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Ultrastructural modifications in the mitochondrion of mouse Sertoli cells after inhalation of lead, cadmium or lead–cadmium mixture

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Abstract

CD-1 mice inhaled 0.01 M lead acetate, 0.006 M cadmium chloride or Pb–Cd mixture during 1 h twice a week during 4 weeks. Testes were processed for transmission electron microscopic analysis. The percentage of damaged mitochondria was related to exposure time and the type of metal inhaled, noticing more damage when the mixture was administered. A dose–time relationship was found. Cadmium chloride caused the most severe mitochondrial alteration compared to lead acetate, whereas the mixture was more aggressive compared with each metal alone. Our results suggest that the changes in Sertoli cell could lead to a transformation process that may interfere with spermatogenesis.

Keywords: Sertoli cell; Cadmium; Lead; Mitochondria; Metals; Testes

1. Introduction

As a consequence of increasing industrialization, lead (Pb) and cadmium (Cd), which are highly toxic metals, have increased in the atmosphere [1]. These metals enter the lung through respirable size particles (diameter $\leq 2.5\,\mu m$), and are distributed through the circulation to other organs [2,3]. Reports have indicated that the average concentration of Pb in urban areas is from 5 to 10 $\mu g/m^3$, while for Cd, is 0.06 $\mu g/m^3$ [4]. Where Pb or Cd pollution has increased, or in occupational exposures, a higher number of cases from infertile or sterile subjects have been reported [5,6]. Tobacco smoke is another important source of Cd exposure and it has been reported that one cigarette contains about 1–2 μg [7] or more, and that this concentration is related to tobacco leaf origin [8,9]. Some alterations in smokers' reproductive system could be attributed to the metal content of the tobacco leaf [10].

There are a variety of studies to suggest that reproductive health is adversely affected by exposure to these heavy metals (Pb, Cd) and by cigarette smoking. Cd exposure induces loss of tight junctions in Sertoli cells and ischemia and necrosis of the testes. In females, a decrease in estrogen production has been reported, as well as interference with ovarian–uterine function and embryo implantation [11]. Cigarette smoking during pregnancy is associated with reduced birthweight of the fetus [12] and some reports suggest an association with breast cancer [13]. Likewise, it has been noted that Pb exposure leads to decreased fertility in humans and rats [14]. Whether or not these changes reflect heavy metal components in tobacco smoke remain unclear. Suppression of the hypothalamic–pituitary–testicular axis has been reported in rats and possibly in men occupationally exposed to Pb [15]. As well there has been a report of decrease in sperm counts morphological and motility alterations in sperm from men [16]. Taken together, these findings suggest that some toxic metals, including Cd and Pb, directly perturb male and female reproductive function.

Metal-induced testicular dysfunction may arise from disturbances in Sertoli cells [10] that support spermatogenesis or Leydig cells responsible for androgen production under control from the hypothalamic–pituitary–testicular axis [11]. Sertoli cells have a well-defined ultrastructure, with a vast cytoplasm and a large convoluted nucleus, numerous spherical or elongated mitochondria with tubular cristae. The mitochondrion is sensitive to the toxic actions of Pb or Cd exposure [17–20]; however, no reports are available regarding the potential effects when a metal mixture in the atmosphere is inhaled. Heavy metal exposures may

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decrease glutathione levels and interfere with mitochondrial sulfhydryl and thiol groups, leading to the permeability transition and cell death [21].

The research described here was designed to study the effects that inhalation of Pb, Cd or Pb–Cd mixtures have on mitochondrial ultrastructure of Sertoli cells in the mouse as well as the relationship between exposure time and metal interaction.

2. Materials and methods

2.1. Animals

Seventy-two CD-1 adult mice (32 ± 2 g) were randomly divided in three groups of 24 animals each. Group I inhaled lead acetate 0.01 M (Sigma Chemical Co., St. Louis, MO, USA) for 1 h twice weekly during a 4-week period [22]. Group II inhaled cadmium chloride 0.006 M for the same duration (Sigma Chemical Co.) [23,24] and Group III was exposed to a mixture of cadmium chloride and lead acetate with the same concentrations indicated above. Each group had eight control animals that inhaled deionized water with the same protocol. Inhalations were performed in a closed acrylic box (50 cm × 30 cm × 20.98 cm) connected to an ultra-nebulizer (Ultra Neb 99 DeVilbis), with 10 l/min continuous flux. After the inhalation the animals (10 each time) were returned to their cages and fed with pelleted diet and tap water ad libitum [25]. The exposure program was repeated for 1, 2, 3 or 4 weeks and the experimental animals (four exposed, two controls from each group) were euthanized for ultrastructural analysis at the end of each weekly treatment period.

2.2. Electron microscopy

Experimental animals were anesthetized with sodium phenobarbital and perfused through the left ventricle with cacodylate-buffered saline (0.1 M, pH 7.4) followed by the fixative (2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4) [25]. Testes were removed and cut into small blocks (1 mm^3), washed with cacodylate buffer, postfixed in 1% OsO_4, dehydrated in ascending ethanol series, and embedded in Araldite 6005. Thin sections (60–80 nm thick) were contrasted with uranyl acetate and lead citrate and analyzed in a Zeiss EM10 electron microscope [26]. For assessing the extent of the Sertoli cell mitochondrial damage, 30 cells were randomly selected in 100 μm^2 to count those cells with damaged mitochondria, identified as no crests seen in the mitochondria from each Sertoli cell, and reported on a percent basis.

2.3. Statistical analysis

Data analysis used one-way analysis of variance (ANOVA). After raw counts the data were log-transformed

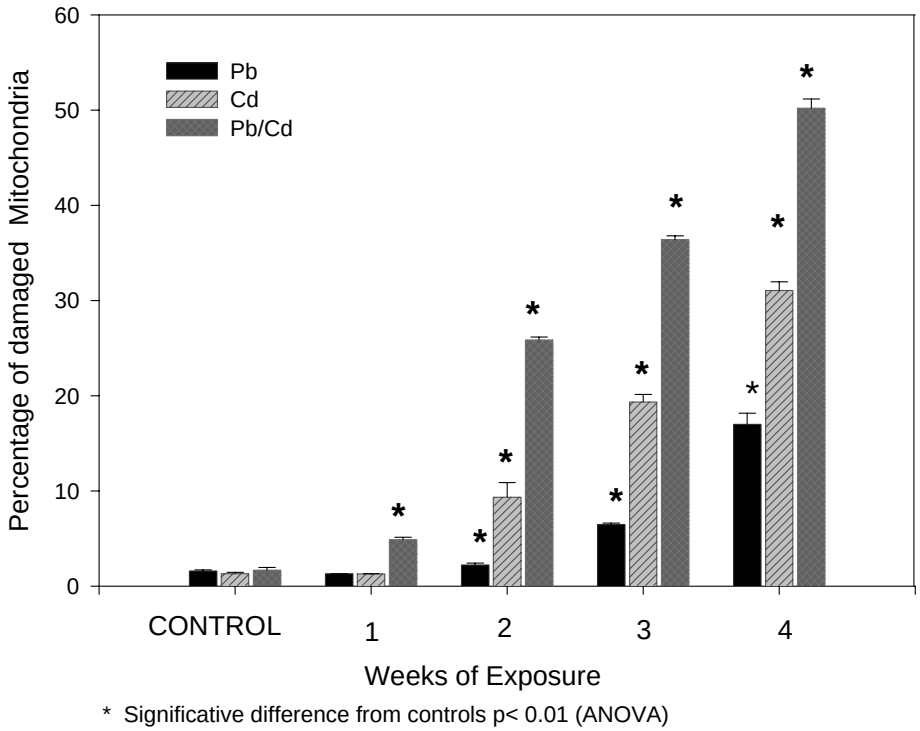

Fig. 1. Percentage of damaged mitochondrial after inhalation of lead (Pb), cadmium (Cd), or the Pb–Cd mixture. Asterisk (∗) denotes effects of exposure that were significantly different when compared with control animals from the same group ($P \leq 0.01$).

into a normal distribution. Calculations were carried out using JMP v.5 (SAS) software. The differences in treatment were considered significant at $P \leq 0.01$.

3. Results

Damaged mitochondria were scored in Sertoli cells from mice exposed by inhalation to either Cd (0.006 M) or Pb (0.01 M) alone or in combination. The percentage of damaged mitochondria increased significantly as a function of exposure duration and metal contaminant (Fig. 1). In general, exposure to Cd had a stronger effect than Pb at any duration and the highest damage percentage was observed in mice that inhaled the Pb–Cd mixture in the 4-week exposure scenario ($P < 0.01$). The effect that Cd or Pb alone had became significant after 2 weeks whereas the effect of the Pb–Cd mixture was significant after only 1-week exposure. Differences in the number of damaged mitochondrion remained significantly increased versus controls at all subsequent times for each metal treatment group.

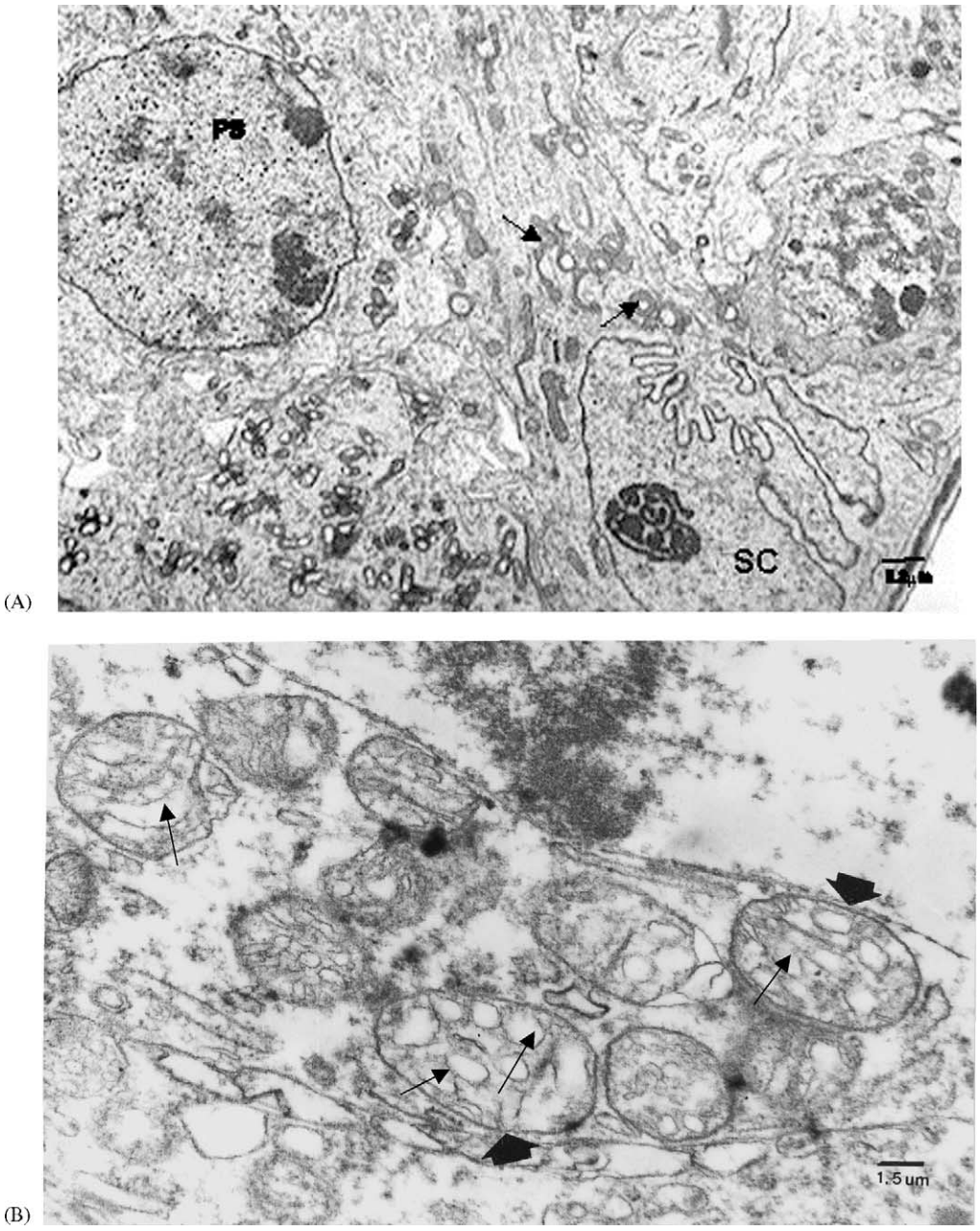

Fig. 2. (A) Section of a seminiferous tubule in which a classical Sertoli cell (SC) is observed with its characteristic irregular nucleus and tubular cristae in the mitochondrion (thin arrows). The nucleus of an adjacent primary spermatocyte (PS) is identified. (B) Electron micrograph showing mitochondria from control Sertoli cells (arrowhead). The tubular cristae are evident (thin arrows).

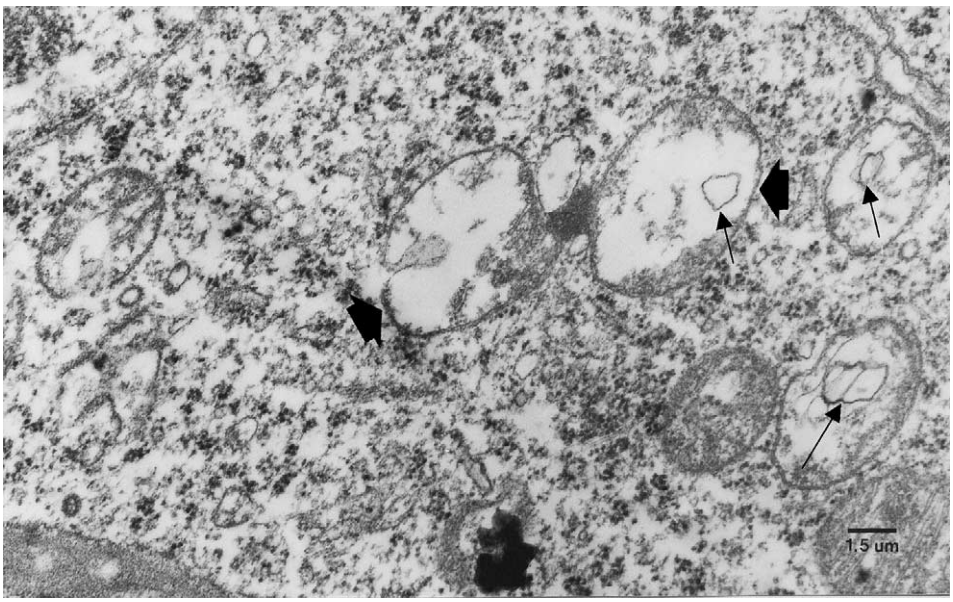

Fig. 3. Disruption of mitochondrion (arrow) was observed in Sertoli cells from mice inhaling only 0.006 M $CdCl_2$. Some cristae remained in the swollen mitochondria at 4 weeks (thin arrows).

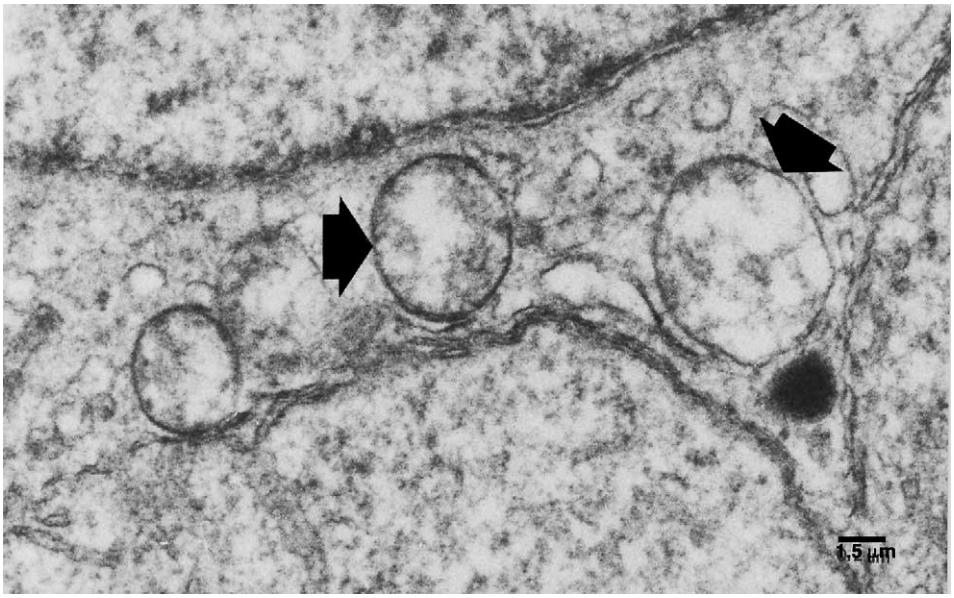

Fig. 4. Mice inhaling only Pb acetate 0.01 M, exhibited mitochondrion cristae loss at 4 weeks (arrow).

Sertoli cells from control mice were identified as large structures with an irregular shaped nucleus some lipid droplets and numerous spherical or elongated mitochondria with tubular cristae sometimes referred to as “tubular crest” or “transverse cristae” (Fig. 2). Changes in Sertoli cell mitochondria included swelling and loss of cristae when in animals exposed to Cd alone (Fig. 3). In contrast, animals exposed to Pb for 4 weeks had some cristae remaining and showed flocculent density inclusions (Fig. 4). When the Pb–Cd mixture was inhaled, we observed cristae distortion, increased matrix density and dense core spherical inclusions (Fig. 5). Other changes included the presence of vacuoles in the cytoplasm, an increase in the quantity of residual bodies, basal membrane detachment of the Sertoli cell and intercellular swelling.

4. Discussion

The present study observed metal-induced changes to the mitochondrion of Sertoli cells in mice exposed to Cd, Pb or Pb–Cd mixture by inhalation, an increase in the extent of

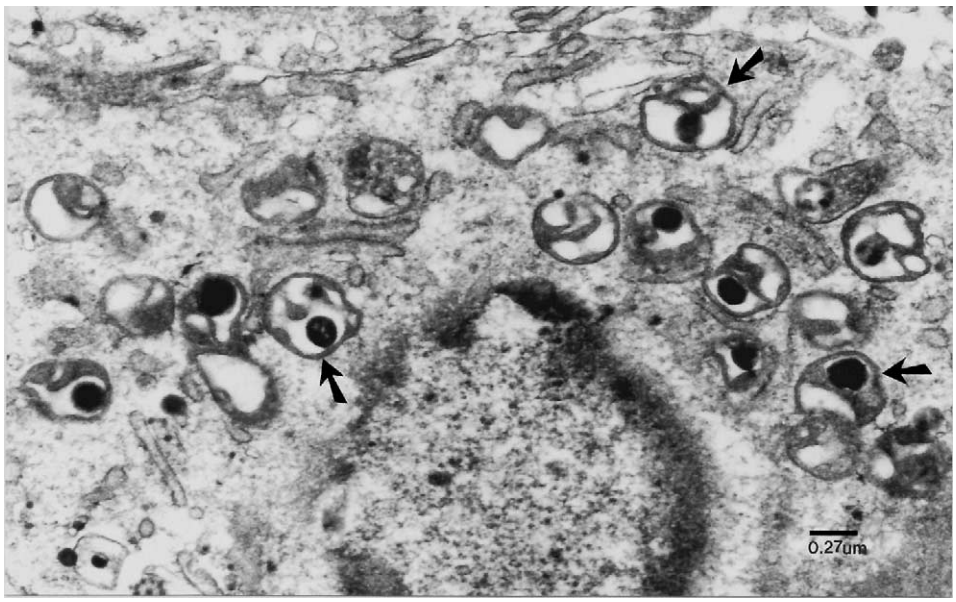

Fig. 5. Mitochondria of Sertoli cells showed cristae distortion with increased density and dense core spherical structures (arrows) in the case of mice exposed to Pb–Cd mixture at 4 weeks.

damage as a function of exposure duration and time. The most striking change involved distortion of mitochondrial cristae and an unusual arrangement, like inclusions, which were more evident when the mixture was inhaled. These changes suggest an irreversible damage with a decreased synthesis of ATP and uncoupled oxidative phosphorylation [27].

Mitochondrial changes induced by Cd or Pb exposure have been described in other species [27,28]. Mainly the effects involve cristae loss and swelling for one metal tested. In the current study model, the effects of either metal alone was clearly weaker than when the two metals, Pb and Cd, were used in combination as judged by the degree and frequency of damaged mitochondria in Sertoli cells. The effect that the inhaled mixture had on mitochondrial ultrastructure was most likely an additive one because the incidence of mitochondrial damage in the combinatorial exposure was twice that observed for each metal inhaled alone.

Three types of intra-mitochondrial inclusions have been reported in general, including small granular deposits of calcium, amorphous matrix inclusions that correspond to aggregates of denatured organic material, usually proteins that might be the case in the images shown in Cd and Pb exposures, and filamentous inclusions [29]. The round inclusions found in the present study did not fit any of the aforementioned definitions. Dense matricial granules such as those observed here have been reported in adrenocortical carcinoma [30]. On this basis we speculate that the affected Sertoli cells could be at an early stage of disease processes that could end in severe health consequences such as cancer [30].

Metals perturb multiple organs and systems. The targets of these toxic effects are enzymes and/or membranes of cells and organelles, especially the mitochondria. Several biochemical processes occur in the mitochondrion that utilize trace metals such as Fe, Cu, and Mn [31–34]. Perhaps these processes are sensitive to changes induced with Pb and Cd, resulting in the generation of reactive oxygen species (ROS) [35,36], oxidative stress, and ultimately ultrastructural modifications in the mitochondrion. ROS production can increase membrane permeability allowing calcium entry accompanied by uncoupled respiration and osmotic swelling [20], which in turns breaks inner mitochondrial membrane and a general collapse of energy production [20,36,37].

Spermatogenesis is a complex process in which the interaction of diverse cells such as Sertoli, Leydig and germinal cells is tightly regulated by hormonal signal and receptor stimulation. If one specific cell type is damaged by pollutants, then the whole process could be disrupted leading to reproductive failure, infertility or sterility [38]. In some countries metal pollution is a severe problem as is concern for increased rates of infertility or sterility. Semen volume density, total counts of motile sperm, low levels of Zn, acid phosphatase, citric acid and an increase in pathologic sperm have all been reported, as well as an increased rate of abortions [10,39]. In Mexico, a population of ceramic folk art workers is still exposed to Pb [40] and a more detailed study should be done in order to decrease the exposure and the inherent health disorders.

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