Effects of cadmium on photoreceptors and ganglionic cells of retinal layer in mice embryo — An ultrastructural study

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Cadmium (Cd) is one of the environmental contaminant and because of its non-decomposable character, it can damage nature. In this study, TEM was used in order to assess the ultrastructural effects of Cd on photoreceptor and ganglionic cells of mouse retinal layer. Apoptotic nuclei, heterochromatic nuclei, deletion of nucleus membrane, invisible nucleolus, and apoptotic cells with mitochondrial changes were observed in mice embryo (days 15 of gestation) following CdCl₂ injection to mothers on day 9 of gestation. Cadmium exposure caused apoptotic changes both in photoreceptors and ganglionic cells.

Keywords: Cadmium, Mice, Retina. TEM, Ultrastructure

Environmental contamination by cadmium (Cd²⁺) results from its industrial use and its presence in agricultural fertilizers. Polluted surface water runs off into streams and rivers which often also contain effluent from metal surface-treatment plants, which use large quantities of water. Cd²⁺ contamination of the environment is a subject of serious international concern since the metal is known to enter the food chain and can undergo bioaccumulation, endangering human health²,³. Cd³⁺ could be absorbed through skin, gastrointestinal and respiratory tract⁷. Cd²⁺ enters circulatory system and binds to a low molecular weight protein, metallothioneine (MT), and is transferred to body tissues. MT synthesis is activated by Cd²⁺ exposure¹⁻³.

Since Cd²⁺ is a resistant metal ion, which cannot be degraded by bacteria, its accumulation in body organs causes a series of toxic effects²,⁴,⁵. After a single dose administration of radioactive Cd²⁺ (2mg/kg) most of it is absorbed by liver (liver primary absorption). By then, Cd²⁺ is transferred to other tissues specially kidneys³,⁶. Cd²⁺-MT complex is purified in glomerular system of kidney and discharged from body. Non-absorbed Cd²⁺ is eliminated via defecation⁶.

Among the heavy metals, Cd²⁺ is considered an important toxicant; its mutagenic, embryotoxic, apoptotic and teratogenic potentials have been extensively investigated²,³,⁷. Several authors demonstrated teratogenic effects of Cd²⁺ on skeletal system, neural tube, limb bud, renal system, heart and liver formation and immune system²,⁴,⁸⁻¹⁵. Thus, it may indicate that Cd²⁺ affects on eyes development, which originate from neural system¹⁶. The present study reports electron microscopic qualitative changes in nucleus and mitochondria of retinal photoreceptors and ganglionic cells during organogenesis in Cd²⁺- treated mouse embryo.

Materials and Methods
Female albino BALB/C strain mice, of either sex (30), aged 3 months were obtained from Iran University of Medical Sciences Tehran, Iran. They were housed in an air- conditioned room (25°C, 12: 12 hr L:D cycle) for acclimatization to the environment until use. Commercial laboratory chow and chlorinated tap water were available ad libitum. Females weighing 250-350 g were mated in the
evening with male mice (3 females:1 male). The females were checked for vaginal plugs on the following morning to determine onset of gestation. The day of plug observation was defined as the first day of pregnancy. Pregnant females had been divided randomly into three groups: experimental I, experimental II and control group.

On day 9th of gestation, both experimental group I and II were treated intraperitoneally with 3 mg/kg and 5 mg/kg of CdCl₂ dissolved in distilled water (2 mg/ml), respectively. The control group received a proportionate volume of physiological saline on the same day. They were sacrificed on day 15 by lethal dose of ether and the fetuses were collected by cesarian section. Each head, as a whole, fixed for 1 week in karnowsky fixative. For Transmission Electron Microscopy study four embryos from each group were selected randomly. Specimens were prepared as described by Bancraft. Semi-thin (500nm) sections were cut in the transverse axis and stained with Toluidine blue. The semi-thin sections were inspected with respect to their appropriate axial position and subsequently ultra-thin sections (50-70nm) were cut and counterstained with uranyl acetate and lead citrate. The ultra-thin sections were inspected and photographed using a Zeiss EM-900 Transmission Electron Microscope.

**Results**

Transmission Electron Microscopy was used to study nucleous, mitochondria, and endoplasmic reticulum of photoreceptors and ganglionic cells in control and experimental groups. Qualitative findings are described below:

Photoreceptor and ganglionic cells of embryos in the control group showed normal neural cell’s ultrastructure, such as euchromatic nucleous, one or two visible nucleolus and evenly distributed cytoplasm (Figs 1 and 2).

No clear destructive change was observable in ganglionic cell’s cytoplasm of embryos in the experimental group I, but density of nucleous was increased. Additionally, subplasmalemal chromatin started to be clumping (Fig. 3). Photoreceptor nuclei also demonstrated different stages of apoptotic changes. Swollen endoplasmic reticuli occupied some part of cellular volume (Figs 4 and 5).

Remarkable apoptotic appearance of photoreceptors and ganglionic cell’s nuclei were found in embryos of experimental group II. Highly condensed nuclei lost their membrane and in later stages characterized by rounded dense chromatin masses through cytoplasm. Furthermore, dense-matrix mitochondria with swollen cristae were abundant in these affected cells (Figs 5 and 6).

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![Electron micrograph](image-url)

**Fig. 1**—Electron micrograph shows parallel and regularly arranged nuclei of photoreceptors in control group. Several nucleoli (arrows) can be seen (×4400).
Apoptotic cells in experimental groups were prevalent. Cd\(^{2+}\) promotes apoptosis, which was manifested by typical morphological changes and internucleosomal DNA fragmentation. Even if the exact mechanisms by which Cd\(^{2+}\) exerts its apoptotic effects are not completely understood\(^1,3,7\), Cd\(^{2+}\) has been demonstrated to disturb Ca\(^{2+}\) homeostasis in
embryonic cells. While, Ca\(^{2+}\) ions are essential element for embryo development stages such as: mitosis, organogenesis, cell cohesion and morphogenetic processes. Cd\(^{2+}\) may affect the cell membrane permeability to Ca\(^{2+}\) ions and disturb Ca\(^{2+}\)-channel dynamic and intracellular Ca\(^{2+}\) transport. Cd\(^{2+}\) and Ca\(^{2+}\) are competitive ions. once Cd\(^{2+}\) is accumulated, the Ca\(^{2+}\) uptake efficiency decreases

Fig. 4—Electron micrograph of one normal (NO) and one apoptotic cell (AP) from experimental group I. The condensed chromatins (C) represent early stage of apoptotic changes (×7000).

Fig. 5—Electron micrograph of three ganglionic cells (G), in experimental group II. G\(_1\), G\(_2\) and G\(_3\) represent early to late stages of apoptotic changes, respectively (×4400).
leading to further deterioration of subsequent Ca\(^{2+}\) uptake. In a Ca\(^{2+}\) - free system, DNA fragmentation is increased by Cd\(^{2+}\) (refs 1,2,5,9,18).

CaCl\(_2\) induces the synthesis of a specific set of stress proteins in embryos exposed to this metal. They are metallothionein (MT) and heat shock proteins (hsps). It is known that together with hsps and MT genes, a member of the BAG-3 gene family is over-exposed in cells exposed to Cd\(^{2+}\) and appears to play a dual role in preventing cell death and contributing to the cellular defense response to stress. MT and hsps increase resistance to Cd\(^{2+}\)-induced apoptosis. Over-exposure of these genes has been observed in nematodes Drosophila, sponges, mammals and humans. MT content increases in embryos at the blastula stage. Changes in metal level are not correlated to MT content in the embryo, whereas DNA methylation is the factor regulating MT expression\(^{2,19}\). Because of rapid cell differentiation DNA strands are susceptible to DNA lesions, on the other hand cells have mechanisms to remove and repair the DNA lesions as soon as possible. These mechanisms prevent transcriptional errors, mutations and cell death. Embryonic exposure to DNA repair inhibitors such as Cd\(^{2+}\) may increase the probability of teratogenesis since Cd\(^{2+}\) exposure may cause lesions to persist\(^{2,20}\).

The inhibition of nutrient transfer to the embryo may be one possible mechanism of developmental anomalies and vascular damage could be a key physiological mediator of Cd\(^{2+}\) toxicity. As to how Cd\(^{2+}\) would affect the generation of blood vessels remains obscure. In cultured human umbilical vascular endothelial cells, capillary network was inhibited by Cd\(^{2+}\) via its impairment on the proliferation, migration and tube formation. Acute exposure to Cd\(^{2+}\) caused changes on the integrity and permeability of the vascular endothelium. All the Cd\(^{2+}\)-treated embryos had markedly less complex cranial vessels with fewer vessels perfusing the craniofascial regions and impaired branching and anastomosis of the cranial vessels\(^{21-23}\).

Assessment of the ultrastructure of photoreceptors and ganglionic cells by TEM revealed too many large vacuolated mitochondria with low cristae. Further, we defined mitochondria condensation of the cells. In chromatolysis stage, before the onset of apoptosis, the quantity of mitochondria increase and they localize around the cell nucleus. These mitochondria are important sources of free oxygen radicals and apoptotic proteolytic activating factors which cause DNA lesions. But at the later stages, apoptosis starts, there is progressive decrease in mitochondrial quantity, especially during neural cell

Fig. 6—Electron micrograph of an apoptotic photoreceptor from experimental group II. A cluster of condensed chromatin (C) in the center of cell, disrupted nuclear membrane (arrows) and integrated nucleoplasm with cytoplasm are detectable (×12000).
apoptosis. Elimination of C-cytochrome enzyme activity, mitochondrial swelling and christolysis synchronize with cytoplasmic apoptotic changes. The present observations seem to support this assumption.

Cd\(^{2+}\) is supposed to involve mitochondria as a target, since micro molar concentrations have a direct effect on increasing permeability of the inner membrane of mitochondria. However, little is known about this harmful effect of Cd\(^{2+}\). (refs. 2, 7).

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