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PRACA ORYGINALNA
ORIGINAL ARTICLE

ULTRAMICROSCOPIC CHANGES OF RATS PARATHYROID GLANDS AND THYMUS AFTER SINGLE ADMINISTRATION OF CYCLOPHOSPHAMIDE AT THE DIFFERENT PERIODS OF OBSERVATION

ULTRAMIKROSKOPOWE ZMIANY W PRZYTARCZYCACH I GRASICY U SZCZURÓW PO POJEDYNCZYM PODANIU CYKLOFOSFAMIDU W RÓŻNYCH OKRESACH OBSERWACJI

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ABSTRACT

Introduction: Cyclophosphamide has wide spectrum usage as first-line drug in cancer chemotherapy that is why a detailed study of its effect on individual cell populations is of great interest for science and practice. The interaction of the nervous, immune and endocrine systems plays essential role in the homeostasis maintaining.

The aim: This study aimed to investigate the ultramicroscopic changes that occur in the parathyroid glands and thymus of male rats after cyclophosphamide administration.

Materials and methods: Twenty-four WAG matured male rats were divided randomly into two groups. The first group served as control and was provided 0.9% soluble sodium chloride. The second group received cyclophosphamide in a dosage 200 mg/kg of body weight of animal by intramuscular single injection. All animals were sacrificed on the 7th and 30th day after injection. Parathyroid gland and thymus specimens were dissected out and processed for electron microscopy.

Results: The results showed that cyclophosphamide exposure caused marked ultramicroscopic changes in rats parathyroid glands and thymus. On the 7th day after immunosuppression, the nuclei of parathyrocytes have deep wavy invaginations, amount of the organelles that participate in the protein synthesis is reduced to a minimum in the cytoplasm of the chief cells. Characteristic feature is the appearance of numerous plasma cells and active macrophages in thymus. There is a tendency to normalization of the parathyroid structure on the 30th day after administration of cyclophosphamide and reduction of mitotic activity of lymphocytes in thymus, which points to the development of involution process.

Conclusions: This data can be successfully extrapolated from experimental animals to humans.

KEY WORDS: Immunosuppression, Parathyroid glands, Thymus, Ultrastructure, Cyclophosphamide

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INTRODUCTION

The concept of the parathyroid glands' structure has been formed during hundreds of years due to the development of microscopic technique and biological sciences [1]. Modern technologies have helped to obtain information about the ultramicroscopic structure of the parathyroid glands and their numerous compounds at different levels of the organization [2]. However, there are plenty of questions, the answers to which have not yet been found.

Parathyroid glands are aimed at sustaining one of the main components of the normal functioning of the body - calcium [3]. The interaction of the nervous, immune and endocrine systems plays an essential role in the homeostasis maintaining [4]. On the one hand, hormones affect the immune reaction rate; on the other hand, the activity of immunocompetent cells depends on the endocrine status of the organism [5]. This is a reason to assume that

the disturbance of one of the components of this complex status can lead to the imbalance of others. Moreover, *the interaction between the immune system and bony tissue is beyond the doubt* [6]. However, the morphogenetic influence of the immune system on the parathyroid glands *has not been extensively studied yet*. Therefore, in the case of treatment of patients with bone and joint pathology by immunotropic medications the effect of this group of drugs on the condition of the parathyroid glands must be taken into account.

Recent studies show that different types of immunotropic medicines have a significant role in the reducing endocrine diseases' risk factors. The parathyroid glands have a pronounced ability to rebuilding of their structure under the influence of various endogenous and exogenous factors [7,8]. However, the authors mostly focus on immunological and immunohistochemical studies of the parathyroid

glands while the morphological aspects remain practically unexplored [9].

The thymus is a primary lymphoid organ that provides the microenvironment required for the development of T-lymphocytes. Many investigators have reported that thymus actively respond to a huge variety of exogenic and endogenic factors [10-12]. Therefore, one of the most promising directions in the problem solving of maintaining homeostasis is the study of the immune system due to the influence of immunotropic medications [13-14]. In the last few decades, chemotherapeutic drugs are widely used in healthcare settings as well as in the treatment community of cancers and other diseases. Unfortunately, acute and chronic dosing of chemotherapeutic agents is associated with numerous side effects. Cyclophosphamide has wide application as first-line drugs in cancer chemotherapy for various tumors that is why a detailed study of the drug's effect on individual cell populations and the timing of their recovery under various administration schemes is of vast interest for science and practice [15].

Proceeding from all above-mentioned electron microscopic study of the immune and endocrine system organs' structural features is essential for the development of new approaches for effective immunocorrection of the immune and endocrine systems [16-17].

THE AIM

This research aimed to investigate the ultramicroscopic changes that occur in the parathyroid glands and thymus of male rats after cyclophosphamide administration.

MATERIALS AND METHODS

ANIMALS

The following experimental work was conducted on 24 WAG matured male rats with initial body weight 180-200 g. White rats were used in the experiment because the structure of their organs of the immune and endocrine systems is not fundamentally different from those of humans. Keeping and manipulation of animals were carried out in compliance with national and world norms of bioethics [18, 19]. Experimental protocols were approved by the ethical committee of the Faculty of Medicine, Kharkiv National Medical University.

EXPERIMENTAL DESIGN

Rodents were randomly divided into two groups. *The first group served as a control* and was provided 0.9% soluble sodium chloride. The second group of animals received cyclophosphamide in a dosage 200 mg/kg of body weight of animal by intramuscular single injection. Rats used in the study were culled by cervical dislocation of the neck and tissue samples were collected on the 7th and 30th day after the injection. The parathyroid glands were extracted according to the original method, which has been described in detail

in the patent [20]. The thymus was dissected with adjacent adipose and connective tissue in order to save its structure for further investigation.

ELECTRON MICROSCOPY

Materials for electron microscopy studies were further immersed in Karnovsky's fixative, postfixed in 1% osmium tetroxide for 1 hour, dehydrated in an increasing ethanol series, infiltrated and embedded in EponAraldide resin. Ultrathin sections were cut with UMTP-4 ultramicrotome. The ultrathin sections were mounted on copper grids, double-stained with uranyl acetate and lead citrate and observed in EM-125 transmission electron microscope (TEM).

RESULTS

The parenchyma of the parathyroid glands of the control group of animals is *represented mainly* by chief cells, which contain numerous membrane-limited secretory granules as well as usual cytoplasmic organelles. Chief cells play an essential role in calcium homeostasis by releasing the appropriate amount of parathyroid hormone to maintain normal blood calcium levels. Chief parathyrocytes are organized as *cords* or clusters of cells supported by a loose connective tissue in which capillaries are embedded. Membrane *interdigitations* are *well developed* and form extensive complexes. Desmosomes also occur, but less frequently than interdigitation. In addition, the chief cells are classified into light cells and dark cells showing different functional phases of a single cell type. It is presumed that individual cells undergo periods of active secretion followed by periods of physiologic rest.

The cytoplasm of active chief cells in the parathyroid glands of the control group animals is finely granular and contains an abundant narrow rough endoplasmic reticulum, numerous dictyosomes of Golgi apparatus and occasional granules of glycogen, numerous secretory granules, and rare cilia. The nuclei are oval or spherical with occasional shallow invaginations. Inactive chief cells contain rare cisterns of rough endoplasmic reticulum and small Golgi apparatus, few secretory granules, and abundant glycogen. Lipid inclusions are membrane-limited and they are also prominent in many of inactive chief cells (Fig. 1).

The oxyphilic parathyrocytes derived from chief cells as aging or some metabolic derangements. They are observed either singly or in small groups between chief cells. Electron microscopy of the control series rats has shown that oxyphils are found in less number. The size of oxyphils is larger than size of chief cells. The cytoplasm of oxyphils is filled with numerous rod-shaped mitochondria. Organelles involved in protein synthesis are reduced to a minimum. Glycogen and lipid droplets are found in almost all cells, in some, there are single secretory granules.

On the 7th day after immunosuppression, most of parathyrocytes have irregular shape, clear boundaries and well-defined intercellular contacts. Active chief cells are fewer than in rats of the control group. Some cells shrink and contain

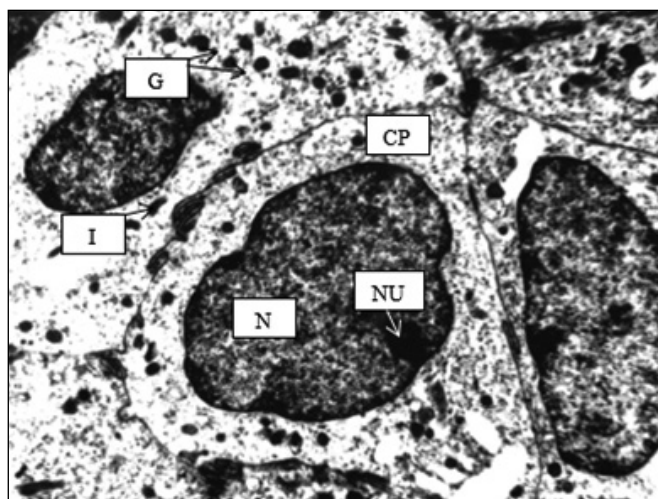


Fig. 1. Parathyroid gland of the control rat (7th day). TEM. Magnification x8000. CP – chief parathyrocyte, N – nucleus, NU- nucleolus, I – interdigitation, G – secretory granules.

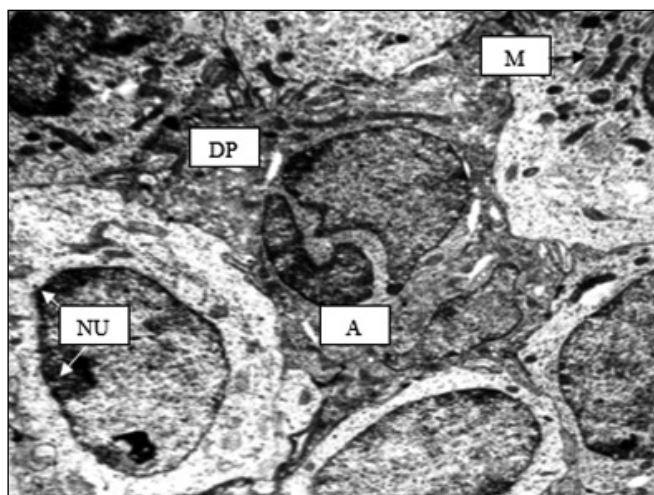


Fig. 2. Parathyroid gland of the experimental rat (7th day). TEM. Magnification x8000. DP – dark parathyrocyte, A – signs of apoptosis, NU- nucleolus, M – mitochondria.

signs of apoptosis. The nuclei of chief parathyrocytes are shifted to the periphery. They are irregular in shape and contain deep wavy invaginations, chromatin condenses. The electronic density of the nuclei increases in comparison with the control group of rats. The chromatin is located in the form of clumps on the periphery of the nucleus.

The cytoplasm of the chief cells contains poorly developed organelles and infrequent secretory granules. The Golgi apparatus is small and relatively inconspicuous. There are numerous lipid droplets and lysosomes. The number of mitochondria increases; they have a bean-shaped or oblong form with deep transverse cristae and an increased electronic density of their matrix. The capillaries are distended, *slightly curved*, and *narrowed* along the whole length. Additionally, some capillaries are marked with erythrocyte aggregations (Fig. 2).

Lastly, the oxyphills increase dramatically in number on the 7th day after immunosuppression. General structure of the oxyphills has no significant changes in comparison with control rats.

On the 30th day after the administration of cyclophosphamide to rats, the morphological pattern of the parathyroid glands at the ultramicroscopic level of the organization is different. At the same time, there is a tendency to normalization of the shape of cells and their nuclei. In the field of vision there are single dark cells. Some parathyrocytes have electronically dense nuclei with invaginations. The number of secretory granules in the cytoplasm is visually reduced in comparison with control animals. Lipid droplets of various electron densities are located on the periphery of parathyrocytes. There are single blood capillaries with a smaller diameter than in the slides of the control animals (Fig. 3).

The cellular composition of the cortex of the intact matured rats' thymus is represented by two main populations - lymphocytes and REC (reticuloepithelial cells). Lymphocytes of the control group animals' thymus have a regular

rounded or slightly elongated shape with a smooth outer surface and characterized by high nuclear-cytoplasmic ratio. Small lymphocytes predominated in the inner layer of the thymus cortex. Their large, rounded nuclei are localized somewhat eccentrically, sometimes are invaginated, and have compact chromatin. The narrow electron-enlightened cytoplasm contains single spherical mitochondria and many free ribosomes.

Well-developed agranular endoplasmic reticulum is represented mainly by tubules and cisterns. The elements of the smooth endoplasmic reticulum are connected with the cisterns of the granular endoplasmic reticulum and the cell membrane. The mitochondrial matrix of lymphocytes is characterized by a significant electron-optical density. The lysosomal apparatus is represented by primary lysosomes, secondary lysosomes, and residual bodies.

The medium-sized lymphocytes were detected, the nuclei of some of them had 2 nucleoli. In the subcapsular zone of the thymus, lymphoblasts, the precursors of T lymphocytes, were detected. In subcapsular and, less, in the cortical zones are located mitotically dividing cells, a characteristic feature of which is the presence of dilated tubules of the endoplasmic reticulum near the plasmalemma (Fig. 4).

Within 7 days after the administration of cyclophosphamide, the ultrastructure of lymphocytes has a typical structure: chromatin in the form of lumps located along the periphery of the nucleus, a thin cytoplasm contains ribosomes and polysomes, sometimes there occur mitochondria.

Along with this, a large number of dark lymphocytes appear, representing a special pool of cells. They have smaller dimensions, vague boundaries, an electronically dense nucleus with condensed chromatin, a narrow cytoplasm rim around it with practically indistinguishable organelles.

Characteristic feature of the ultramicroscopic structure of the thymus after the application of cyclophosphamide is nu-

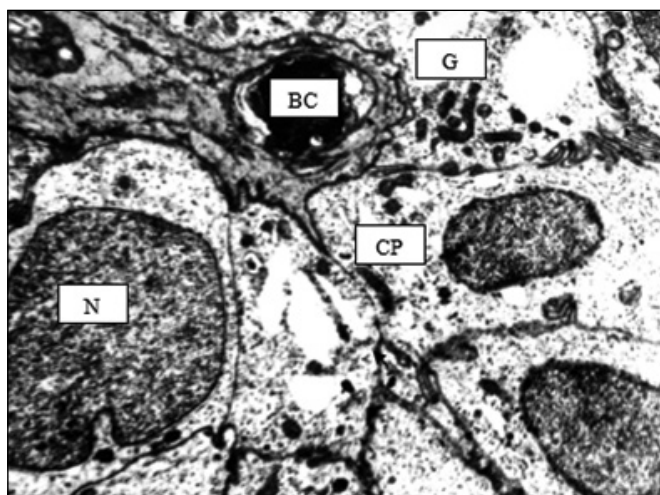


Fig. 3. Parathyroid gland of the experimental rat (30th day). TEM. Magnification x8000.
CP – chief parathyrocyte, N- nucleus, G – secretory granules, BC – blood capillary.

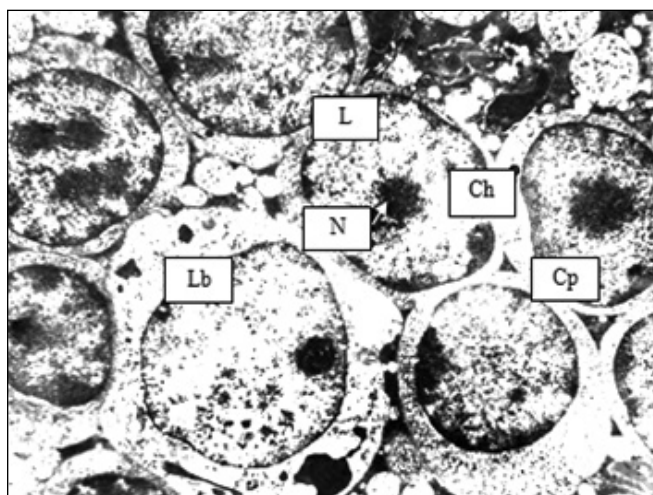


Fig. 4. Thymus cortex of the control rat (7th day). TEM. Magnification x8000.
L- lymphocyte, Lb – lymphoblast, N – nucleus of the lymphocyte, Ch – chromatin, Cp – cytoplasm.

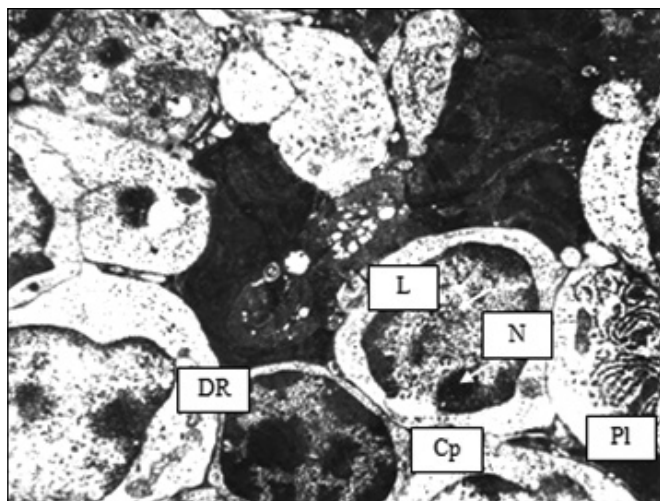


Fig. 5. Thymus cortex of the experimental rat (7th day). TEM. Magnification x8000.
L – lymphocyte, DR – dark lymphocyte, PI – plasma cell, N- nucleus, Cp – cytoplasm.

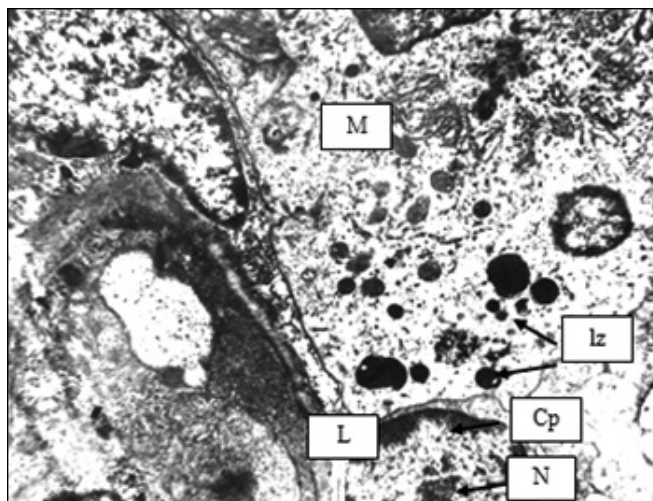


Fig. 6. Thymus cortex of the experimental rat (30th day). TEM. Magnification x8000.
L – lymphocyte, M – macrophage, Lz – lysosomes, N- nucleus, Cp – cytoplasm.

merous plasma cells, apparently migrating to the parenchyma of the organ from perivascular spaces. They have a rounded shape, large size, smooth contours. Nucleus of various sizes, irregular shape, invaginated, often located eccentrically, electronically dense, contain condensed chromatin. The cytoplasm is filled with dilated tubules of a granular endoplasmic reticulum with electronically dense contents. Only a light perinuclear region, poor in organelles, is preserved. Sometimes rounded mitochondria with destroyed cristae occur.

Many active macrophages are noted, as can be seen from the presence of secondary lysosomes and residual bodies in their cytoplasm. This picture is similar to electron microscopic changes in the structure of the thymus in involution, which indicates its active response to the use of an immunosuppressant (Fig. 5).

30 days after the administration of cyclophosphamide, the general features of the ultramicroscopic structure of the thymus are similar to those of the control group of animals. The presence of a large number of plasma cells is observed, the distinctive feature of which is the presence of dilated and deformed tubules of the granular endoplasmic reticulum filled with electron-dense contents, which indicates a disturbance of the metabolism of immunoglobulins with a shift towards intracellular accumulation. Distinctively seen their clock-face chromatin pattern where small dots symmetrically rim the nuclear membrane according to numbers on a clock.

Within eyeshot, the number of large macrophages increases, a large round or oval nucleus with a predominance of decondensed chromatin, numerous mitochondria, and lysosomes in

the cytoplasm. Sometimes appear eosinophils and neutrophils of a typical structure. There is an increase in the number of connective tissue elements of the thymus stroma, in particular, collagen fibers, in comparison with the control group of animals (Fig. 6).

DISCUSSION

The principal change in the parathyroid glands of experimental rats compared to control animals of a similar age is the transformation of the population of active chief cells into inactive cells in less active stage of their secretory cycle. Ralph Y.C. reported that after the administration of methotrexatum active chief cells are fewer than in rats of the control group [21]. Moreover, cells were characterized by an electron-transparent cytoplasm which contained few organelles and secretory granules. A similar condition was reported by Toneto M. G. et al. [22]

Fournier C. et al. suggested that administration of high doses of cyclophosphamide is based on its ability to inhibit the proliferation of any metabolically active cells [15]. This allows us to explain the tendency to a decrease in all the organelles that are involved in protein synthesis. Kafetzis I. D. observed that the initial response (within 24 hours) to parathyroid suppression caused by heavy metals in rabbits was depletion of secretory granules, however, chronically stimulated chief cells had similar numbers of granules to cells in control rabbits [1].

Ge Q. and Zhao Y. were the first who discover that the administration of immunosuppressive drugs could lead to transformation of the nuclei and formation of the numerous invaginations of the nuclear envelope [21].

Avilova et. al. investigated the impact of xenobiotics on the thymus ultramicroscopic structure and revealed similar immunosuppressive effect as during administration of cyclophosphamide. Was observed thymocyte loss with more active process of involution due to activation of apoptosis and appearance of degenerative changes [10].

In research of Elmore S. [11] thymus has been shown to be a sensitive target organ following exposure to immunotoxicants and endogenous corticosteroids, and a decrease in size or weight is often one of the first noted measures of compound-induced effects with cortical lymphocytes being especially susceptible. Therefore, changes in thymus histopathology and architecture are considered to be of particular relevance for immunotoxicity screening.

CONCLUSIONS

1. The results showed that cyclophosphamide exposure caused marked ultramicroscopic changes in the rats' parathyroid glands and thymus. On the 7th day after the administration of cyclophosphamide parenchyma of the parathyroid glands consists of mainly inactive chief cells. The nuclei of the parathyrocytes are shifted to the periphery and have deep wavy invaginations. Amount of the organelles that participate in the protein synthesis is reduced to a minimum. The outcome of thymus investigation allows to trace clearly the propensity to accidental

involution development: there is a large number of dark lymphocytes, plasmocytes, macrophages.

2. There is a tendency to normalization of the shape of parathyrocytes and their nuclei on the 30th day after the administration of cyclophosphamide to rats. In the field of vision, there are active dark chief cells, which may indicate the resumption of active synthesis of the parathormone. Reduction of lymphocytes mitotic activity, the presence of a large number of plasmocytes and macrophages with a characteristic structure, proliferation of connective tissue indicates the development of involutive processes in the thymus.
3. Investigating dynamic ultrastructural changes of parathyroid gland and thymus indicating intensive reactions of this organs to exogenous effects, cause huge interest in further study of their structure in immunostimulation, the results of which will be reflected in our future publications.
4. The results of a comprehensive evaluation of the morphological structure of rats' parathyroid glands and thymus at the ultramicroscopic level complement and extend the theoretical understanding of the interaction between endocrine and immune system. Revealed changes may be related to the generality of embryonic development of the parathyroid gland and thymus.

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