Toxicity of cadmium on hepatic and myocardial cells.

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ABSTRACT: Cadmium (Cd) is a heavy metal, a prevalent environmental containment, which can harm many organs after environmental exposure and has been linked to many disorders, diseases and carcinogenesis in human. The aim of the present study was to investigate the ultrastructural alterations of mice myocardial and liver cells induced by exposure to Cd. Exposure of cells to Cd leads to significant transmutations of the cellular structures. The ultrastructural changes were investigated in all of the above mentioned cells after 4 weeks at a dose corresponding to human environmental exposure of Cd. In Cd induced group, ultrastructural alterations included formation of intracellular vacuoeles and significant disruption of the outer membrane of the mitochondria. Many transformations in cellular structures are in a casual relationship with the increased oxidative stress, aberrant gene expression and inhibition of cell apoptosis of the exposed cells. The evidence of the ultrastructural alterations in our observed Cd group indicates that the exposure plays a central role in the disturbance of organ's homeostasis and damages of cellular structures.

Key Words: Cadmium, Liver, Heart, Oxidative stress, Toxicity.

INTRODUCTION

Cadmium is a heavy metal that is linked to carcinogenesis and many other harmful disorders in human body. It is a classified carcinogen by the International Agency for Research on Cancer (IARC) and therefore it's exposure is restricted in industry[1]. Cadmium is used in electroplating, pigments, paints, in batteries, in agricultural fertilization and it is co-product of zinc mining.

Workers in certain occupations are exposed to Cd in significantly higher levels than general public. Food, water and polluted air, are sources for human exposure to Cadmium. It is etimated that the average European consumes 30 mg per day[2]. Another source for cadmium is cigarette smoking, as tobacco plants accumulate Cd from soil[3]. The type of Cd intake plays a significant role in absorption level because the inhaled Cd is much higher than the absorption level from the gastrointestinal system[4]. After absorption, Cd is transferred by blood to all organs of the body, where a small protein called metallothionein (MT) with a high binding affinity to Cd, stores the heavy metal. Cadmium has a half-time of 20-30 years in the human body. The highest concentration of Cadmium in the body (approximately 50%) is found in liver and kidneys because of the high Mt concentration[5,6].

Cadmium has a high toxicity, because it induces cancer by multiple mechanisms. Some of them are induction of oxidative stress, aberrant gene expression, inhibition of DNA repair, and inhibition of apoptosis[7].

As stated above, Cd is considered to be a dangerous metal with a wide range of actions and mainly responsible for acute inflammatory response and carcinogenesis. The first inflammatory response is caused by the oxidative stress that is caused by Cd accumulation in large amounts in small time period, whereas the second inflammatory response is caused by small and repeated doses for a long time period. The prime impact of the Cd, which is oxidative stress, is a result of the displacement of iron and cooper from intracellural compartments, something that leads to the Fenton reaction (:$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\cdot + OH$-),

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but also of the inhibition of the mitochondria electron transfer chain[8].

Moreover Cd influences various genes' expression, that is involved in carcinogenetic procedures. Five categories of genes are influenced by the impact of Cd on DNA: 1. immediate early response genes (c-fos, c-jun, c-myc:protooncogenes), 2. stress response genes, 3. translation and transcription factors 4. miscellaneous genes. When Cd is accumulated in large doses, stress response proteins are produced such as metallothionein, glutathione and heat shock proteins. These proteins are responsible for the adaptation of the human cells to low and repeated doses of Cd. Metalothionein is a binding protein to Cd as well as to the oxidative products of Cd's impact, glutathionin and catalase are the most antioxidant molecules and play major role in the detoxification of Cd and ROS and finally heat shock proteins as another class of stress response proteins[7].

Furthermore, Cd has to do with the involvement of secondary messengers, such as ROS and Ca+2, in the alteration of several genes' expression. Also, according to Benbrahim-Tallaa et al. exposure to Cd can lead to an epigenetic modification: methylation of certain CpGs. Cd- induced DNA hypermethylation decreases the expression of tumor suppressor genes, which could be important in Cd-induced malignant transformation in human prostate cells[9].

When it comes to the impact of Cd on human organs, there are many alterations of them that have to be mentioned, especially these that concern the muscle heart and the liver.

It is estimated that Cd plays a significant role especially in carcinogenesis of lung tissue, as the absorption rate is significantly higher than in other human tissue[10].

Our understandings of the harmful mechanisms are mainly derived from in vivo animal models. The aim of the present study was to investigate the ultrastructural alterations in myocardial and liver cells of mice after Cd exposure and not to lungs, a tissue that have been studied a lot in the past.

MATERIAL AND METHODS

Six adult mice, weighing 23-25 gr, were divided into two groups, one of four animals and one of two. The first group orally received CdCl2 dissolved in tap water, at dose 5 mgr/Kgr/day for 4 weeks. The second group received tap water free of Cd and used as control group. At the end of the treatment, the animals sacrificed under anesthesia, the liver and the heart were dissected and tissue samples were fixed in 3% glutaraldehyde in phosphate buffer pH 7,3 for 2 hours and then were postfixed in 2% osmium tetroxide. After staining with 1% aqueous solution of uranyl acetate, the tissue pieces were dehydrated in a series of alcohol solutions of increased density each time and then embedded in EPPON. Thin sections were stained with lead citrate and were observed in a transmission electron microscope.

RESULTS

Many myocardial cells presented extensive myofilament degeneration (Figure 1) and dilated cisternae of sarcoplasmic reticulum (Figure 2). In some places, the absence of glycogen granules and the intracellular oedema was striking (Figure 3). Of interest was the presence of swollen mitochondria with electron lucent matrix and disorganization of cristae (Figures 4, 5). In the control group, the histological appearance of the myocardial cells was normal (Figure 6). In the hepatocytes, the most prominent feature was the presence of many mitochondria with flocculent matrix and the absence of glycogen granules (Figure 7). In some areas the mitochondria were fully oedematous with disruption of their outer membranes (Figure 8). However the presence of small electron dense particles was also striking (Figures 7, 8). Also in liver tissue sections, the sinusoids were dilated (Figures 8, 9, 10). In the control group, the histological appearance of hepatocytes presented no deviations from normal pattern (Figure 11).

DISCUSSION

Many studies have previously identified high toxicity and carcinogenic effect of Cadmium, on the exposed cells. Cellular alterations were primarily observed in renal cells[11], myocardial cells, hepatocytes and pancreatic cells[2,12,13].

As far as hepatocytes' alterations are concerned, the mitochondria are a primitive target of Cd, as it affects mitochondrial permeability, inhibits respiratory chain, and then generates ROS. Cadmium is shown

to inhibit mitochondrial complex III, resulting in accumulation of semiubiquinones at the Q0 sites. The unstable semiubiquinones are prone to transfer one electron to molecular oxygen to form superoxide anion. Cd effects on mitochondrial electron transfer are the major origin for Cd generated ROS. These features of Cadmium's exposure lead to generation of ROS, which obviously disrupt normal function of mitochondria[14,15].

In our observations the hepatocytes have mitochondria with flocculent matrix and disruptions of the outer mitochondrial membrane, whereas the observed mitochondria in the heart-muscle cells were swollen with electolucent matrix and disorganization of cristae. Excessive ROS could directly react with unsaturated fatty acids on the surface of the mitochondrial membrane, thus resulting in the damage of its structure. Also, Cd seems to influence the energy dependent transport of K+, something that leads to destabilization of membrane's electric dynamic and to the malfunction of ATP-synthetase, resulting in reduction of ATP[16].

Furthermore, Kayama et al. (2000), Yamano et al (2000), proved the intense activation of Kuppfer cells, which are an important source for Cd-induced inflammatory mediators such as IL-1β, TNF-α, IL-6, and IL-8, the presence of which detected through immunoassay methods. Thus the inflammatory response to Cd exposure may lead to liver fibrosis and increased hepatotoxicity[17].

Another dangerous factor for the liver's homeostasis is the lipid peroxidation, which is induced by oxidative stress and indicates the level of oxidative stress.

Moreover, Trabesi H. et al. (2013) examined the potential of Cd's toxicity to generate nanoparticles. After granting to rat CdCl2 intraperitoneally, they discovered, by using fluorescence imaging, the presence of Cd sulfide (CdS) and/or Cd selenide (CdSe) nanoparticles following Cd injection in the liver (6.52 nm) and kidneys (56.30 nm). The above result may be correlated to the assumption of vacuoles in the cytoplasm that we detected in the cytoplasm of the hepatocytes[18].

Finally, the absence of glycogen granules was striking in our observations. This result is owned to the oxidative stress caused by Cd, since it is known that oxidative stress diminish the ability of cells to reserve glycogen.

Another important organ influenced by Cd toxicity is the heart muscle. Our samples showed intracellular oedema and myofilament degeneration among others, as well as the samples of Ferramolla ML et al. (2012) who found hypertrophic cardiomyocytes and myocardial fiber necrosis.[19]

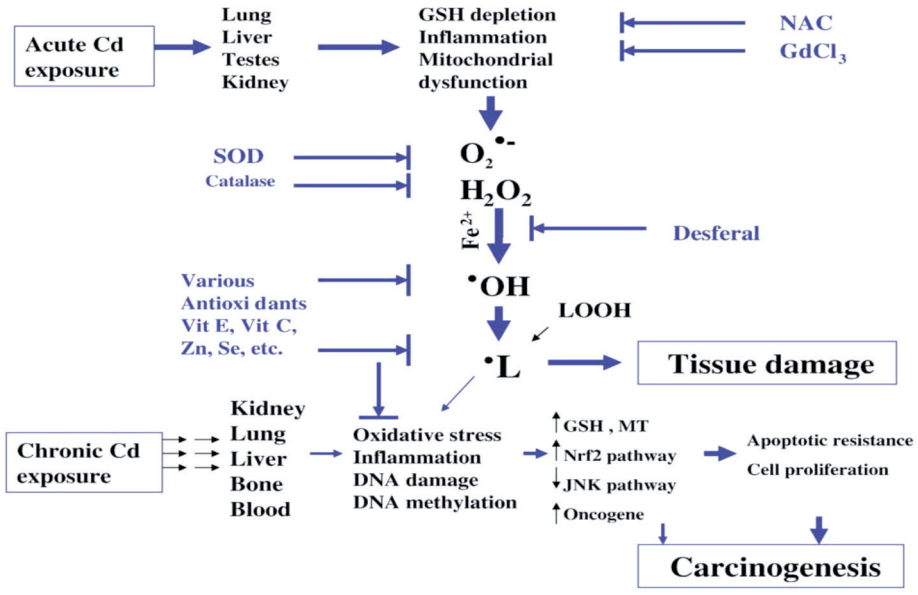
Cadmium also has an inotropic action, since at concentrations of 1, 10, and 20 microM is established to decrease dose-dependently (21.3, 50.3, and 72.0%, respectively) the muscle contraction amplitude. This is explained by its competitive action on the potential-controlled Ca2(+)-channels of the L-type (Ca 1.2). However, the decrease in heart's contraction amplitude is caused by the dysfunction of mitochondria. It was shown that Cd2+ at concentrations of 15 and 25 microM produces swelling, something that we also observed, of non-energized and energized mitochondria in isotonic (with KNO2 and NH4NO3) and hypo-osmotic (with 25 mM CH3COOK) media, and the disturbance of the activity of respiratory chain at 20 mM of Cd.[16]

Moreover, Cd toxicity was further correlated with the cause of cardiovascuar diseases according to S.Milton Prabu et al (2013). An increase of activities of marker enzymes such as creatine kinase-MB, aspartate transaminase, alanine transaminase, alkaline phosphatase and lactate dehydrogenase in serum were observed in his studies. In addition, the levels of lipid peroxidation products and protein carbonyl contents in heart were significantly ($p < 0.05$) increased and the activities of enzymatic antioxidants such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase in the heart and non-enzymic antioxidants such as glutathione, vitamin C and E in the heart were significantly ($p < 0.05$) decreased in Cd intoxicated rats. The levels total cholesterol (TC), triglycerides (TG), phospholipids (PL), free fatty acids (FFA), LDL and VLDL were significantly ($p < 0.05$) increased and the level of HDL was significantly decreased in the serum of Cd-treated rats. Cd intoxication also increased the levels of TC, TG and FFA and decreased the level of PL in the heart tissue. Further Cd treatment significantly ($p < 0.05$) decreased the levels of membrane bound ATPases in heart[20].

Finally all the above mentioned studies suggest the Cd is also responsible for carcinogenesis to the types of cells mentioned above. More precisely carcinogenesis caused by Cd follows a common pattern to all type of cells. Only small and chronic doses of Cd can lead to cancer, in contrast to higher and single doses that lead to apoptosis due to intense oxidative stress. Although Cd-induced oxidative stress causes the production of typical oxidatively generated mutagenic lesions such as 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) adducts in the DNA of human cells, Cd-induced DNA strand breaks and the formation of 8-oxodG adducts can be suppressed by DMSO. DMSO is a scavenger of hydroxyl radicals[21,22].

Antioxidant enzymes such as superoxide dismutase and catalase were able to block the DNA strand breaks, chromosomal aberrations and gene mutations induced by Cd[29]. This activation of antioxidant enzymes takes place because of the adaptation of cells to chronic small doses of Cd, due to the activation of metalothionine, γ-glutamyl-transferase, heat shock proteins that act competitively to the ROS. An experiment approach on rats will clarify the adaptation mechanism. Qu et al. showed that fluorescence intensity in control liver cells was dramatically higher, when intoxicated with 50μM Cd, than in Cd-treated liver cells[23,24].

Thus Cd carcinogenesis is conducted through genes' expression alterations. Many studies show the ability of cadmium to activate MAP, bcl-2, c-fos, c-jun, c-myc genes and to eliminate the activity of anti-apoptotic genes such as p53[25].



CONCLUSION

According to our findings and the literature, Cadmium is a dangerous metal for human health, causing harmful cell alterations and possible carcinogenesis depending on the amount of Cd 's exposure and the time period in which it is being accumulated. Even though many studies have revealed the pathophysiology of Cadmium's exposure in human cells, there are still significant impact of Cadmium in human cells for further research.

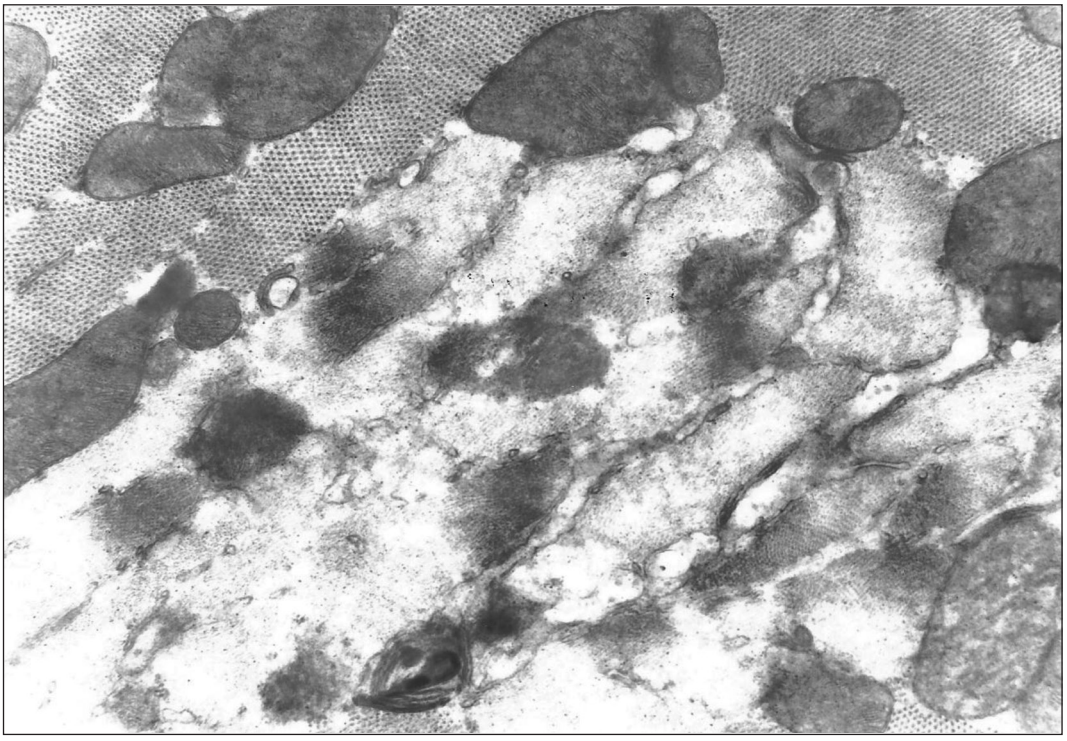


Figure 1. Myocardial cell-Group A. Extensive degeneration of myofilaments, mitochondria with flocculent matrix, disordered cristae and disrupted outer membrane x 28,500.

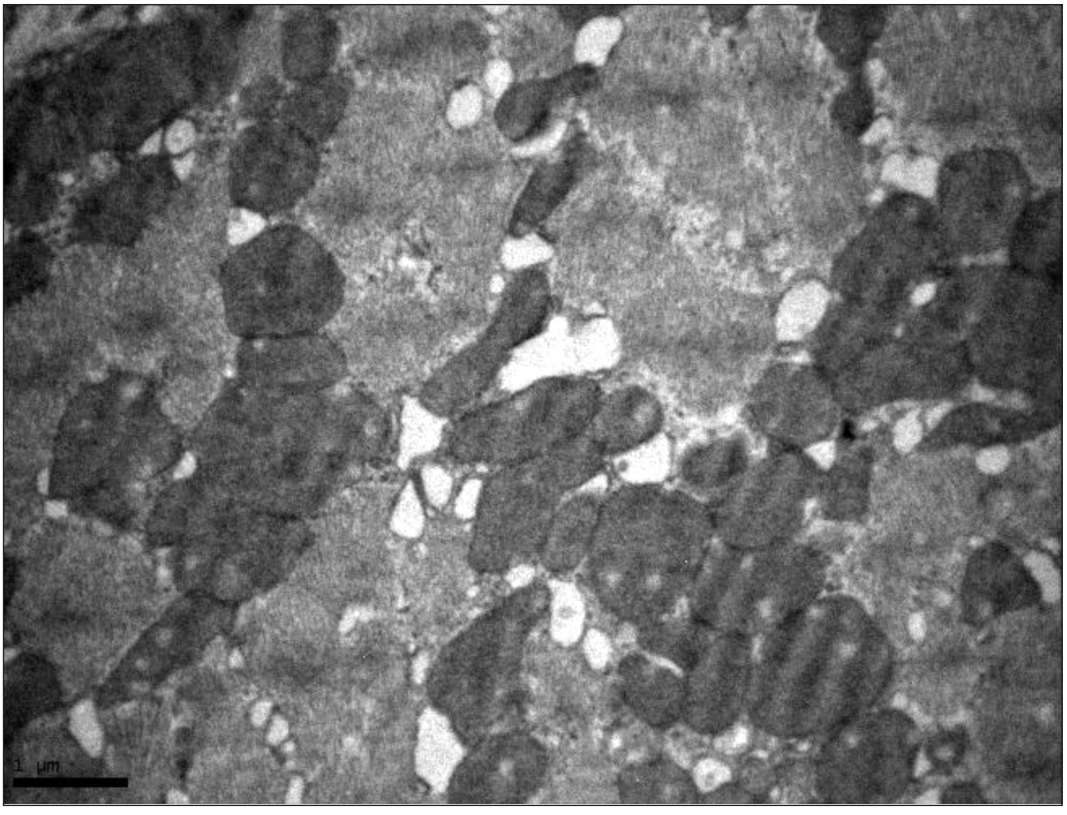


Figure 2. Myocardial cell-Group A. Dilated cisternae of sarcoplasmic reticulum X 15000.

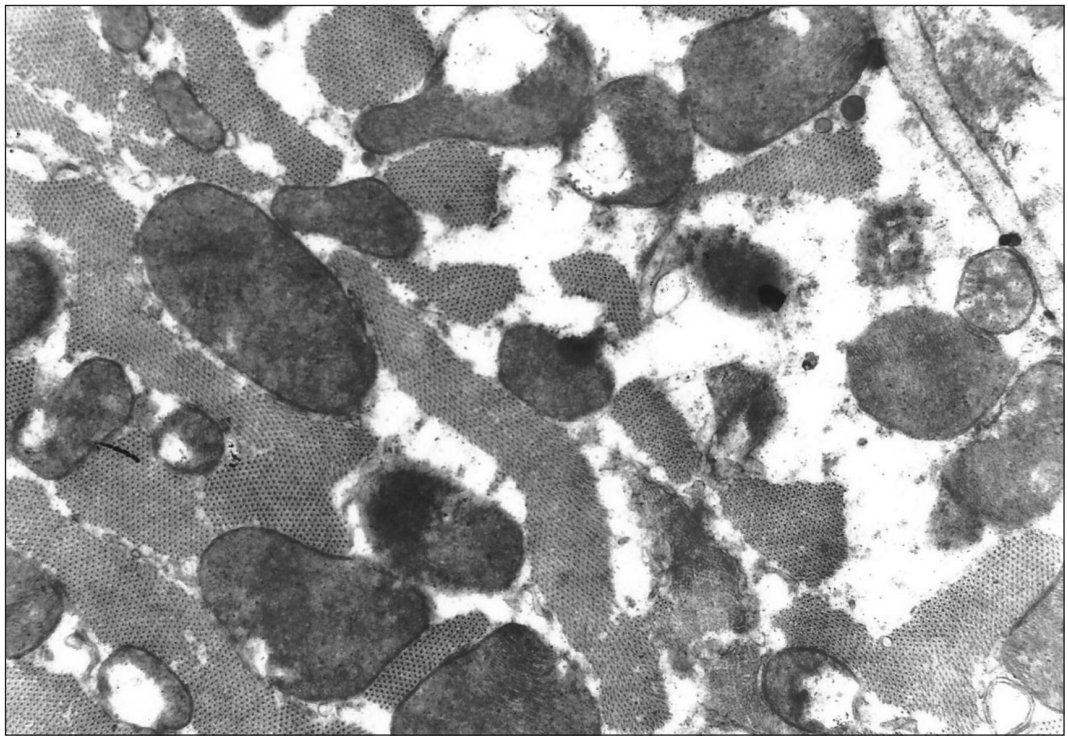


Figure 3. Myocardial cell-Group A. Intracellular oedema, myofilament degeneration, mitochondria with flocculent matrix x 20,000.

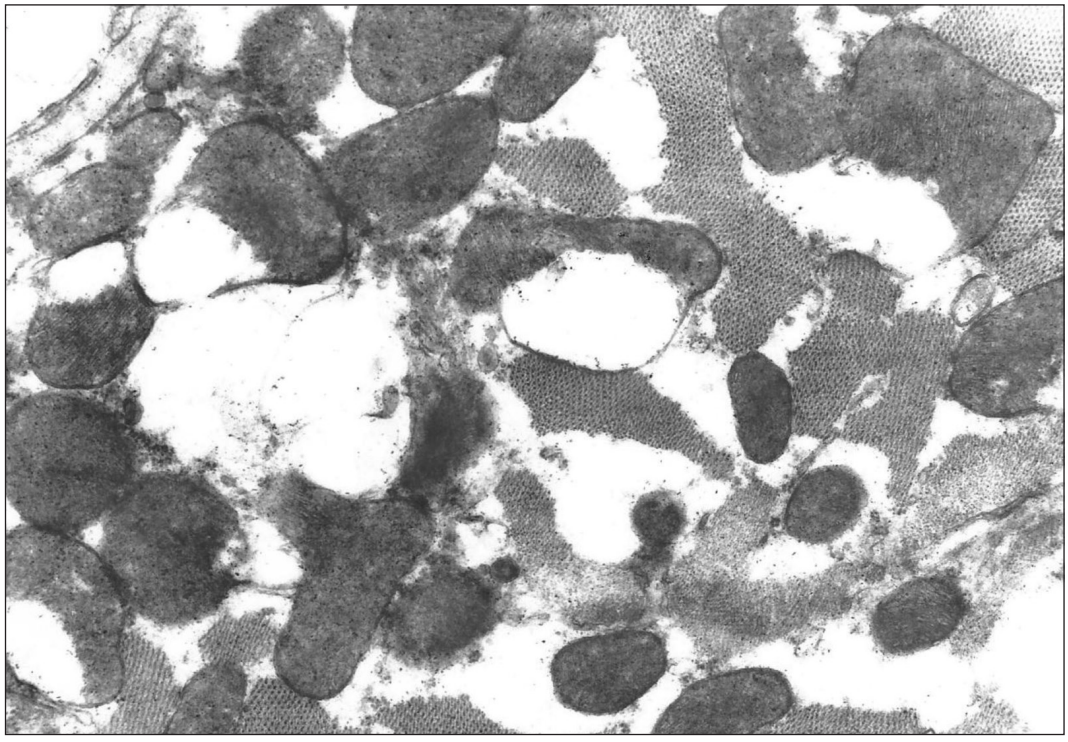


Figure 4. Myocardial cell-Group A. Swollen mitochondria with disrupted outer membrane and degenerated myofilaments x 24,000.

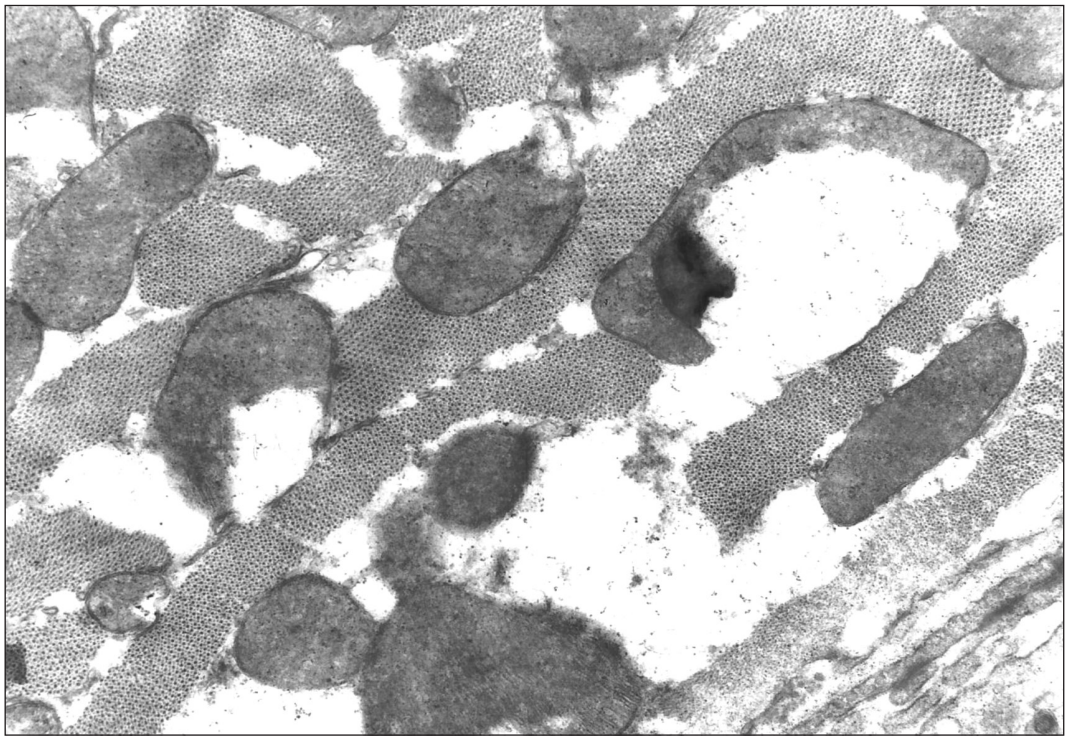


Figure 5. Myocardial cell-Group A. Swollen mitochondria with disrupted outer membrane and degenerated myofilaments x 24,000.

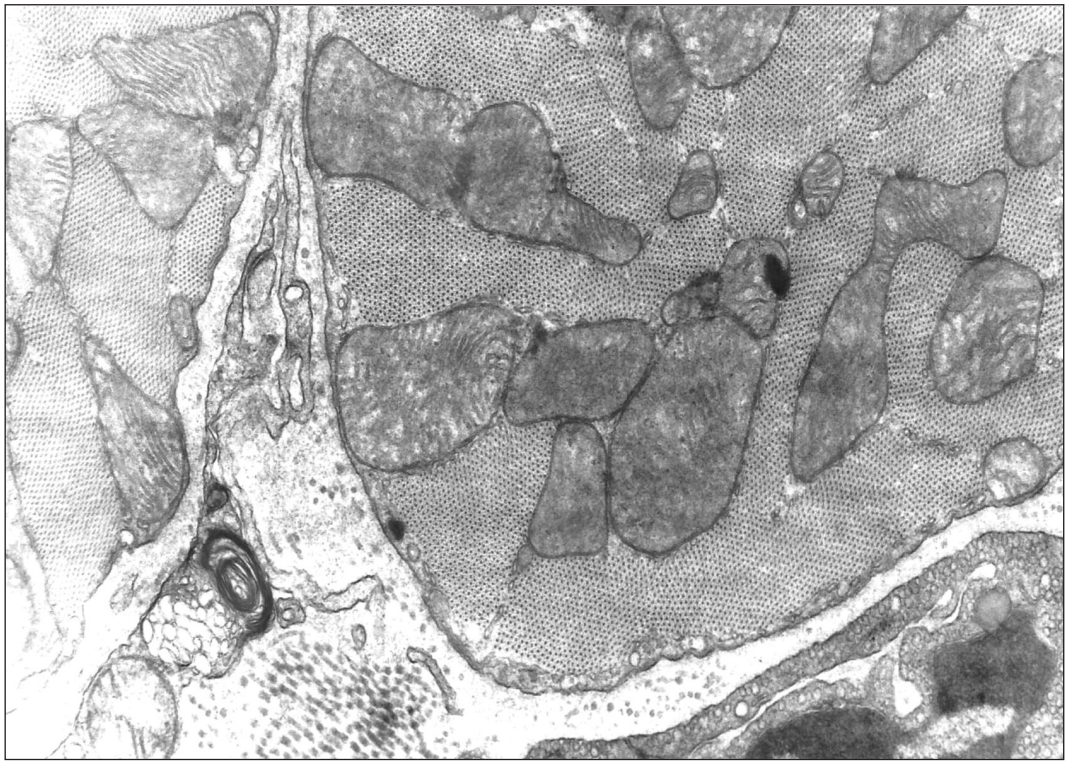


Figure 6. Myocardial cell-Control group. Normal arrangement of myofilaments, mitochondria with intact cristae and outer membrane x 24,000.

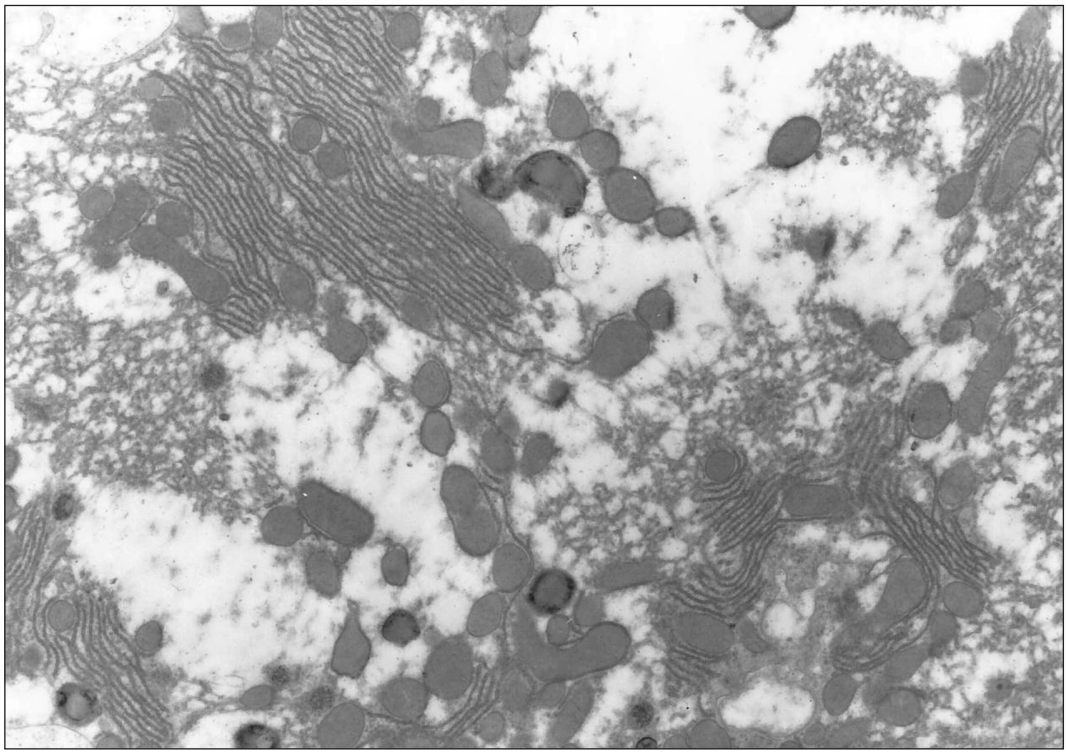


Figure 7. Liver cell-Group A. Absence of glycogen granules, mitochondria with flocculent matrix x 8,000.

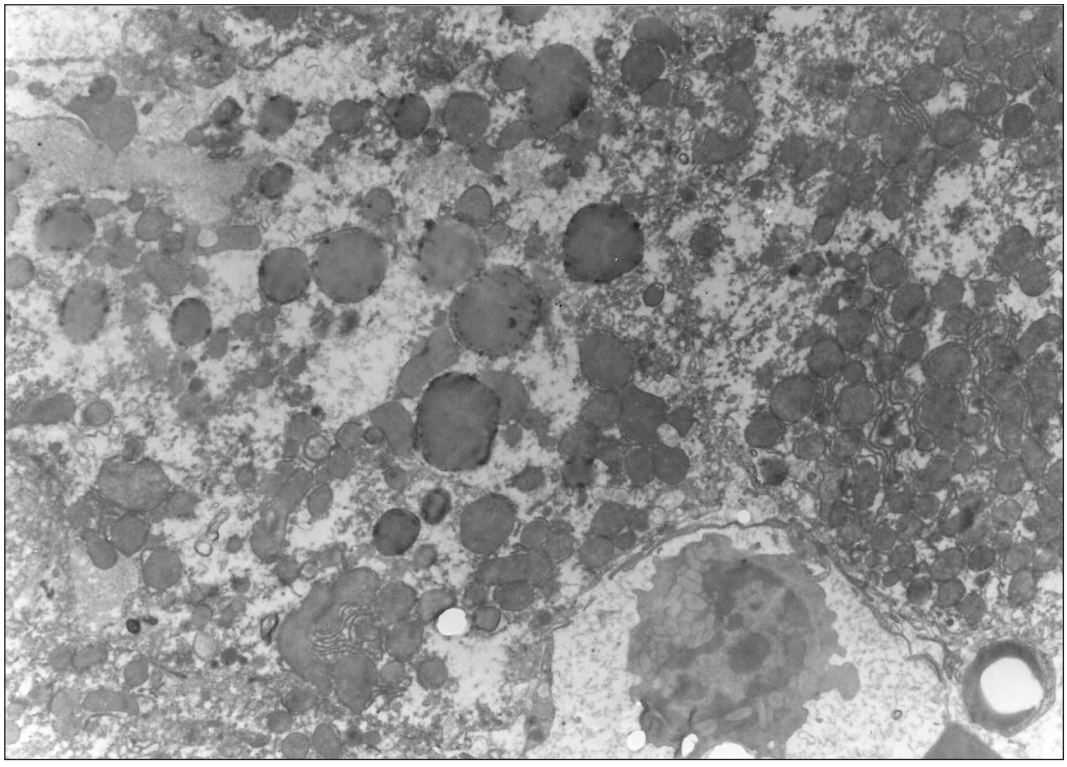


Figure 8. Liver cell-Group A Normal nucleus, cytoplasm with absence of glycogen granules, dilation of a sinusoid x 5,000.

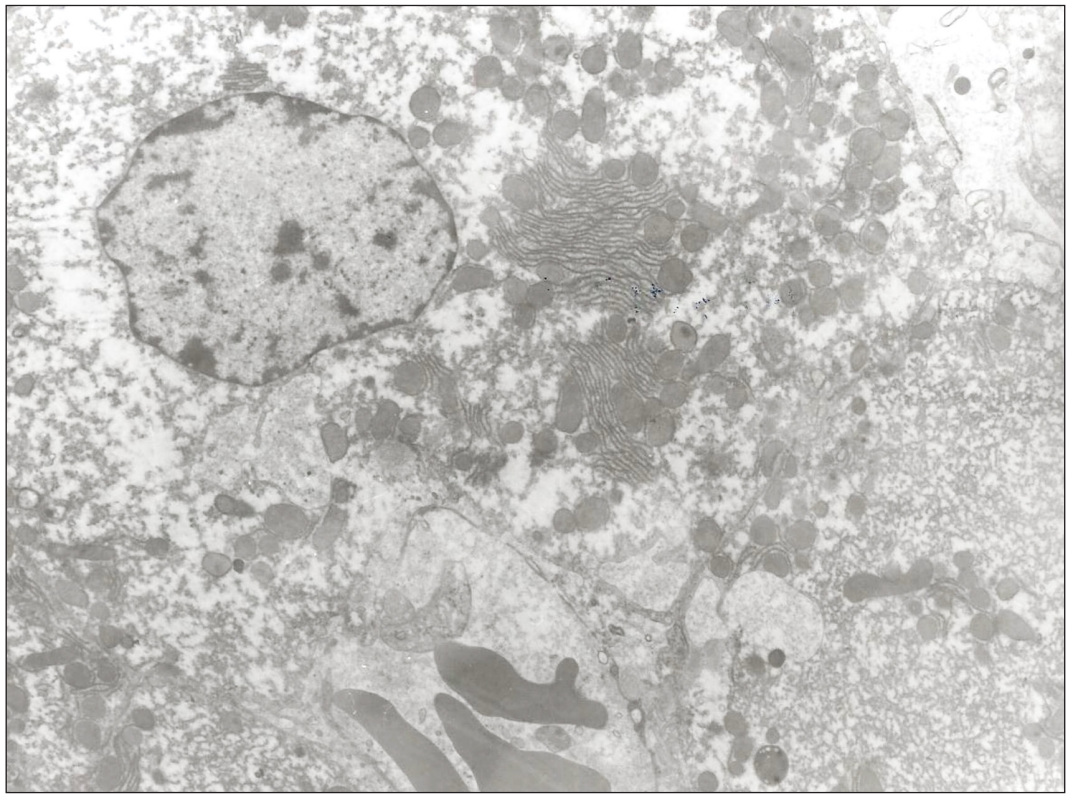


Figure 9. Liver cell-Group A. Dilated sinusoid, mitochondria with flocculent matrix x 9,900.

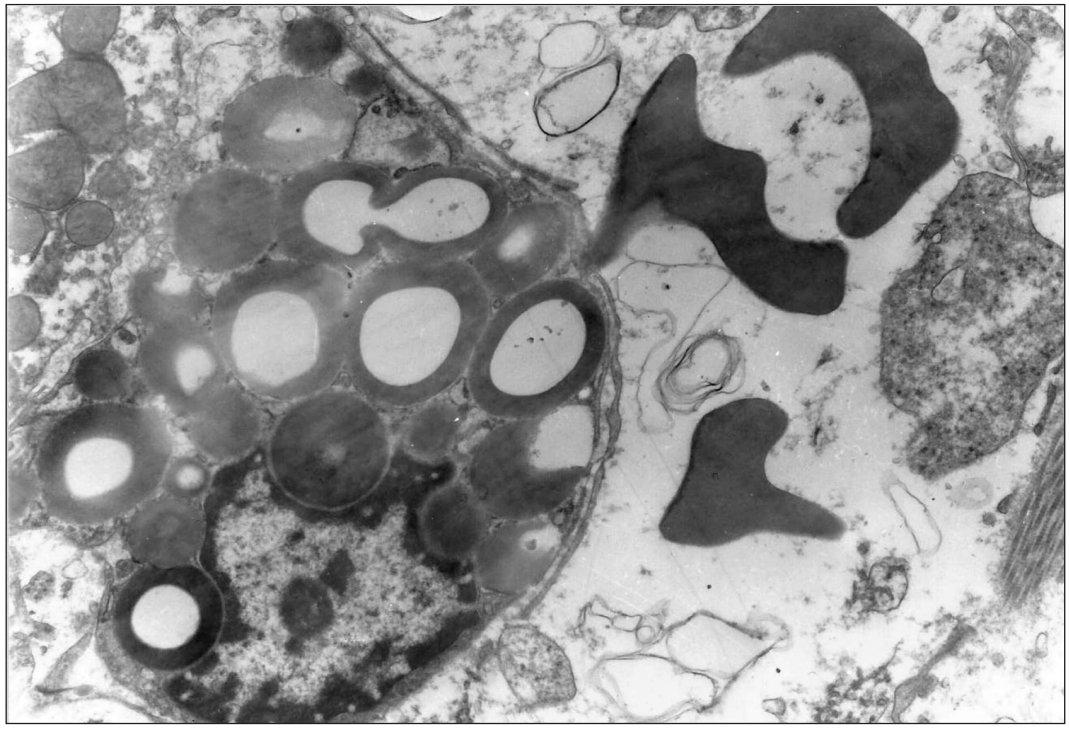


Figure 10. Liver-Group A. Ito cell in a dilated Disse space x 11,500.

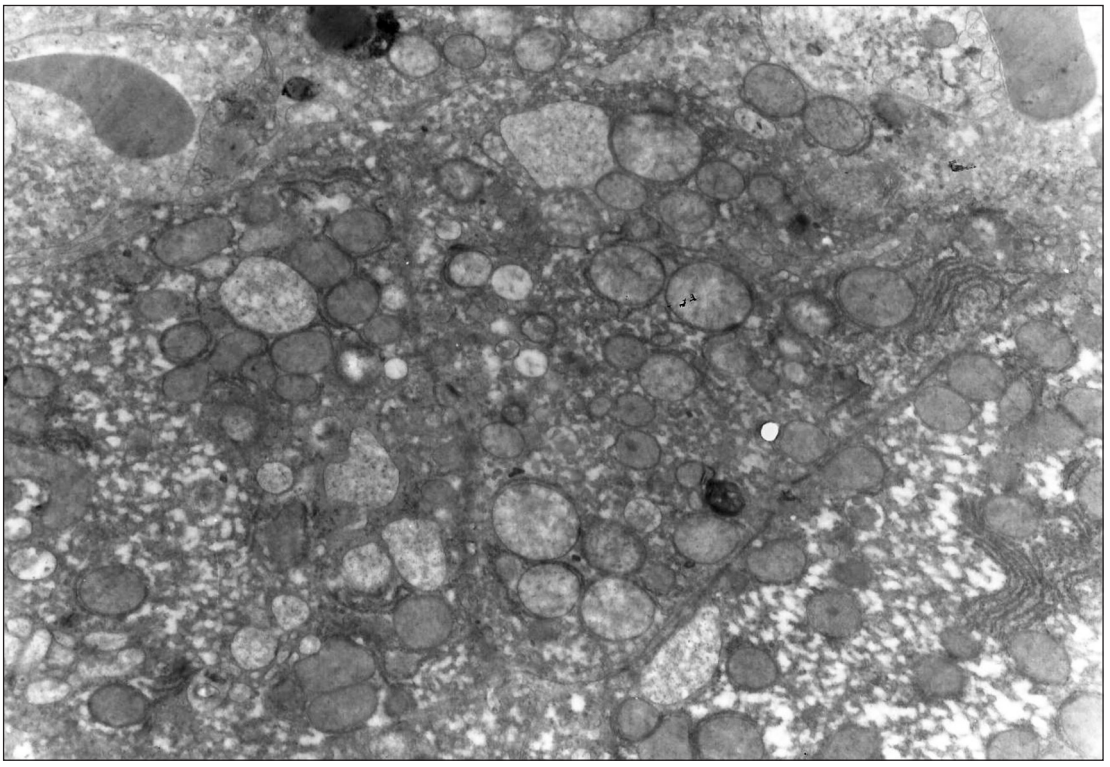


Figure 11. Liver cell- Control group. Normal appearance of cytoplasm and organelles x 10,000.

Τοξικές επιδράσεις του καδμίου στα ηπατικά και μυοκαρδιακά κύτταρα.

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ΠΕΡΙΛΗΨΗ: Το κάδμιο(Cd) είναι ένα βαρύ μέταλλο, ένα διαδεδομένο στη φύση στοιχείο, το οποίο μπορεί να αποβεί επιβλαβές για πολλά όργανα μετά από περιβαλλοντική έκθεση, ενώ έχει συνδεθεί με πολλές διαταραχές, ασθένειες και καρκινογέννεση στον άνθρωπο. Στόχος της παρούσας μελέτης ήταν η διερεύνηση των δομικών αλλαγών, που προκλήθηκαν από την έκθεση στο κάδμιο, σε ηπατικά και μυοκαρδιακά κύτταρα ποντικών. Η έκθεση των κυττάρων σε κάδμιο οδηγεί σε σημαντικές αλλαγές των κυτταρικών δομών. Οι δομικές αυτές αλλοιώσεις των παραπάνω κυττάρων παρατηρήθηκαν μετά από τέσσερις εβδομάδες έκθεσης των κυττάρων σε δόσεις ανταποκρινόμενες σε αυτές, τις οποίες εκτίθεται ο άνθρωπος στο περιβάλλον. Στην ομάδα κυττάρων που εκτέθηκε σε κάδμιο, σχηματίστηκαν κυτταροπλασματικά κυστίδια, ενώ παρατηρήθηκαν επίσης σημαντικές αλλοιώσεις στην μιτοχονδριακή μεμβράνη. Πολλές αλλαγές στις κυτταρικές δομές σχετίζονται με το αυξημένο οξειδωτικό στρές, την ανώμαλη έκφραση γονιδίων και την αναστολή της απόπτωσης, κάτι που τονίζεται επίσης και στην διεθνή βιβλιογραφία. Οι κυτταρικές αλλαγές που παρατηρήθηκαν και στη δική μας ομάδα κυττάρων (εκτεθειμένων σε κάδμιο)αποτελεί απόδειξη ότι η έκθεση σε κάδμιο παίζει σημαντικό ρόλο στην διαταραχή των κυτταρικών δομών και κατ' επέκταση της ομοιόστασης των οργάνων.

Λέξεις Κλειδιά: Κάδμιο, Ήπαρ, Καρδιά, Οξειδωτικό στρες, Τοξικότητα.

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