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MORPHOLOGICAL STUDY OF MOUSE (BALB/c) THYMUS AFTER HIGH AND LOW DOSE DEXAMETHASONE TREATMENT

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ABSTRACT

Dexamethasone induces thymic atrophy and thymocyte apoptosis. In the present study histological and ultrastructural changes which occur in the thymus of the mouse (BALB/c) following treatment with high (20 mg/kg) and low (8 mg/kg) doses of dexamethasone were investigated. In low dose treated mice, apoptotic cells were observed focally and localized mainly in thymic nurse cells (T.N.C.). A zone of intact thymocytes was formed in the medulla of animals receiving 20 mg/kg of dexamethasone as well as an increase in trans-endothelial vesicles and a decrease in the size of the vesicles in the cortical capillaries. The enveloped thymocytes within thymic nurse cells respond to dexamethasone through apoptosis, and these changes were seen to be more severe in mice treated with high doses of dexamethasone. The formation of apoptotic cells in the thymus caused by low dose dexamethasone mimics the physiological process of cell death. Differential effects of low dose and high dose dexamethasone may have pharmacological and immunological implications.
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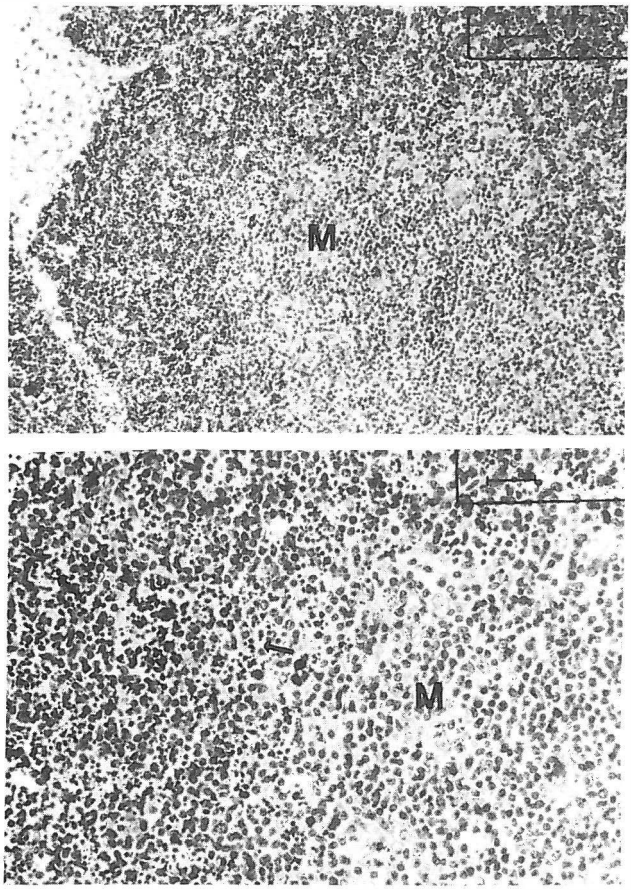
INTRODUCTION

The thymus is the central organ responsible for the generation of immunocompetent T-lymphocytes.[1] The thymic microenvironment appears to have an important role in differentiation of thymocytes. The non-lymphoid cells of the thymus mainly comprise the intrathymic microenvironment. Among non-lymphoid cells, the thymic epithelial cells have both functional and structural roles.[12,27,29] One class of the epithelial cells are thymic nurse cells (T.N.C.) which are localized in the cortex and corticomedullary regions of the mouse thymus.[1,2]

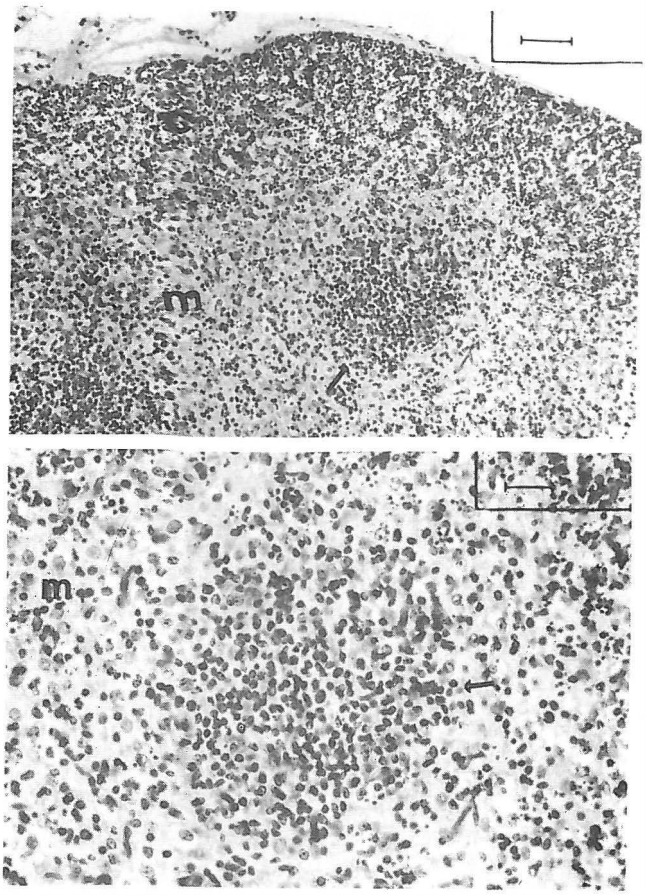
The T.N.C. enclose several thymocytes, and the enclosed cells display the characteristics of cortical thymocytes at various stages of the maturation cycle.[3,18,19,20] Brelinska and Hiramine found that some of the enclosed thymocytes in isolated T.N.C. show death.[3,19] Glucocorticoid hormones are secreted physiologically by the adrenal gland. One of the potent synthetic glucocorticoids is dexamethasone which has pharmacological uses. Various glucocorticoid hormones induce atrophy and apoptotic cell death in the thymus. It is believed that most of the cortical thymocytes are sensitive to glucocorticoids.[4-6,13-15,21-24,26]

The apoptosis of cortical thymocytes are normally involved in the elimination of autoreactive or unselected immature thymocytes during the maturation process. It is also involved in normal physiological regression of the gland.[10,11,31,32]

In this present study, light and electron microscopic changes of the thumus were demonstrated after using two different doses of dexamethasone and compared with each other.



Figs. 1,2. Light micrograph of thymus in 8 mg/kg dexamethasone treated sample. The clustered pyknotic cells in the cortex (arrow) and normal medulla (m) are shown (H&E, 100×). Same field with high power magnification (H&E, 200×).



Figs.3,4. Light micrograph of thymus in 20 mg/kg dexamethasone treated sample. It is seen with increased pyknotic cells in the cortex (c) and the two sites of medulla (M), one site appears focally with normal cells (arrow) (H&E, 100×, 400×).

MATERIALS AND METHODS

Four-week old BALB/c mice were intraperitoneally injected with 8mg/kg and 20 mg/kg of dexamethasone sodium-phosphate (Co: sicor, Italy). The control group received only P.B.S. After 16 hours, the mice were sacrificed by cervical dislocation and the thymus glands were removed and processed for routine light and transmission electron microscopy. Five mice per group were analyzed. One lobe was used for light microscopy which was fixed in Bouin solution and embedded in paraffin. Sections five microns thick were stained with hematoxylin and eosin. The sample from the other lobe was processed for electron microscopy by the following method: the tissue was cut to a $1mm^3$ cube and fixed in 2% glutaraldehyde buffered with cacodylate sodium pH 7.3 for 4 hours at 4°C by immersion. Then the tissue was washed with the buffer, post-fixed in 1% OSO_4 in the same buffer, dehydrated in acetone and embedded in Araldite-Epon 812 (Polyscience). Ultrathin sections were prepared and stained with uranyl acetate and lead citrate and examined with a Zeiss electron microscope.

RESULTS

Based on the results observed with several doses of dexamethasone (1,3,6,8,10,15 and 20 mg/kg) by light microscopy, two different doses considered as low (8 mg/kg) and high (20 mg/kg) were selected for further studies.

The rate of cell death was very different between the low and high doses as evaluated by light microscopy (Figs. 1,3).

Histology

Both normal and apoptotic cells were observed in the thymic cortex of mice treated with low dose (8 mg/kg) dexamethasone under light microscopy. The apoptotic cells were seen in clustered form and resembled pyknotic cells. The pyknotic cells were small with condensed nuclei and without differentiation between cytoplasm and nucleus. In contrast, normal cells had clear nuclei and eosinophilic cytoplasm (Figs. 1,2).

At high doses of dexamethasone (20 mg/kg) several empty spaces associated with increased apoptotic cells and depleted normal cells were observed in the cortex. The

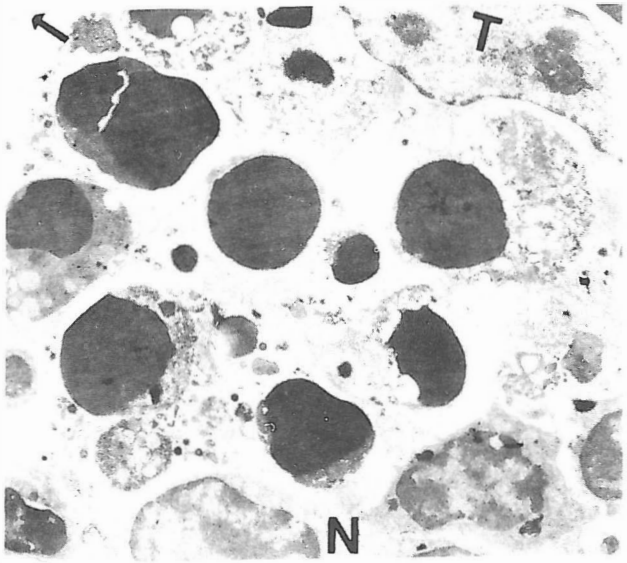


Fig. 5. Electron micrograph shows the T.N.C. (8 mg/kg) with apoptotic cells and intact cells around the T.N.C.(N). Desmosome junction (arrow) and nucleus of T.N.C. are seen. (4400×).

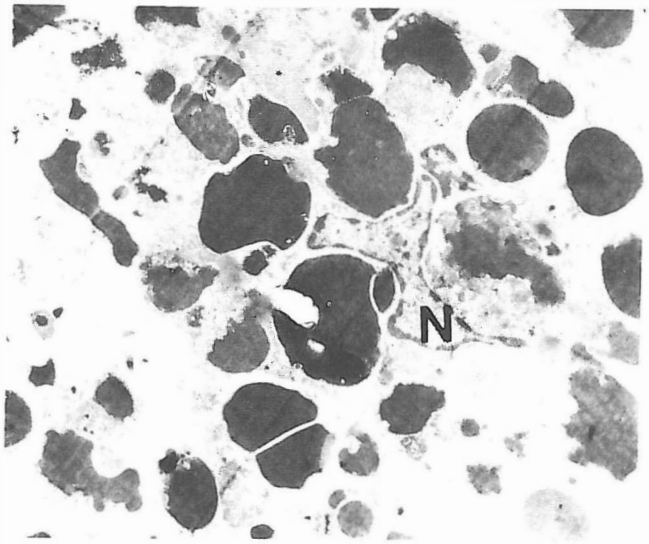
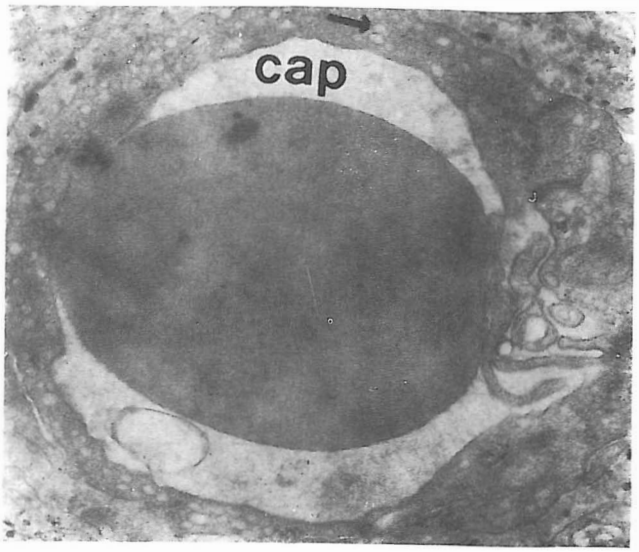


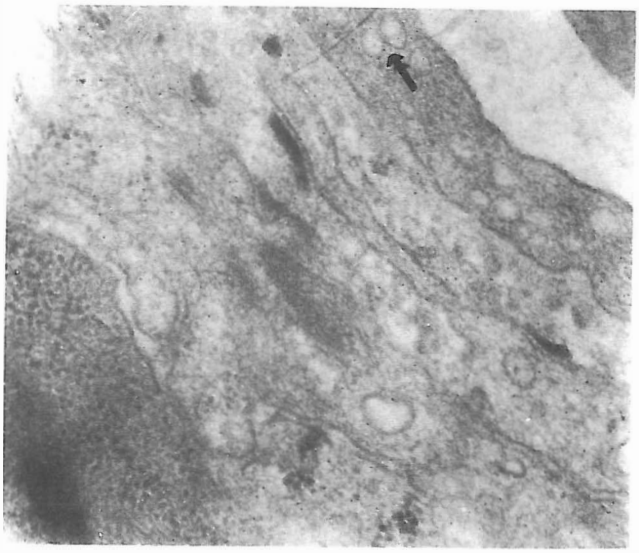
Fig. 6. Electron micrograph shows the T.N.C. (20 mg/kg) with many apoptotic cells. Nucleus of T.N.C. (N) (4400×).

medulla exhibited two distinct regions, one resembling normal medulla containing some apoptotic cells whereas focal aggregation of normal thymocytes was observed in the other part (Figs. 3,4). No changes were observed in the thymic medulla of animals treated with low dose dexamethasone (8 mg/kg) (Figs. 1,2).

Ultrastructural analysis

At 8 mg/kg of dexamethasone, a T.N.C. with euchromatin nucleus, clear nucleolus, tonofilaments under the cell membrane and desmosome junctions which enveloped some apoptotic cells were seen, while intact thymocytes were seen around the T.N.C. The enveloped apoptotic cells were





Figs. 7,8. Electron micrograph of capillary in 8mg/kg dexamethasone treated sample (cap) (20000×). Arrow indicates transendothelial vesicles with less vesicles. (50000×).

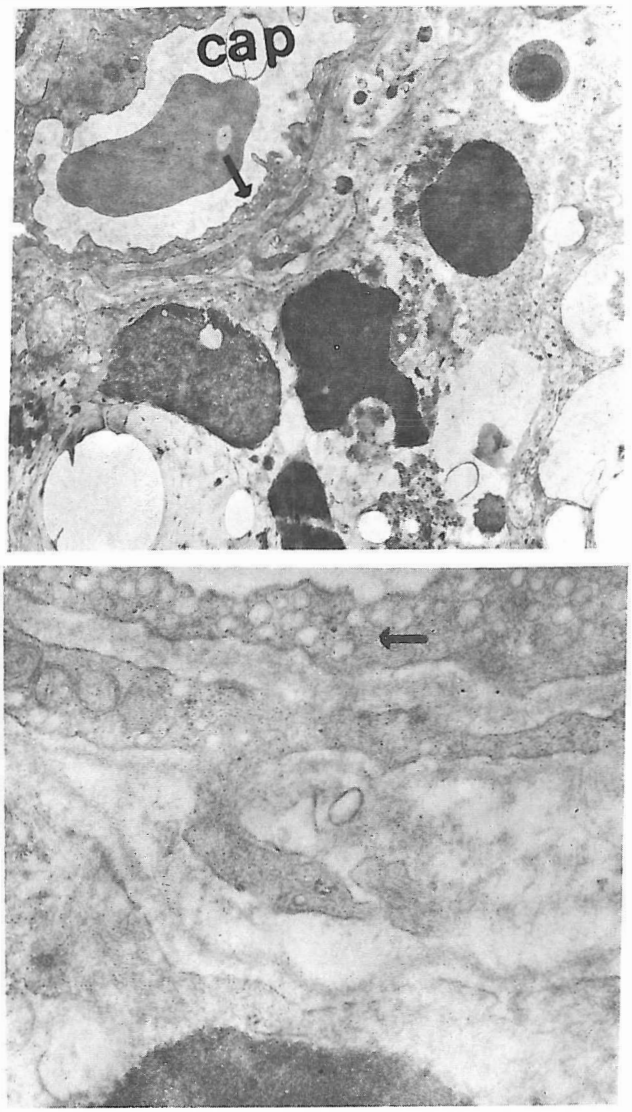
at various stages of cell death (Fig. 5).

In addition to the changes described for dexamethasone treatment at low doses (8 mg/kg), empty spaces were seen around the apoptotic cells in animals treated with 20 mg/kg of the drug (Fig. 6).

Increased numbers of transendothelial vesicles together with decreased vesicle size were characteristics of cortical capillaries in animals which received high dose dexamethasone (20 mg/kg). The control group and low dose-treated group showed no such changes (Figs. 5,7,9,10).

DISCUSSION

In the present study we have investigated the effect of two different doses of dexamethasone on thymic morphology



Figs. 9,10. Electron micrograph of capillary in 20 mg/kg dexamethasone treated sample (cap), 7000×. Transendothelial vesicles are noticed with small size and are increased in number (arrow). (30000×).

in immature mice. According to our results, the thymus was involuted by two different doses of dexamethasone with extensive cell death. The type of cell death was apoptotic and was confirmed by electron microscopy, which is in agreement with other reports.[8,13,22-26]

With respect to our results, the thymic apoptosis was dexamethasone dose-dependent, which is in agreement with the results reported by Sun (1992)[8]. The rate of apoptosis was low and limited to the cortex in animals receiving 8 mg/kg of the drug, while in mice treated with 20 mg/kg the rate of apoptosis was high and extended from the cortex to the medulla (Figs. 3,4). The rate of thymic cell death after treatment by dexamethasone *in vivo* at 2-16 hours was time-dependent,[8,17] while the cell death of thymocytes *in vitro* was independent of the dexamethasone dose but was dependent on time.[6,22-25]

According to other studies, the enveloped thymocytes by T.N.C. display the characteristics of cortisone and radiation-sensitive cells[2,16,18,29] Data demonstrated all thymocytes within T.N.C. were induced to apoptosis by dexamethasone whereas the intact cells around the T.N.C. show no response to the drug (Fig. 5).

The vacuole formation within epithelial cells was observed following acute treatment with high dose (125 mg/kg) dexamethasone in old mice (Leen et al., 1988) and chronic treatment with dexamethasone (2.5 mg/mL) divided in two injections. Furthermore, in high dose dexamethasone treated immature BALB/c mice (Fig. 6), the destruction of the enveloped thymocyte, with membranes around those cells, looked similar to vacuolation. The high dose of dexamethasone increases the rate of cell death and increases empty spaces within T.N.C.

The results presented in this study show that high dose dexamethasone (20 mg/kg) causes depletion of normal thymocytes from the cortex and formation of normal thymocyte zones in the medulla. Another study shows that high dose dexamethasone induced depletion of lymphocytes from the thymus.[16] Kato reported that depletion of lymphocytes from thymic postcapillary venules and lymphatic vessels was increased by glucocorticoids.[9] It is well established that the lymphatic vessels in the cortex are absent.[30] Therefore, it seems that the depletion of normal thymocytes from the cortex due to dexamethasone treatment occurs through medullary lymphatic vessels, and the formation of normal lymphocyte zones in the medulla further substantiates these results (Fig. 4).

It is observed that in mice receiving 20 mg/kg of dexamethasone, the transendothelial vesicles are increased in number but decreased in size, the reason for such changes not being clearly understood. The cortical capillaries of the thymus are involved in the blood-thymus barrier, and dexamethasone reaches the cortex by crossing the capillary endothelium.[7] Therefore it is possible that the above observation may be due to the effect of high dose dexamethasone on the capillary-blood barrier.

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