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Ultrastructural Alterations in Proximal Tubule Cells Induced by Lead

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Abstract: Four groups of 24 male Wistar albino rats (*Rattus norvegicus*) were exposed to lead acetate trihydrate (0, 0.5, 1.0, and 2% for 1 to 6 months) in drinking water to investigate the ultrastructural alterations in the renal cortical tissues due to lead intoxication. In comparison with respective control rats, chronic exposure to subtoxic doses of lead had produced adverse alterations mainly in the mitochondria, endoplasmic reticulum, lysosomes and nuclear chromatin with basal membrane infoldings. These changes were mainly seen in the proximal convoluted tubules and increased severely with increasing dose and/or time interval of lead exposure.

Key words: Lead, renal cells, ultrastructure, mitochondria, lysosomes, endoplasmic reticulum, *Rattus norvegicus*

Introduction

Lead is a nonthreshold multi targeted toxicant causing effects in different organs of the body specially the kidney. The absorbed lead is conjugated in the liver and is passed to the kidney, where a small quantity is excreted and the rest accumulates in and interferes with the functioning of body organs specially the kidney as a target site for lead toxicity (Fowler *et al.*, 1980; Friberg *et al.*, 1980; Bader *et al.*, 1981; Vicitry *et al.*, 1986).

Continuous environmental and occupational lead exposure can lead to chronic nephropathy (Fowler *et al.*, 1980; Chang *et al.*, 1980; Khalil-Manesh *et al.*, 1992), which is a characteristic manifestation of lead toxicity and characterized by tubular, interstitial and glomerular damages including renal lesions, tumors and cancer risks (Vyskocil *et al.*, 1989; Schraishuhn *et al.*, 1992; Waalkes *et al.*, 1995; Papaioannou *et al.*, 1989). Limited ultrastructural investigations have been done on the renal tissues of experimental animals, exposed to lead with special attention to lead intranuclear inclusions and cytoplasmic fibrillar bodies (Richter, 1976; Colle *et al.*, 1980; Fowler *et al.*, 1981; Stiller and Friedrich, 1983; Vicente-Ortega *et al.*, 1996). The alterations in the ultrastructural levels in the renal tissues due to chronic lead exposure is rather limited and have not yet been identified.

With this objective, a detailed ultrastructural study was undertaken on the kidney of male Wistar albino rats killed at 2 months intervals up to 6 months after lead acetate trihydrate treatment.

Materials and Methods

A total of 24 male Wistar albino rats (*Rattus norvegicus*) of the same age weighing 110-130 gm of the King Saud University colony were used. Animals were randomly divided into four groups, caged at room temperature and they received food and water *ad libitum*.

Following a period of stabilization (7 days), lead acetate trihydrate was administered in drinking water at the rate of 0.0, 0.5, 1.0, and 2%. Lead acetate was made soluble in water by addition of 1-2 drops of acetic acid. The rats were maintained on standard laboratory animal diet pellets (Grain Silos and Flour Mills Organization, Riyadh). One animal from each group was killed by dislocation of the neck after 1, 2, 3, 4, 5, and 6 months of treatment.

Small piece of left kidney cortex from each experimental rat of all groups were transferred immediately to a pool of cold (4°C) glutaraldehyde fixative on a glass surface for 10-15 min to attain a suitable hardness and then chopped into pieces approximately 1 mm³ in size and transferred to vials containing 2% buffered glutaraldehyde for 2 hr, rinsed in 3

changes of 0.1 M cacodylate buffer (pH 7.4) at 2 to 4°C one of which was over night.

The tissue blocks of kidney were post-fixed in 1% osmium tetroxide in the buffer for 2 hr at 2-4°C, then washed overnight in 3 changes of cacodylate buffer (pH 7.4) at 2-4°C. Then specimens were then dehydrated in graded concentrations of ethyl alcohol, cleared in 3 changes propylene oxide and infiltrated gradually in resin and embedded in plastic capsules in fresh full strength Agar 100 epoxy resin before being cured at 60-70°C for 2 days. Semithin sections (1µm) were cut, using Jung ultramicrotome, while ultrathin sections (700 Å) were cut using glass knives and Lica ultramicrotome UCT and mounted on carbon-coated grids. Semithin sections (1 µm) were stained with 1% toluidine blue, while the ultrathin ones were stained with saturated solution of urinal acetate in 50% ethanol followed by Reynold's lead citrate stain, then examined and photographed by Jeol Jem-100 CX electron microscope at 80 Kv.

Results

Renal cortical tissues of control rats revealed the normal ultrastructural pattern of proximal convoluted tubule cells. The ultrastructural changes induced by lead chronic exposure in the proximal tubules of the treated rats were as follows:

Mitochondrial alterations: The mitochondrial (M) ultrastructural abnormalities became evident at 2 months and more of 0.5% lead acetate trihydrate treatment. There was a general reduction in the number of mitochondria in the epithelial cells lining the proximal convoluted tubules in comparison with those of the respective control rats. The mitochondria became swollen and tended to be oval or rounded in shape rather than elongated (Figs. 1&2). Occasional rupture of the outer membrane and vacuolation of the inner compartment of the mitochondria were seen (Figs. 3&4). Lipid transformation of mitochondria was also observed in some injured cells. In addition, flocculent densities were observed in the inner compartment of most injured mitochondria.

The mitochondrial cristae have become marginated, short disoriented with unusual interdigitating pattern and some mitochondria in some injured cells appeared with complete disorientation of their cristae in respective to well-preserved parallel ones of the control rats (Fig. 5). Disruption, fragmentation and occasional granulation of the cristae became evident in the mitochondria of all lead-treated rats, some of these organelles became heavily laden with electron dense materials. Most of the mitochondrial cristae fragmentation was seen at their luminal ends. Fine granulation in the swollen mitochondria matrix was also detected.

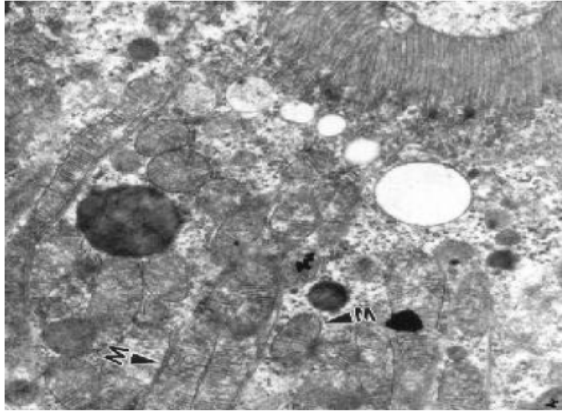


Fig. 1: Control rat received 2% sodium acetate for 6 months demonstrating normal ultrastructural pattern. X7200.

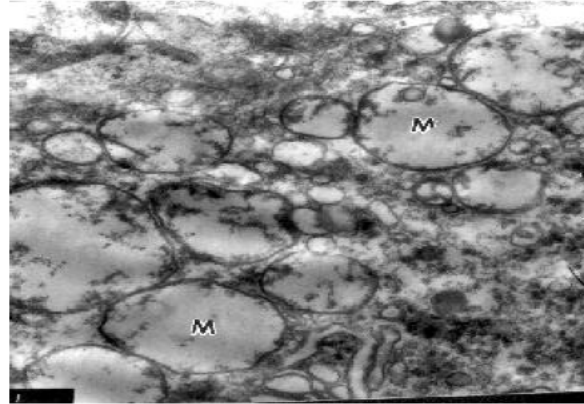


Fig. 4: Lead-treated rat received 2% lead acetate trihydrate for 6 months. The mitochondria are with partial or complete loss of cristae. X10,000

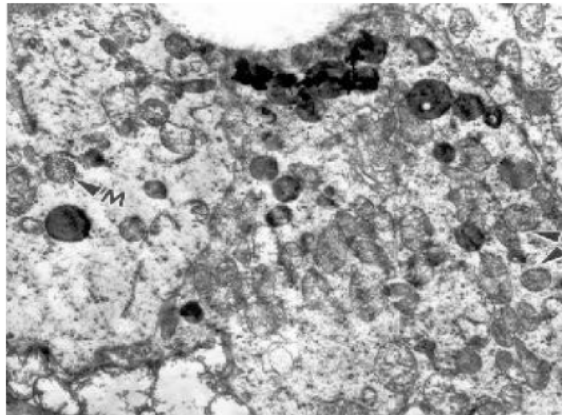


Fig. 2: Lead-treated rat received 0.5% lead acetate trihydrate. The mitochondria are swollen with oval or rounded in respective the elongated ones of the control rat. In addition, a focal accumulation of lysosomal-related structures is seen. X4,800

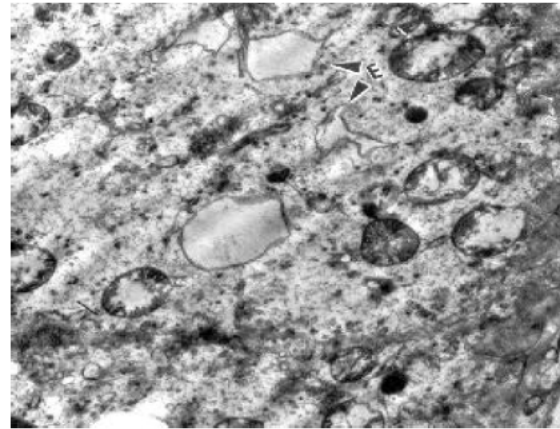


Fig. 5: Lead-treated rat received 0.5% lead acetate trihydrate for 2 months. The mitochondria have fragmented and disoriented cristae while the endoplasmic reticulum is almost unremarkable. X10,000.

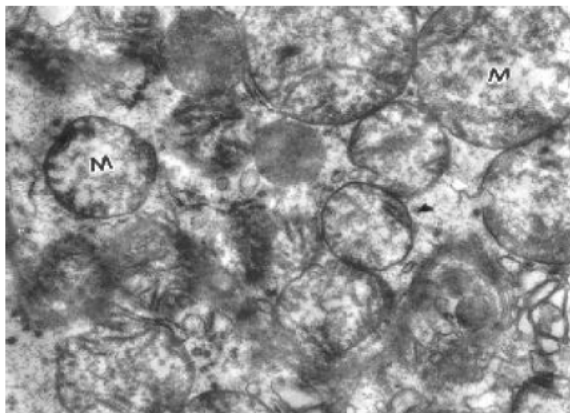


Fig. 3: Lead-treated rat received 1% lead acetate trihydrate for 3 months. The mitochondria are swollen and heavily laden with electron dense materials. X 10,000.

Endoplasmic reticulum alterations: The endoplasmic reticulum (E) of the lining epithelia of the proximal convoluted tubules was markedly swollen and were dilated and distended showing cloudy swelling phenomenon with numerous fragmental rough endoplasmic reticulum (Fig. 6). Proliferation and vesiculation of the endoplasmic reticulum were also seen, in respective to well preserved rough endoplasmic reticulum in the cells of control rats.

Lysosome alterations: A general increase in the number of lysosomal-related structures (L) within the epithelial lining cells of the proximal tubules in lead-treated rats was seen. Some of the autophagic lysosome-related structures contained crystalline structures (Fig. 7).

Cytoplasmic vacuolation: Many proximal tubule cells of the lead-treated rats showed clear cytoplasmic vacuoles (CV) surrounded by a single layered wall together with numerous lysosomal vacuoles (LC). This change was accompanied with an increase in the pinocytic vesicles with ill-defined cytoplasmic bodies (Fig. 8).

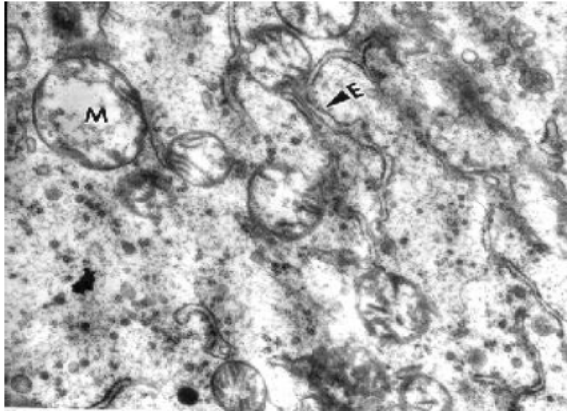


Fig. 6: Lead-treated rat received 1% lead acetate trihydrate for 4 months demonstrating marked dilation and numerous fragmentation of the rough endoplasmic reticulum. X 10,000

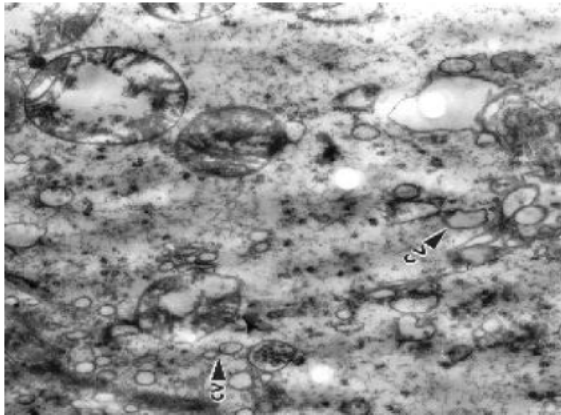


Fig. 7: Lead-treated rat received 2% lead acetate trihydrate for 6 months. Some of the lysosomal-related structures containing crystalline structures. X 10,000.

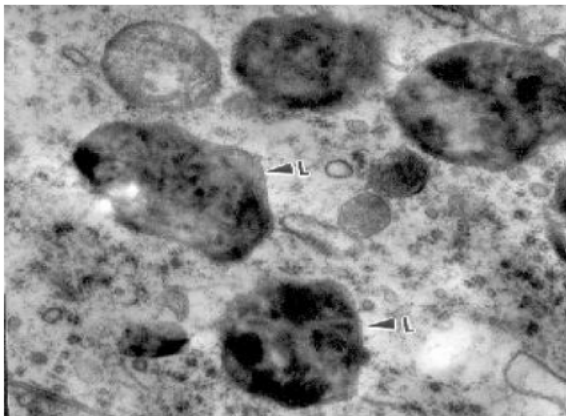


Fig. 8: Lead-treated rat received 1% lead acetate trihydrate for 5 months. There are cytoplasmic vacuoles and scattered pinocytotic vesicles. X 10,000.

Nuclear alterations: The proximal tubular epithelial cells revealed nuclear irregularity. Blebbing in the nuclear membrane seen in epithelial lining of the proximal tubule. Interchromatin granulation and clumping of nuclear chromatin together with fibular structures were also observed in the nuclei of the proximal tubule cells as an indication of pyknosis and hyperchromasia.

Discussion

The marked ultra structural alterations due to lead intoxication were seen mainly in mitochondria. These organelles have shown toxic effects and the results indicated clearly that the mitochondria are highly susceptible to toxic injury of lead. The swollen mitochondria due to lead intoxication might be an indication to the overall swelling of the injured cells. Mitochondria swelling is thought to be related with a change in osmolarity that leads to an influx of salts and water into the inner mitochondrial membrane, which becomes distended while the outer membrane eventually ruptures due to osmotic swelling (King *et al.*, 1983).

The disorientation and fragmentation of the mitochondria cristae might indicate a special lead affinity for mitochondria membranes, which play a key role in the functional integrity of this organelle (Fowler *et al.*, 1981 and Oskarsson *et al.*, 1992). The marked ultrastructural changes in the mitochondria due to lead intoxication suggest that cells with such a degree of mitochondrial injury are unable to perform efficient functions, specially the oxidative phosphorylation and ATP production. These findings are consistent with those of Kendall and Scanlon (1985), although such long-term subtoxic level exposure have not been previously reported.

The increase in the number of lysosomal-related structures due to lead intoxication as seen by the results might be an indication to an increase in the proteolytic activity in the injured cells and related to the degradative of the cytoplasmic inclusion bodies produced through lead detoxification mechanism as a defensive action of renal cells during the progress of lead poisoning. The increase in the lysosome number might also reflect an increase in the synthesis of hydrolytic and detoxifying enzymes. Some previous studies showed that lead poisoning increases the number of lysosomes in the epithelial cells of the pars convoluta and induces the loss of brush border in the pars recta of renal tubule (Spit *et al.*, 1981, Vicente-Ortega *et al.*, 1996).

The proliferation and dilation of the endoplasmic reticulum due to lead intoxication, as seen might reflect a need by the injured cells for oxidative enzymes which are required for detoxification while numerous vacuoles due to lead intoxication might indicate cell swelling due to lead intoxication.

The present study shows that chronic exposure to subtoxic doses of lead produces adverse alterations mainly in the mitochondria, endoplasmic reticulum and lysosomes and is increased severely with increasing dose and interval of lead exposure.

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