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ORIGINAL ARTICLE

Which type of Apoptosis or Necrosis occurs in Thymus Gland after Application of Cadmium chloride?

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ABSTRACT
Cadmium chloride (Cd) is a toxic agent that is also a potent immunotoxin. The cadmium and dexamethasone (Dex) induce apoptosis on immune cells by different speed. While the different effects of Cd and Dex treatment and the changes due to each of them were not evaluated in vivo conditions. In this study, the morphology of cell death in thymus gland was determined. 4-6 weeks male Balb/c mice were divided into three groups, Control, Cd (CdcI2) and Dex group. Three groups were injected intraperiteonaly by saline, 1.8mg/kg Cd and 8mg/kg Dex, respectively. After 16-hours, thymus glands were extracted and were studied by light and electron microscopy. The morphological changes were similar in both Cd and Dex groups by light and electron microscopy. The size of cortex was decreased and the cortical thymocytes were seen pyknotic. As well as electron microscopy revealed that thymocytes were observed with heterochromatin of nuclei and cytoplasmic changes. The majority of thymocytes showed apoptosis. There was no changes in thymic nurse cells, but ultrastructure of the apoptotic cells in two treated groups were similar Cadmium chloride like dexamethasone had immunotoxic effects on thymus gland and induces programmed cell death in immune cells. However, the type of cell death due to Cd and Dex, both is apoptosis, but because of different morphology of apoptotic cells it is possible that the apoptosis mechanism of them was different. On the other hand all thymocytes was not undergoes of this changes and TNCs were normal.
Key Words: *Thymus, Apoptosis, Cadmium, Necrosis.*

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INTRODUCTION

Cadmium is a chemical element that is used in industry. The cadmium could contaminant human, animals and plants tissues by industrial productions and smoking [6].
A toxic effects of cadmium on kidney, lung, liver, spleen and hematologic cells was previously determined [2, 3, 11, 19]. Sensitivity of various human immune cells line, for apoptosis induction differes. As some cells rapidly undergo apoptosis by cadmium [2, 11, 15].
The thymic microenvironment has important role in differentiation of thymocytes. The thymus mainly comprises the lymphoid cells and non-lymphoid cells. One class of the non-lymphoid cells is thymic nurse cells (T.N.C.). The T.N.C. encloses several thymocytes that it produces a niche for them. The enclosed cells show various stages of maturation cycle [1, 16, 17].
Exposure to cadmium causes thymic atrophy and thymocytes death [18]. Although the thymocytes cell deaths were reported as necrosis and apoptosis, but the percentages of apoptotic cells are higher compared to necrotic cells. The apoptogenic properties of cadmium on thymocytes have been determined by electrophoresis, ELISA and flow cytometery under *in vitro* and *in vivo* cell culture system [4, 9, 10]. The mechanisms of thymocytes apoptosis are exactly characterized after using dexamethasone [14]. The mechanisms of apoptosis by cadmium and dexamethasone may be different from one each other. The speed of apoptosis induction by cadmium is slower than that caused by dexamethasone [4]. As far, ultrastructural findings have not been elucidated to confirm similarity or difference of cell death patterns

by dexamethasone and cadmium administration. Furthermore, the used techniques have not determined which the type of the thymic cells has undergone dying. As well as, the type of cell death occurrence due to cadmium are under debate *in vivo* [4, 9-11].
The main objective of the present investigation was to characterize and to compare morphological changes of thymic cells after administration of cadmium and dexamethasone by light and electron microscopy. Furthermore, the cell death type was determined (the *in vivo* system).

MATERIAL AND METHODS

Male Balb/C mice, 4-6 weeks old, were used in this study. The mice were divided into three groups; positive and negative control and experimental group. Nine mice were studied in each group. The mice in the positive control group were injected Intraperiteonally with 8mg/kg dexamethasone. The mice in experimental group were administrated intraperitoenally with cadmium chloride solution in physiological saline at the concentration of 1.8mg/kg. The negative control group was injected only with vehicle (physiological saline). After 16 hours of administration time, the thymus was extracted and cut to small pieces. The pieces of thymus were prepared for light and electron microscopic techniques. For light microscopy, Pieces of thymus were fixed in Bouin's solution and embedded in paraffin and then the paraffin block was sectioned at 5µM thickness. The sections were stained with Hematoxylin and eosin (H & E) and studied under light microscopy.
For electron Microscopy, Thymus pieces were fixed overnight in 2% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2). They were subsequently post-fixed for 1 hour in 1% (w/v) osmium tetroxide in 0.1 M sodium cacodylate buffer. All tissue pieces were dehydrated through a series of ethanol concentrations and embedded in Epon 812. Ultrathin sections were supplied by ultramicrotome. Ultrathin sections were stained with lead citrate and uranyl acetate and then examined by a electron microscope (Zeiss).

RESULTS AND DISCUSSION

Histological changes (light microscopy):

In negative control group, each lobule of thymus contained cortex and medulla; cortex was full of thymocytes and medullary was rich in reticular epithelial cells. Also the medulla contained degenerating epithelial cells called Hassall's corpuscles. All thymocytes were appeared intact (figure 1).
In experimental and positive control groups, the cortex was affected more than medulla. Thickness of thymus, separately medulla and cortex, has declined, and also cortex diameter decrease was more than medulla. Pyknotic cells were more in cortex. Unaffected cells were also observed among other pyknotic cells (figure2).

Ultrastractural changes:

In negative control group, intact thymocytes contained normal organelles and large nucleus with more euchromatin (figure 3). The thymocytes were seen besides the thymic nurse cells (TNC). TNC with large euchromatin nucleus and nucleolus inside it were enclose thymocytes (figure 4).
In cadmium and dexamethasone treated groups; the morphology of cytoplasm and nucleus of TNC were intact. Thymocytes have been seen in different morphological changes besides of TNC. The thymocytes were appeared in both alive and dead forms. The dead cells were seen as apoptotic cells. Apoptotic cells were observed in the two early and late stages (figure 5).
The early stages have been characterized by chromatin condensation by capping formation and compaction of cytoplasm. At the late stage, nucleus appeared by margination of compacted nucleus and then its fragmentation. The cytoplasmic changes were continued by the destruction of organelles and then separation of the whole thymocytes from TNC, the morphological changes seemed to be similar in Cd and Dex treated groups.
Thymus gland is a central lymphoid organ in which a complex process of maturation and differentiation of T-cells is happened. Many heavy metals affect the immune system; like thymus (3, 6). Some heavy metals as cadmium and also dexamethasone caused atrophy of thymus. Our results indicated atrophy in thymus tissue following cadmium chloride and dexamethasone administration. Comparison tissue changes following cadmium chloride administration with dexamthason because of three dominant reasons: a) sensitivity to cell toxicity, b) depletion of thymocytes, c) cell death of thymocytes.
1) **Sensitivity to cell toxicity**; Resistance and sensitivity of lymphoid cells line to cadmium are different (15, 18). Cellular and humoral reaction to cadmium except thymus was seen in other parts of lymphatic system. Still the type of thymocyte cell which resistant into cadmium was not distinct. Whereas T-cell differentiation was in cortex of thymus gland, hence any toxic agents like cadmium chloride could affects thymus glands, perhaps disturb this process. Although our results showed that the number of pyknotic

cells was high in the cortex, but intact T-lymphocytes in both cortex and medulla were seen. Thus the reaction of the cells was not the same and some of t-cells were resistance to cadmium toxicity.

It may conclude that the mature T-lymphocytes may resist against cell death when exposed to cadmium. There are controversial results concerning humeral and cellular reactions following cadmium exposure (8, 12). Since T-cells differentiation and selection of mature T-cells are carried out in thymus, any toxic agents that affect the thymus tissue can impact its function.

In addition, oxidative stress and GSH play an important role in the process of cadmium toxicity,they induce apoptosis. Intracellular Ca and ROS levels are increased by cadmium [9-11]. The enveloped thymocytes by TNC have undergone cell death by Dex, though it is dependent of dose of dexamethasone. Some thymocytes within TNC were induced to apoptosis whereas the TNC and some other thymocytes were enveloped by the TNC did not show any response to the cadmium and dexamethasone. All cells have not undergone to apoptosis and the cells in cortex of thymus have not been shown the same reactions to cadmium exposure. It seems the response of thymocytes to inducers is different.

2) **Lymphocyte Depletion**; Depletion of T-lymphocytes from thymus are previously reported [9, 10, 18]. The results showed that histological changes of thymus including decrease in the thickness of medulla and cortex by cadmium and dexamethasone were similar. Histological changes by cadmium are confirmed by other researchers [3, 9, 10, 18]. There may be a similar mechanism involved in histological changes of cadmium and dexamethasone. The decline of thickness of thymus may result from lymphocytes depletion. Although the many cells are depleted and then may be released to blood circulatory system, the released cells are not characterized by now. Another studies show that dexamethasone induced depletion of lymphocytes from thymus [7, 18]. Dexamethasone causes depletion of lymphocytes through thymic postcapillary venules and lymphatic vessels [5]. Our results demonstrate size and volume of thymus was decreased. Therefore, it seems that the depletion of thymocytes from the cortex due to dexamethasone and cadmium treatment may concern as phenomena that it impacts on thymus function. Regards to similarity of thymus morphological changes in cadmium and dexamethasone groups, it seems likely the similar mechanisms responsible for these changes. Therefore cadmium like dexamethasone could deplete lymphocytes from thymus through lymphatic and post-capillary venules.

3) **Cell death of thymocytes**; The cadmium administration lead to cell death of thymocytes [4, 13]. Base on our results the type of cell death was characterized as apoptosis, because ultarstructural changes in cadmium and dexamethasone was similar. Also different stages of apoptosis in two groups were same almost.

The reports have been defined based on ELISA, Electrophoresis and the flow cytometery techniques, indicated that cadmium induces both apoptosis and necrosis in thymocytes [4, 10]. Recent studies demonstrated apoptosis *in vitro*. While based on electron microscopy results of current study necrosis type of cell death was not seen. Controversy of results in this study and others might relate to the differences of techniques by which were employed in this study and others. Although it was reported that the speed of apoptosis by dexamethasone and cadmium different (4). In our study no differences were found in the speed of dexamethasone and cadmium treated groups morphologically.

The apoptotic cells were divided into two stages; early and late. The reviews of the mechanisms of cell death have shown that ROS generation is seen only in cadmium exposure groups. Activation of endonuclease and DNA fragmentation, elevation of intracellular Ca^{2+} and activation of caspase-3 that causes cell death, are common mechanistic factors that are seen in both dexamethasone and cadmium treatment groups [4, 9-11, 13]. Recently, the apoptosis process is defined by two pathways; caspase-dependent and caspase-independent pathways [11]. Although that study carried out by non-morphological technique and *in vitro* system.

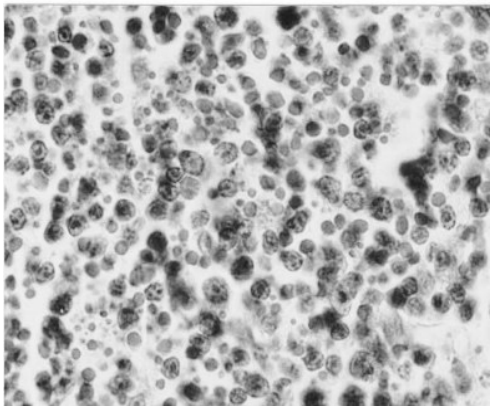

Figure 1: Light micrograph of thymus in control group (mag: X 400, H&E)

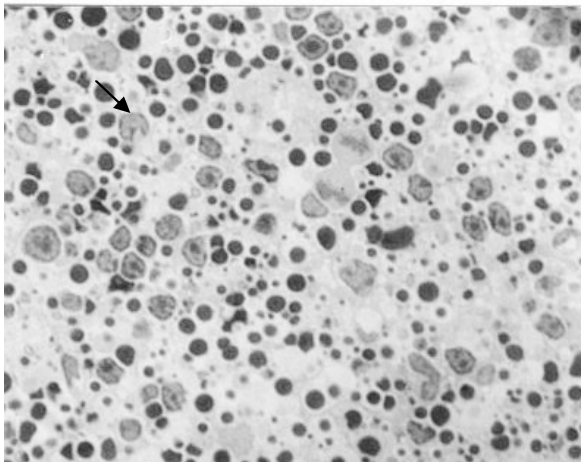

Figure 2: Light micrograph of thymus in cadmium treated group. The pyknotic cell is seen (arrow). (mag:X 400, H&E)

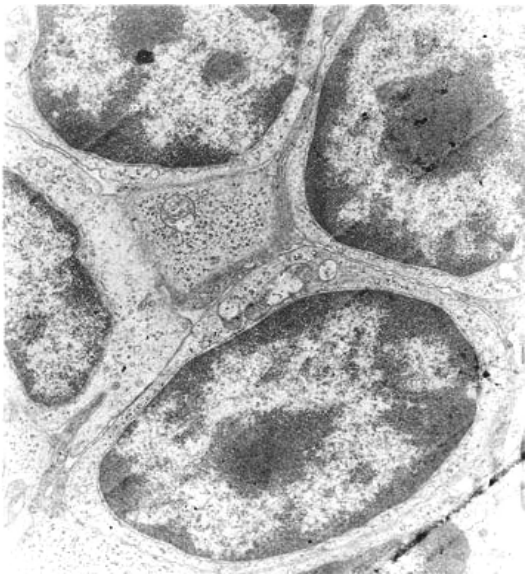

Figure 3: Electron micrograph of normal thymocytes (Mag:X 20000)

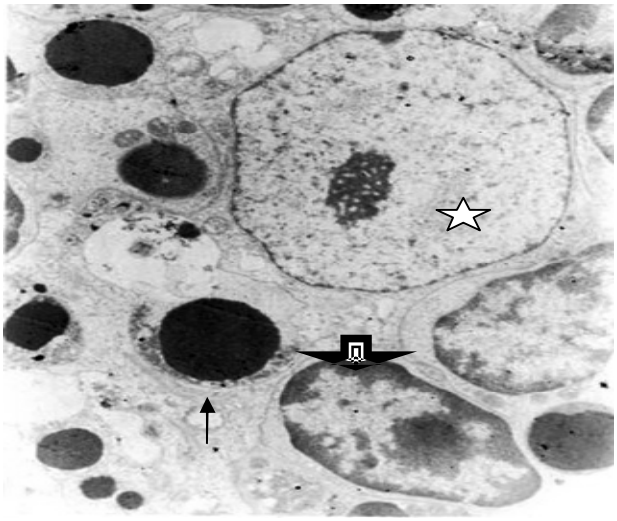

Figure 4: Electron micrograph of T.N.C. (asterike) shows with intact thymocytes (arrow head) and apoptotic cell (arrow), (mag X 4400)

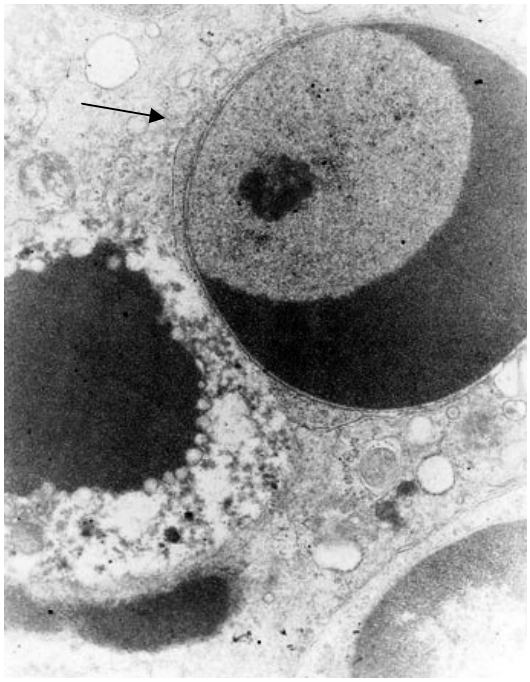

Figure 5: Electron micrograph shows a typical apoptotic cell in both dexamethasone and cadmium treated groups (mag X 20000)

CONCLUSIONS

In conclusion, uptake of cadmium compound can be threat the health of human. The acute and chronic effects of cadmium have shown different signs during of life. As activity of thymus is high at infant and childhood age, the toxicity of thymus in active phase may cause changes of the immunological reaction at adolescence. The sensitivity of lymphoid cells to cadmium toxicity, depletion of T-lymphocytes from thymus and cell death occurrence is discussed in this study. Ultrastructural changes of thymus tissue after cadmium treatment were similar to dexamethasone treatment. Cadmium and dexamethasone induced apoptosis in thymocytes of thymus tissue. Apoptosis appeared into the early and late stages. However, in this study the necrosis has not been observed in both dexamethasone and cadmium administration.

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