well differentiated and well developed interstitial cells. Moreover, it is believed that gonadotropin facilitated the release of the spermatozoa into the lumen which was clearly observed in Fig. 5 and in Fig. 6a. Review of Fig. 6b again clearly showed that the interstitial cell consists of three regions-basal, middle and apical regions. The nucleus, granula, mitochondria, Golgi complex, transition vesicle that indicate transport of lipid, condensing vacuoles, small cytoplasmic pieces as a result of pinocytosis to get rid of excess cytoplasm and lipids granules. It was clear from Fig. 6b, that apical region contained a variable dense round fat granules and a few Golgi complexes. In addition, the apical surface has a few irregular microvilli or depressions of a size consistent with the lipid granules. Clearly, the number of the microvilli and width of the apical surface is related to the functional state of the cell. Both features showed hypertrophy indicating that active secretion of sex hormone was underway. The figure also showed that the mitochondria were abundant and evenly distributed and lying close to Golgi complex indicating increased metabolic activities.

Cadmium effects on cell differentiation: Cadmium treatment had resulted in impaired cell differentiation in a number of ways. Spermatocyte cell division resulted in uneven chromatin allocation in the daughter cells (Fig. 7). Evidently, as can be seen from the same figure early spermatids were not developed. Thus nuclear deformation initiated early cellular impairment and differentiation process. Moreover, there was no embeddment of the spermatozoa in the Sertoli cells (Fig. 7). Thus cadmium treatment inhibited early spermatogonial development which in turn was responsible for the absence of spermatozoa development. Under normal condition, the interstitial cells differentiation and growth that follow the normal pattern of excretory cell differentiation in which lipid droplets were observed within the cytoplasmic space, with drift towards Golgi apparatus as presented in Fig. 6.

Nuclear' shape in control group: Fig. 8 showed normal nuclear shape during spermatogenesis being massive, slightly coarse, with homogenous chromatin distribution and oval shape nuclei. The nucleoplasm was noted homogeneously dense and apparently without abnormal structures. Condensation of the chromatin towards the nuclear pole increases and hence the shape of the nucleus changes accordingly that ultimately led to an oval shape of the head of the sperm.

Nuclear shape in cadmium treated group: In comparison with Fig. 8, a deformed nuclear envelope associated with uneven condensation of chromatin was noted (Fig. 9). The overall shape was irregular with poor regression towards head formation as well as other accessories on the head. Also, the nuclear pole was not clearly identifiable.

Acrosomal shape in the control group: During differentiation of the acrosome the vesicles fuse into a single large vacuole. The acrosomal granules are attached to the nucleus and had grown and spread symmetrically over the surface. In addition, the acrosomal granules had lied against the vacuole membrane on the side towards the nucleus and intending it (Fig. 8).

Acrosomal shape in the cadmium treated group: Figure 10 showed that the acrosome had grown in skewed shape and was flattened over the nuclear pole. This had resulted in lateral indention by acrosomal granules and dislocation of the AG. There were no machetes, which were supposed to develop in the posterior part of the head and the middle piece of the cytoplasm (Fig. 10). Normally, the machete is a transient structure which present just before the onset of nuclear condensation of the spermatid and disappears later when the condensation and the formation of the middle piece is complete.

DISCUSSION

In the present study, fine structure examination revealed that cadmium resulted in injured endothelial blood vessel and related effects on cellular differentiation and spermiation. Setchell[19] and Waits and Setchel[26] indicated that cadmium impaired testicular vascular bed and testicular blood flow. Our findings provided evidences that endothelial cellular dysfunction was a major cause for these vascular changes induced by cadmium treatment. The ultrastructure examination of the present study showed that cadmium induced hypertrophy of the nucleus and swelling of the cytoplasmic extension of the endothelial cell. These findings were supported by histological changes that were reported by Massanyi *et al.*[15]. Apparently the major maturation changes occur in the sperm, as they traverse along the tubules, depends upon improved metabolic capacity that was acquired from

sertoli cell during spermiation. As sertoli cells represent blood testes barrier, the lack of blood supply is a critical factor that impairs their abilities to deliver metabolic needs before and during spermeation. Furthermore, blood flow, which is regulated by testicular arterioles, is critical for oxygen supply in serniniferous tubules. Our observations confirmed that cadmium resulted in cellular damage in endothelial and in muscle cell of the middle layer of the arterioles that was associated with irregular vascular structure as well as irregular nuclear shape.

Clearly the major developmental changes occur in the testicular cells as spermiation proceeds, depends upon improved blood supply in order to fulfill the energy demands to all cellular activities interstitial, sertoli and germinal cells. It has been demonstrated that cadmium is testicular tumorigen and produces high injury incidence in Leydig cell tumors[2,21,25]. In addition, results of the present study revealed that cadmium caused abnormal elongation of the middle piece. These findings are consistent with in vitro experiments, electron-dense deposits consisting of cadmium - oxine complexes were preferentially found in swollen mitochondria of the enclothelial cells[10,24]. Thus necrotizing germinal cells which evacuated into the lumen of the tubule suggested that administration of cadmium may imply the disturbance of respiratory function of the endothelium due to the primary toxic effects of this metal on mitochondria. Thus decreased sperm motion referred in this study may be caused by degeneration of mitochondria in spermatozoa, so the energetic source in male reproductive cells was severely affected.

Furthermore, results of the present study showed that the interstitial cells maturation did not follow the normal pattern of excretory cell differentiation in response of cadmium treatment. Even though we did not measure hormonal production in the present study, the fine morphological abnormalities induced by cadmium in the interstitial can not be isolated from testicular functional abnormalities, in terms of, cellular differentiation and spermiation abnormalities. As interstitial cell undergoes necroses secondary to vascular damage, the inhibin and testosterone production is disturbed. Thus LH production remains elevated mainly due to low circulating androgen as a result of degenerative and hypofunctioning interstitial cell on the testes[9]. Previous studies [18,20] had indicated that gonadotropins release the sperm by activating a mucolytic enzyme in the region of the apical cytoplasm of Sertoli cells. The spermatozoa are then transported in the rete testis by muscular activity and the rete system may also aid in this process. Our findings provided evident that the spermatozoa attachment and release were both severely impaired as a results of cadmium treatment.

The results of the present study suggest that cadmium react with endothelial vascular layer, which play a role in the pathology of testicular vascular injury in mice. Further research is needed to investigate the effects of cadmium on the respiratory chain of the mitochondria of the endothelial cell.

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