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MORPHOLOGICAL INVESTIGATIONS OF THYROTROPIC ENDOCRINE CELLS OF RATS ADENOHYPOPHYSIS UNDER EXPERIMENTAL IMMUNOSUPPRESSION

ABSTRACT

Anterior pituitary hormones can change the metabolic activity and function of immune cells; have expressed influence on the immune responses. However, the immune system is able to influence on the endocrine and nervous systems by a principle of feedback. The concept of cellular and tissue mechanisms that underlie the reorganization of the anterior pituitary at immunosuppression, is necessary for a possible correction of the therapy in patients receiving cytostatic drugs for the treatment of autoimmune diseases, cancer, in organ and tissue transplantation. The study of adaptive reserves of the adenohypophysis, depending on age is also actual.

The aim of research was to study the features of structural reorganization of thyrotropic endocrine cells of distal adenohypophysis of rats of puberty at experimental immunosuppression.

Study was carried out on 60 white outbred male rats of puberty. Immunosuppressive condition was modelled in animals by administration of a cytostatic drug cyclophosphamide.

The material was studied by light, electron microscopy, morphometry using the computer appliance, which includes a microscope the Olympus CX-41 and a digital camera Olympus.

It was established that in 1 and 7 days after administration of cyclophosphamide structural changes of thyrotropic endocrine cells show a decrease in their functional activity, which is accompanied by a decrease of the average area of the cells and their nuclei with the simultaneous increase of nuclear-cytoplasmic ratio, a decrease of the relative nucleoli area, relative mitochondria area, and relative secretory granules area.

At 15 and 30 days there are expressed destructive-dystrophic changes of nuclear and cytoplasmic structures of the endocrine cells, accompanied by a decrease in hormone production.

In 60 days after administration of cyclophosphamide despite the restoration of ultrastructure of significant number of cells their secretory activity is still reduced since the percentage of relative area occupied by secretory granule does not reach the control level.

Thus, administration of cyclophosphamide to test animals of puberty causes an active response of thyrotropic endocrine cells of anterior pituitary, as evidenced by the development of polymorphic morphological changes, the nature and the severity of which depends on the terms after administration of cytostatic.

Keywords: rats, anterior pituitary, thyrotropic endocrine cells, immunosuppression.

Background. Lately it was established that anterior pituitary hormones can change the metabolic activity and function of immune cells, have expressed influence on the immune responses [7]. However, the immune system is able to influence on the endocrine and nervous systems by a principle of feedback [1]. Numerous studies have shown that the anterior pituitary is extremely sensitive to the influence of various exogenous and endogenous factors [2, 3, 4]. The concept of cellular and tissue mechanisms that underlie the reorganization of the anterior pituitary at immunosuppression, is necessary for a possible correction of the therapy in patients receiving cytostatic drugs for the treatment of autoimmune diseases, cancer, in organ and tissue transplantation.

Today in scientific literature is almost no information about the features of cytoarchitectonics, ultramicroscopic structure of the anterior pituitary at immunosuppressive condition of the organism. The study of adaptive reserves of the adenohypophysis, depending on age is also actual.

The aim of research was to study the features of structural reorganization of thyrotropic endocrine cells of

anterior pituitary of rats of puberty at experimental immunosuppression.

MATERIALS AND METHODS

Study was carried out on 60 white outbred male rats of puberty. Immunosuppressive condition was modeled in animals by administration of a cytostatic drug cyclophosphamide at a dose which leads to immune depression (200 mg/kg of body weight) [5]. The control rats were given the same volume of 0.9% of sodium chloride solution only. Animals were sacrificed (by decapitation under ether anesthesia) at 1, 7, 15, 30 and 60 days after the injection of cyclophosphamide. Material was investigated by light, electron microscopy, morphometry using the hardware-software complex, which includes a microscope Olympus CX-41 and a digital camera Olympus SP 500UZ with a computer program «Morpholog» [6]. Morphometric studies of distal part of anterior pituitary included the calculation of percentage of different types of endocrine cells, the average cell area, average nuclear area, average cytoplasm area (μm^2), calculation of the nucleo-cytoplasmatic ratio (NCR), the relative nucleoli area, relative mitochondria area, relative secretory granules (active and inactive) area (%) of thyrotropic endocrine cells. Active granules regarded as full, with typical structure, inactive - light, devoid of electron dense substance, as well as partially or completely destroyed. Granules activity index was calculated by the formula: relative active secretory granules area / relative inactive secretory granules area. Statistical processing of data has carried out by the method of variation statistics using Student's t-test. The results were statistically processed using the software package Statistica 6.0 for Windows. Values were considered statistically significant when p was <0.05 (in the text indicated by *).

RESULTS AND DISCUSSION

At the study of the distal part of anterior pituitary of male rats of puberty by light microscopy was established that the cells are characterized by polygonal shape and larger sizes compared with other endocrine cells. Thyrotropic endocrine cells often form the groups consisting of several cells arranged diffusely throughout the gland, but most often they can be observed in the central parts the anterior pituitary near the blood capillaries. In the cytoplasm eccentrically located nucleus, distinct basophilic granulations are determined.

Quantitative analysis of the population of thyrotropic endocrine cells did not reveal significant changes of their number throughout the observation period after the administration of cyclophosphamide.

However, injection of cytostatic drug causes a statistically significant decrease in the average area of thyrotropic cells and their nuclei while increasing of nucleocytoplasmatic ratio in 1, 7, 15 and 30 days of observation (fig. 1).

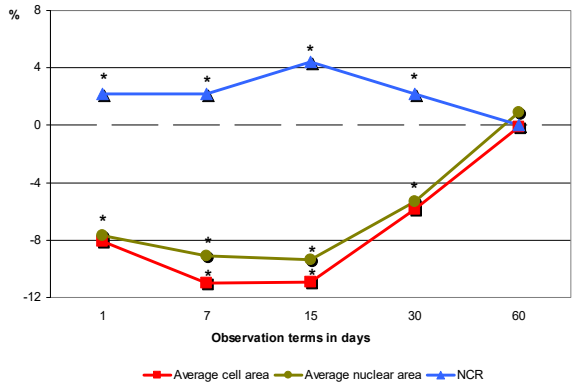

Fig. 1. Dynamic changes in the average cell area, average nuclear area, and NCR (%) of thyrotropic endocrine cells of anterior pituitary of rats of puberty after administration of cyclophosphamide. * p <0.05 (see abbreviations in the text.)

The electron microscopic study found that in one day after administration of the drug in most of thyrotropic cells enlightenment of cytoplasm determined due to a minor amount of the organelles and partial or complete degranulation, which manifested in a decrease of the cytoplasm relative area occupied by secretory granules. Granules activity index is also decreased compared with the data of control group (fig. 2). These signs may indicate a decrease in the functional

activity of thyrotropic endocrine cells in response to cyclophosphamide. There is also a decrease of the relative nucleoli area (1-15 days) and relative mitochondria area in 1-30 days (fig. 3).

Progressive degenerative changes in nuclear and cytoplasmic cell structures, accompanied by a decrease in hormone production and inhibition of intracellular regeneration in the

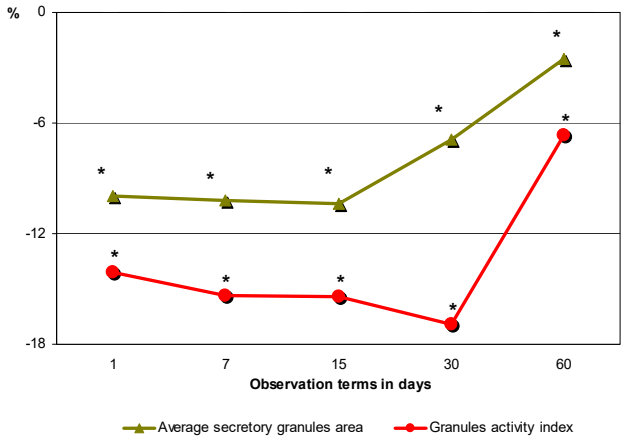

Fig. 2. Dynamic changes in the relative secretory granules area and granules activity index (%) of thyrotropic endocrine cells of anterior pituitary of rats of puberty after administration of cyclophosphamide. * p <0.05.

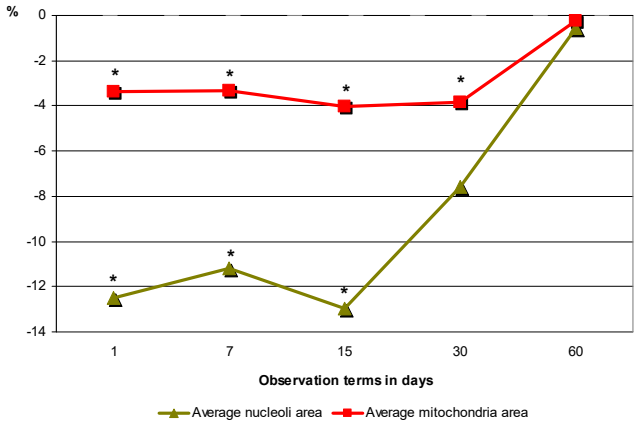

Fig. 3. Dynamic changes in the relative nucleoli area and relative mitochondria area (%) of thyrotropic endocrine cells of anterior pituitary of rats of puberty after administration of cyclophosphamide. * p <0.05.

thyrotropic endocrine cells develop at 7 and 15 day. Typical for ultrastructure of a number of cells in these observation terms is low content of organelles and progressive vacuolization of the cytoplasm. Content of elements of granular endoplasmic reticulum in these cells is greatly reduced; some fragments of cisterns are expanded, are transformed into irregularly shaped vacuoles and contain a small amount of ribosomes on its surface. Extended cistern of remaining dictyosomes of Golgi complex are also involved in the formation of vacuoles of cytoplasm. Reducing the number of mitochondria is accompanied by their significant swelling and destruction of cristae. There are partially or completely damaged mitochondria. The cytoplasm contains a small amount of ribosomes and polysomes, there are isolated lysosomes. Small secretory granules are located as intermittent single-row chains along plasmolemma. By the 30th day after administration of cyclophosphamide progressive vacuolization of the cytoplasm of some thyrotropic endocrine cells results in their transformation into thyroidectomy cells (fig. 4).

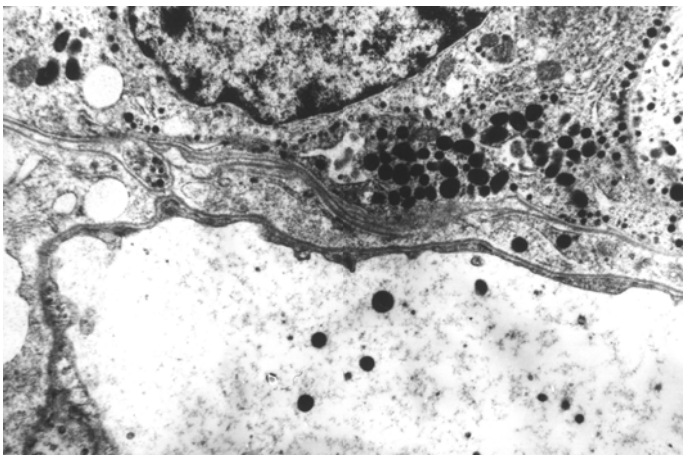

Fig. 4. The distal part of the anterior pituitary of rat of puberty at 30 days after administration of cyclophosphamide: thyroidectomy cell. Magnification x12000.

The merger of small and large cisterns rough endoplasmic reticulum and Golgi complex leads to formation of large cavity filled with flaked material and containing electron dense granules. Along with large vacuoles, in the rest of the cytoplasm revealed many small and medium-sized vacuoles, in which also there are granules. At 60 days after the administration of cyclophosphamide the cells with typical structure of thyrotropocytes are dominated.

CONCLUSION

Introduction of cyclophosphamide to experimental animals of puberty causes an active response of thyrotropic endocrine cells of anterior pituitary, as evidenced by development of polymorphic morphological changes, the nature and the severity of which depends on the observation terms after administration of cytostatic drug.

In the early term of observation (1 and 7 days) after administration of cytostatic structural changes of cells show a decrease in their functional activity, which is accompanied by a decrease of the average cell area, average nuclear area with the simultaneous increase in nuclear-cytoplasmic ratio, a decrease in the relative nucleoli area, relative mitochondria area, and relative secretory granules area.

By the 15th and 30th days there are expressed destructive-dystrophic changes of nuclear and cytoplasmic structures of thyrotropocytes associated with decreased hormone production.

In 60 days after administration of cyclophosphamide despite restoration ultrastructure significant number of cells their secretory activity is still reduced, since percentage of the relative area occupied by the secretory granules does not reach the control level.

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