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Effect of L-carnitin on Cadmium Induced Toxicity in Rat Embryo Hippocampus

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Abstract. Cadmium is a toxic metal which is widely used in industry. This metal exerts toxic effects on multiple organs, including nervous system. The aim of this study have been evaluated the effect of cadmium on weight and development of hippocampus in Wistar rat embryos and then determining if L-carnitin, as an antioxidant, could protect hippocampus from the toxic effects. Female Wistar rats (250-300 g) were used in this study. 24 hours after mating with male rats, the females were separated and their vaginal smears were obtained for sperm detection. This day was considered as embryonic zero day. The females were divided into three groups: Control group which received no injection, Experimental groups which received 1mg/kg B.W cadmium or 1mg/kg B.W cadmium + 500mg/kg B.W L-carnitin in days 7 and 10 of gestation. On day 17 of gestation, the animals were sacrificed by chloroform over dose and their embryos were removed surgically. The embryos were fixed in formalin 10% for 30 days. Then weight of embryos was measured and tissue processing, sectioning and hematoxylin-eosin (H&M) staining were done. Some sections of hippocampus were evaluated using light microscope and MOTIC software. The weights of embryos were significantly decreased in experimental groups. This decrease was significantly greater in the first experimental group. The number of cells and thickness of hippocampus layers were decreased significantly just in the first group. These findings indicate that cadmium has teratogenic effects on embryo's weight and development of hippocampus and at least a part of these effects may be inhibited by L-carnitin.

Key words: Cadmium, L-carnitin, Hippocampus, Embryo, Rat.

1. Introduction

Cadmium (cd) is a toxic metal with long biological halflife which naturally exist in our environment in low concentrations and enters human body trough almost everything we eat or drink and breathe. WHO has estimated safe level for human ingestion at 500 µg/week (1). Cadmium widely used in industry as anticorrosive in plating metals, manufacturing storage batteries, alloys, stabilizer and pigments. Also is absorbed in significant quantities from cigarrete smoke. Therefore cd concentration is increasing in the environment (2).

Cadmium accumulation in the human body is dangerous and may also be linked to osteomalacia, infertillity, hepatotoxicity, renaltoxicity, neurotoxicity and anomalies (3, 4). In chronic poisoning with cadmium it was reported that high concentration of cadmium has been accumulated in hippocampus which induces neurodegeneration in this area (5).

In mitochondria, cadmium enters in structure of phosphorilation-oxidative enzymes and disrupts producing energy cycles. Also it can replace many metal nutrients such as ca^{+2} and reduce ca^{+2} entering to the cell (6). Cadmium induces lipid peroxidation by stimulating the production of superoxid anions and due

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to inhibition of antioxidants such as glotation peroxidase and superoxide dismutase, accumulates free radicals, damage to the cell and produce chronic diseases (7).

It was reported that l-carnitine has an antioxident activity. L-carnitine with chemical formula β-hydroxy-γ-N-trimethylaminobutyric acid, has a substantial role in fatty acid β –oxidation (8). Carnitine transports long-chain acyl groups from fatty acids into mitochondrial matrix therefore has a substantial role to obtain energy. 75% of carnitine enters the human body throughfoods and 25% of that biosynthesizes from aminoacid lysin and methionine in the liver, brain and kidney (9). L-carnitine is necessary for body and has anti-oxidant activity; therefore it seems to have protective effect against cadmium.

The hippocampus is a major component of the brains and belongs to the limbic system. It plays important roles in the consolidation of information from short-term memory to long-term memory and spatial navigation. The hippocampus is located in the medial temporal lobe of the brain. In Alzheimer's disease, the hippocampus is one of the first regions of the brain to suffer damage (10).

As the hippocampus is an important compartment in the memory formation, we want to know what the effect of cadmium exposure during embryonic duration on development of hippocampus is and whether l-carnitine as a protective subestances can eliminate these effects.

2. Materials and methods

Wistar rats weighing 250-300 g were used in these experiments. Animals were housed in colony room: 12/12 hr light/dark cycle at 24 ± 1ºC and had free access to water and food. Cadmium and L-carnitine were obtained from sigma company (USA).

Mice divided into three groups randomly (n=10), control, Cadmium, and cadmium + L-carnitine. Control group was received normal Saline. Cadmium, and cadmium + L-carnitine were received 1 mg/kg cadmium and 1 mg/kg cadmium + 500 mg/kg L-carnitine respectively in 7^{th} and 10^{th} days of pregnancy. Also 2 and 4 mg/kg doses of cadmium were studied but as they induced abortion, we did not continue experiments with these doses. All administrations were intraperitoneally.

In 17^{th} day of pregnancy animals were sacrified by chloroform over dose and embryos were transfered to fixator (10% formaline). After fixation embroy's weight were measured. Head of embryos were embeded in paraffin and blocked after tissue processing with series of alcohol and xylene. Then sagital serial sections, each of 5 µm tickness were made by microtome (FITZ, Germany) and stained with hematoxylin and eosin according to standard method. Histological assessments were performed under light microscopy with MOTIC software. For hippocampus cell counting, we used MOTIC program to prepare images with 400X magnification (cells were counted in 5 square 2 × 2 centimeter in a 10× 10 area).

2.1. Statistical analysis

Data were expressed as mean value ± S.E.M. and tested with analysis of variance followed by the multiple comparison test of Tukey Kramer.

3. Results

Experiments indicated that the weight of embryos was decreased in experimental groups. Thickness of inner and outer layer of hippocampus was significantly decreased in cadmium group in comparison to control but it was not significant in cadmium + L-carnitine group. Thickness of outer layer in cadmium + L-carnitine group was significantly more than cadmium group (Table 1).

Count of cells in inner and outer layer of cadmium group was decreased in comparison with countrol group. Count of cells in two layers in cadmium + L-carnitine group was more than cadmium group.

Table 1: Effect of treatment with cadmium or cadmium + L-carnitine on tickness of layer and count of cells in embro's hippocampus

Treatment	Weight of embryos	Tickness of inner layer (µ)	Tickness of outer layer (µ)	Count of cells in inner layer (μ^2)	Count of cells in outer layer (μ^2)

Control	15.3 ± 0.43	95.7 ± 6.56	114.7 ± 4.56	9 ± 0.63	6.2 ± 0.37
Cadmium	7 ± 0.58***	79.81 ± 1.68*	48.41 ± 1.4***	6 ± 0.56***	3. 6 ± 0.59**
Cadmium+L-carnitine	12.7± 0.49***	86.7 ± 1.4	107.8 ± 5.6***	7.5± 0.48*	5.3 ± 0.21*

*Comparison of experimental groups with countrol ∗ Comparison of experimental groups with each other. One star $p<0.05$, two stars $p<0.01$ and three stars $p<0.001$.

4. Discussion

Results showed that treatment with cadmium decreased weight of embryos. Because the long biological half life of cadmium in the body cadmium can produce detrimental effects on mother and her embryos. It is known that cadmium is embryo toxic in animal model and cause brain and craniofacial malformations (11). Webster and Messerle found that IP injection of 4mg/kg $cdcl_2$ in the mouse produced neural tube defects when given during neuralation (12). One of poisoning symptoms induced by cadmium is decrease blood flow in uterus and placenta which reduce placenta transportation and therefore decrese feeding embryos. The other mechanism can suppress embryo growth in cadmium group is irritating mother gastrointestinal system including: diarrhea, vomiting and nausea, which decrease uptake food and water by mother (13).

A number of mechanisms of cadmium toxicity have been suggested including: 1) Increase corticostrone 2) Inhibition cell proliferation 3) Apoptosis and necrosis.

It was reported that, administration of cdcl2 in drinking water of pregnant rats increased foetal corticostrone hormone (14). Cortisole crosses the placenta and thus may affect the fetus and disturb ongoing developmental processes (15). The development of the HPA axis, limbic system, and prefrontal cortex. The most important parts of the brain which have special corticostrone receptors are frontal cortex, amygdala, and hippocampus. Evidence indicates that increased exposure of the fetus to glococorticoid in mid to late pregnancy may result in adverse outcomes, which include intra-uterin growth restriction and retardation of fetal brain development (16). Also every changes in the cocentration of corticostrone can influence on target genes expression, that are likely important in toxicity responses.

After absorption, cadmiumd is transported to the liver, bound to albumin, where it induces synthesis of metallotionine (MT), a class of small cystein-rich heavy metal binding proteins. MT-bound cd enters the plasma and diffuses to all tissues. Experiments indicated exposure to Cadmium in low doses which did not produce overt histopathological changes, can alter the expression some genes that are related to toxicity responses such as decreased expression of pro-apoptotic genes, particularly Casp3, and DNA repair genes. Cadmium induces oxidative stress and thus exerts abnormal levels of apoptosis in testis by suppression of p53 expression (17). Phosphorylation of P38 (MAPK) mitogen activated protein kinase was enhanced by cadmium. Therefore cadmium disrupts MAPKs pathways and impairs cell viability in developing hippocampus. Also activation of p38 and mitochondria membrane disruption, appear to be the primary targets. In general, mechanisms by which cadmium provokes apoptosis depend upon the dose given (18).

L-carnitine, a vitamine like antioxidant, plays a pivotal role in the prevention cadmium detrimental effects. Researchers have studied effects of treatment by l-carnitine and acetyl l-carnitine on rat testis. Their results have indicated carnitine protects testis from injury induced by cadmium due to its antioxidant activity (19). Also studies have shown entered l-carnitine in human lymphocyte cell culture decrease lymphocyte apoptosis induced by HIV viruses (20). Our results in consistent with these result emphasize on protection effect of l-carnitine following cadmium administration in rat hippocampus.

Our findings indicate that in cadmium group the cell count in both layers of the hippocampus decreased, but this decreasion in external layer was more than internal layer. As internal layer has proliferating neurons which then migrate to external layer this phenomenon is expected.

In conclusion these findings confirm that cadmium can severly distroy hippocampus tissue and decrease weight of embryo. Pay attention to this effect are important for industrial society with high concentration of cadmium in their environment and we propose treatment with l-carnitine as a protective subestances for cadmium poisoning.

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