Original Article

Induction and Determination of Apoptotic and Necrotic Cell Death by Cadmium Chloride in Testis Tissue of Mouse

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Abstract

Background: Cadmium chloride which is potentially toxic is currently used in industry. The toxic effects of cadmium on testes have been reported to range from apoptosis to necrosis, with different effects on fertility. This research aimed to study the effect of different doses of cadmium on testicular tissues at acute stage by light and electron microscopy.

Methods: Cadmium chloride was injected into mature Balb/c mice intraperitoneally in 7 doses. Five mice were studied in each group. After 48 *hr*, the testes were extracted and prepared for light microscopy. Then two concentrations (15 and 25 *μM/kg*) of them were selected for electron microscopic study based on histological changes. The cellular changes of luminal epithelium of seminiferous tubules were studied under an electron microscope. Histological and ultrastructural changes were reported.

Results: The absence of sperm in the tubules was observed at 20 *μM/kg* concentration. At 25 *μM/kg*, histological destruction and epithelial damages were observed. Histological changes were not remarkable at 5 and 10 *μM/kg*. However, ultrastructural changes of seminiferous tubules at 20 *μM/kg* included spermatogonial cell death. At 25 *μM/kg*, vacuolation of Sertoli cells and death of spermatids as well as spermatocytes were observed. Cell death in the tubules was limited to germ cells. However, Sertoli cells exhibited architectural alterations without any cell death.

Conclusion: Both apoptosis and necrosis occurred in testicular tissue by exposure to cadmium in a concentration-dependent manner. Gonadal cells were sensitive to cadmium administration. Supportive cells such as Sertoli cells in seminiferous tubules did not exhibit sensitivity to cadmium.

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Introduction

Environmental contamination with heavy metals, pesticides, fertilizers and industrial chemicals damages organs through different mechanism such as oxidative stress. Cadmium, lead, arsenic and mercury are such heavy metals. Cadmium is an environmental pollutant that is used in manufacturing accessories such as batteries, paints and plastics (1). With an increase in the number of above products, not only workers of manufacturing industries but also the public (by absorption through water and the soil) are exposed to cadmium toxicity (2). Furthermore, the International Agency of Research on Cancer has introduced carcinogenic effects of cadmium on various tissues, including kidneys, prostate, liver, and pancreas (3). Cadmium enters the body through different routes of uptake, for example, through gastrointestinal tract by eating contaminated crops

and meat. The other route of cadmium uptake is through respiration of contaminated air via the respiratory system. According to WHO reports, approximately 60-70 *µm/day* is tolerable for a person weighing 70 *kg*. In addition, the uptake and accumulation of cadmium in smokers and non-smokers is different and the concentration of cadmium in smokers is higher (1).

Some studies have also shown that cadmium can cause cell death by necrosis in testicular tissue (4-6). Cadmium causes necrosis and atrophy in testicular tissue by suppression of glutathione peroxidase activity (7). Apoptosis in germ cells is an essential factor for normal processes of spermatogenesis (8). However, in some cases the balance of cell death in seminiferous tubules, which increase or decrease spermatogenesis, is altered by toxic materials. Factors such as viruses, anticancer drugs, hormones, radiation and toxic materials, including cadmium, induce cell death in seminiferous tubules (9). Control of apoptosis of germ cells in germinal epithelium cells of the seminiferous tubules and the subsequent decline of sperm production are noteworthy from a therapeutic point of view.

Studies on laboratory animals have shown that degeneration of vascular system is the earliest histological change in testes (10). Cadmium has different effects ranging from tissue changes to molecular changes at different concentrations. Sometimes no histological changes are seen but molecular changes occur. The molecular changes include activation of stress genes, tumorigenesis and anti-apoptotic pathways (11). Cadmium chloride does not cause significant damages in the testes at a low dose of less than 3 *mg/kg*; however, it induces mild apoptosis of germ cells and damage to blood-testis barrier (BTB) (12, 13). Furthermore, apoptosis is seen in all the epithelial cells of seminiferous tubules (14).

Due to controversies in the pattern of cell death through necrosis or apoptosis, in this study, the histological and cellular changes as well as the type of cell death in all the epithelial cells of seminiferous tubules were studied by electron and light microscopy.

Methods

In this study, mature 8-10-week-old Balb/c mice were obtained from Razi Serum and Vaccine Manufacturing Institute. The animals were kept in animal houses for 8 *hr* before the test. The mice were divided into eight groups and one group was considered as the control. Different concentrations of 5, 10, 15, 20, 25, 30, 40 *µM/kg* of cadmium chloride were dissolved in normal saline and administrated intraperitoneally. Five mice were studied in each group. Forty-eight hours after administration, the mice were sacrificed by cervical dislocation and their testes were extracted. Then the extracted samples were fixed in Bouin's fixative for 24 *hr*. The samples were placed in paraffin blocks. The blocks were sectioned and stained by hematoxylin and eosin. The doses of cadmium chloride were defined by the presence of injuries and histological changes in seminiferous tubules.

Two effective doses of cadmium (15 and 25 *µM/kg*) were selected for electron microscopy. The groups included the control and cadmium chloride groups (15 and 25 *µM/kg*). These concentrations were selected based on histological data. Furthermore, the non-effective and severe or lethal doses were eliminated from the study. The extracted testes were fixed in 2.5% glutaraldehyde and 1% osmium tetroxide. After fixation and dehydration, the samples were blocked in Araldite -Epon 812 resin. Ultrathin sections were mounted on the grids. The grids were stained by lead citrate and uranyl acetate and then the grids were examined by electron microscopy (Zeiss, 902).

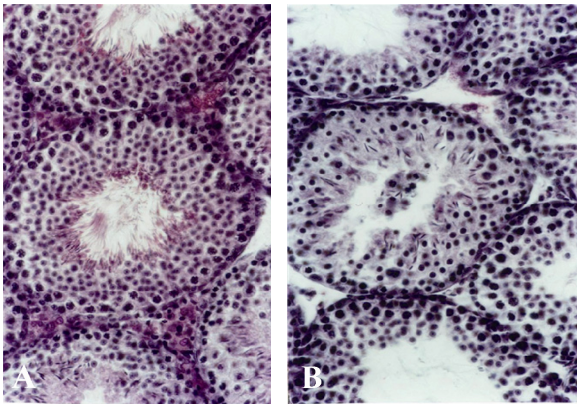
Results

The results were presented in two parts, electron and light microscopy.

Light microscopy: In the control group, seminiferous tubules and interstitial tissue of testes appeared normal. All the cells of epithelium with a variety of spermatogonia, spermatocytes, spermatids and spermatozoa were observed in enveloped Sertoli cells (Figure 1A). Histological changes at 5 and 10 *µM/kg* were similar to the control group and did not show any changes. In addition, the mice receiving 40 *µM/kg* of cadmium died before 48 *hr* and this dose was considered a lethal dose and was excluded from the study.

At 15 *µM/kg*, spermatogenic cells next to the basement membrane were pyknotic. Other epithelial cells of the tubules, including spermatocytes seemed normal. Also the spermatozoa were depleted almost from the center of the tubules and fissures in the epithelium were observed between them from the center to the periphery (Figure 1B).

At 20 *µM/kg*, spermatogenic cells adjacent to Sertoli cells were observed in the pyknotic form. In addition, spermatozoa and some spermatids were depleted from the center of the epithelium



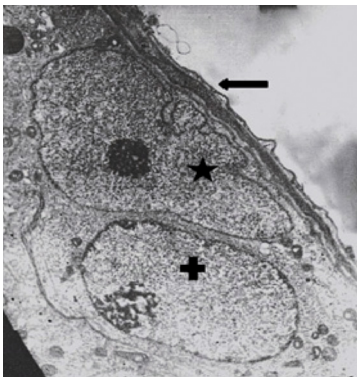
Figuer 1. Histological micrograph of seminiferous tubule in A: control group, epithelial layers from adjacent of the basement membrane to centre of the tubule with presence of spermatozoa; B: group of 15 *μM/kg*, cells adjacent to the basement membrane are seen with pycnotic nucleus and spermatozoa are depleted. Clefts appeare in epithelium. Magnification 400

and the continuity of epithelium of the tubules was disrupted.

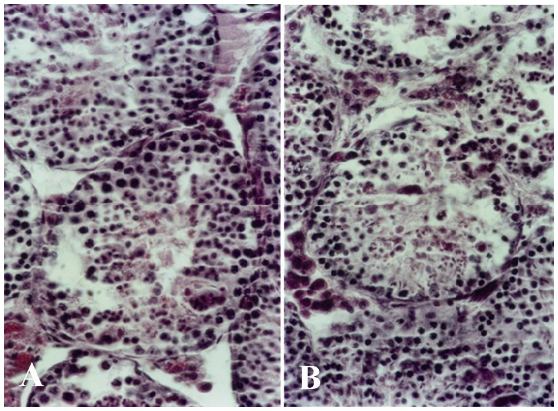
At 25 *μM/kg*, the overall shape of the tubules was disrupted with deep fissures. Cells with pyknotic nuclei were observed next to the basement membrane and also spermatids and spermatozoa were not observed in the tubules (Figure 2A).

At 30 *μM/kg*, the tubules showed severe disorganization. Furthermore, no germ cells were observed within the tubules (Figure 2B).

The electron microscopy: In the control group, the basement membrane was observed with myoepithelial cells. Sertoli cells were connected to each other by intercellular junctions and they were adherent to the basement membrane by hemidesmosomes. Sertoli cells with euchromatin nuclei and intact cytoplasmic organelles were seen. All types of epithelial cells, such as spermatocytes, spermatids and spermatozoa were normal (Figure 3).



Figuer 3. Ultrastructural micrograph of seminiferous tubule in control group: myoepithelium (Arrow), nucleous of Sertoli cell (*) and a spermatogonia (+). Magnification of 4400



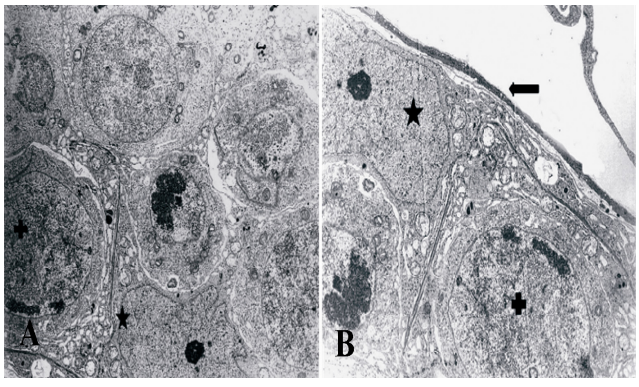
Figuer 2. Histological micrograph of seminiferous tubule in A: group 25 and B: 30 *μM/kg*, disorganization of cell layers in the tubules and presence of more pycnotic cells. Magnification 400

At 15 *μM/kg*, the basement membrane and myoepithelial cells appeared normal. Sertoli cells with the basement membrane and enveloped cells were observed with the intercellular junctions. There were some vacuoles within the Sertoli cells; spermatogonia and spermatocytes were separated from Sertoli cells. The nuclei of some spermatogenic cells were heterochromatin. Although spaces were seen between spermatids, the spermatids seemed healthy (Figures 4A, 4B and 5).

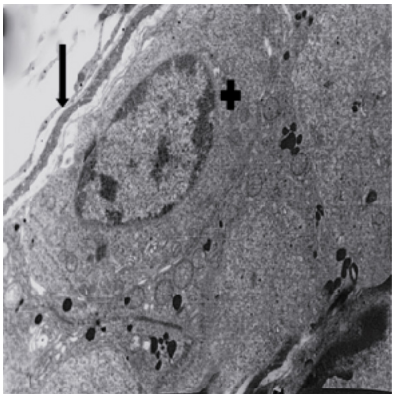
At 25 *μM/kg*, disorganization of tubules was seen within seminiferous tubules. The spermatogenic cells had undergone apoptosis. The germ cells were separated from Sertoli cells. Intracellular injuries were observed in some cells. Spermatids had lost their histological position in epithelium (Figures 6A and 6B).

Discussion

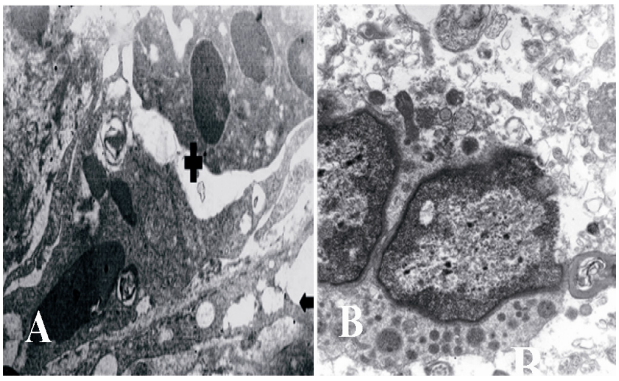
Daily exposure to cadmium is inevitable. Cadmium may influence the reproductive organs (1,



Figuer 4. Ultrastructural micrograph of seminiferous tubule in group of 15 *μM/kg*: myoepithelial (Arrow), nucleous of Sertoli cell (*) with vacuoles within the cytoplasm and spermatogonia (+). A: *3000 magnification; B: *4400 magnification



Figuer 5. Ultrastructural micrograph of seminiferous tubule in group of 15 *µM/kg*: myoepithelial (Arrow), spermatogonia with apoptotic nucleus (+). Magnification 4400



Figuer 6. Ultrastructural micrograph of seminiferous tubule in group of 25 *µM/kg*: myoepithelium (Arrow), necrotic spermatogonia (+), B: necrosis of spermatid. A: magnification 4400, B: magnification 7000

3, 9). According to findings of this research, at 15 and 20 *µM/kg* concentrations, cadmium induced apoptosis in germ cells. Severe disorganization of epithelium and necrosis were seen at 25 and 30 *µM/kg* concentrations. Although the first changes started at a dose of 15 *µM/kg*, tissue changes were not observed with 5 and 10 *µM/kg* doses. Consistent with this study, cadmium has no effects on spermatogenic status at light microscopic level at a dose less than 3 *mg/kg* (15). Ogawa observed damage to blood capillaries in the testes, leading to testicular hemorrhage, loss of continuity of germinal epithelium and interstitial fibrosis in mice with a high dose of cadmium chloride (10 *mg/kg*). Low dose of cadmium chloride (3 *mg/kg*) induces injury in testicular epithelium (15). However, studies show that a dose of 5 *µM/kg* of cadmium induces apoptosis and a dose of 10 *µM/kg* causes hemorrhagic necrosis in the testis (11, 16). In addition, apoptosis in germ cells starts at a dose of 5 *µM/kg* of cadmium (17). In contrast, in this study, histological changes started at a dose of 15 *µM/kg*. The low dose of cadmium increased stress and tumorigenesis in 12 *hr*. During the next 24 *hr*, apoptosis genes were also activated and the repair genes decreased their activity (11). Ogawa showed that the dose of 3 *mg/kg* (16.36 *µM/kg*) weakened the BTB and changed the immunological microenvironment of testis (15). In addition, with regard to the immune system, expression of interleukin-6 (IL-6), IL-1β and tumor necrosis factor-α (TNF-α) increased at the dose of 3 *mg/kg* in mice (15). Therefore, the effective levels of low doses were different, which might be attributed to the type of animal models or other laboratory conditions.

Evaluation with TUNEL method demonstrates that cadmium chloride causes apoptosis of germ cells in seminiferous tubules and these apoptotic cells can be seen in all parts of the epithelium (14). However, at 15 *µM/kg* in this study, apoptosis was confined to the germ cells adjacent to the basement membrane where the first layer of diploid germ cells is located (spermatogonia). Cell death was not observed in germ cells in other layers. There was high sensitivity to cadmium in spermatogonia rather than other epithelial cells. Application of a single dose of cadmium chloride for 4 weeks resulted in the necrosis and degeneration of seminiferous tubules (5). The results are consistent with 25 *µM/kg* concentration in this study and inconsistent with the results of 15 and 20 *µM/kg* concentrations of the study. However, cadmium causes necrosis and atrophy of testicular tissue (4-6), which might be attributed to vascular destruction of seminiferous tubules (10) and a decline in glutathione oxidase (7). Cadmium also disrupts the blood-testis barrier by making changes in the tight junction and Fak that are complex regulators of occluding-zo1 (18-20). One of the reasons for cell death in germ cells by cadmium is a decrease in the activity of antioxidant enzymes (21) that release free radicals (5) and increase lipid peroxidation in testicular tissue (22). The rate of lipid peroxidation is dependent on the concentration of cadmium.

Furthermore, cadmium results in the release of hydrogen superoxide from anions and peroxide of hydroxide radicals *in vivo* (7). Although there were morphological changes in Sertoli cells, cell death was not observed in these cells. Since cell death was limited to the germ cells, these cellular

changes altered the developmental process of germ cells, consequently changing the functional connection of Sertoli cells and germ cells. Cytoskeleton of Sertoli cells is changed by cadmium (7). Cadmium damages cadherin, interferes with DNA repair and call apoptosis (23). Consistent with this study, cadmium causes slight fragmentation of Setoli cell junctions in the absence of edema or necrosis of testicular tissues in rat at 3 *mg/kg* (17).

In this study, cadmium gave rise to depletion of sperm from seminiferous tubules. This phenomenon might be followed by an increase in the contraction of myoepithelial cells. This change was observed earlier than other histological changes. This phenomenon is noteworthy from male infertility viewpoint because cadmium leads to oligospermia and azospermia (24).

Conclusion

The type of cell death induced in seminiferous tubules by cadmium depends on the concentration and duration of treatment (acute and chronic form). Apoptosis and necrosis were seen at low and high concentrations of cadmium, respectively. The morphological changes may be good criteria to determine the confined alterations. Germ cells were more sensitive than Sertoli cells. However, cell death of germ cells might not be the direct cause of cadmium toxicity but might depend on the loss of connection between germ cells and Sertoli cells.

Acknowledgment

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Conflict of Interest

The authors declare no conflict of interest.

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