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STRUCTURAL FEATURES OF A RATS' KIDNEY NEPHRON AFTER THYROIDECTOMY AND PHARMACOLOGICAL CORRECTION

Abstract. *We have studied the structural features of a rats' kidney nephron after total thyroidectomy without hypothyroidism correction and after the L-thyroxine single-drug therapy. The results showed that 100 days after thyroidectomy in the kidney of the rats, which were administered the replacement therapy with L-thyroxine, there was an incomplete resumption of the ultrastructure and some symptoms of degenerative and destructive processes that indicates that the replacement single-drug therapy does not prevent the development of destructive changes in all components of a kidney nephron, but significantly slows their progress.*
Key words: *nephron, hypothyroidism, L- thyroxin, rats, thyreoidectomy.*

Introduction. The relevance of hypothyroidism problems in clinical practice of doctors of various specialties is due to the fact that the deficiency of thyroid hormones necessary for normal functioning of virtually each cell, severe disturbances in all the organs and systems develop, and the prevalence of this disease is about 2%, and in some age groups (elderly women) can reach 6-8% [1, 2]. Therefore, the problem of preventing the development of pathological changes in hypothyroidism acquires an increasing importance. The replacement pharmacotherapy based on a treatment with L-thyroxine, does not provide a full quality of life of these patients [3,4]. Experimental studies in recent years have shown that a treatment of thyroidectomized rats with L-thyroxine does not prevent the formation of morphological and functional changes in the heart, ovaries, brain, and only prolongs the process [5,6]. There are not any data in the literature about the structural changes in the kidney in hypothyroidism and after its correction with L-thyroxine.

Objective: to study the ultrastructure of the kidney nephron after total thyroidectomy in the rats that did not receive replacement therapy and after the single-drug therapy with L-thyroxine.

Materials and methods. The study was conducted on 20 white outbred female rats weighing 180-200 g. The experimental animals were kept and used in accordance with "common ethical principles of animal experiments." The animals were simulated a condition of overt hypothyroidism through the total thyroidectomy [7]. The thyroidectomized animals were administered L-thyroxine (Pharmak, Ukraine), at a dose of 10 mg / kg per os. Their hypothyroidism was controlled by a determination of free thyroxine rate in the blood plasma of animals using ELISA. The animals were decapitated 100 days after the operation under light ether anesthesia according to the requirements for the removal of animals from an experiment.

Portions of the renal cortex, fixed in 2.5% glutaraldehyde solution buffered with phosphate, with final fixation in 1% OsO4 solution and treated in accordance with the conventional technique served as a material for electron microscopic studies. The morphometric analyze was performed using a semi-automated device for processing graphic images. The resulting digital data were processed by the method of variation statistics using Student's t test. The results were considered reliable at $P < 0.05$.

Results and discussion. A prolonged deficiency of thyroid hormones causes changes in all structural components of the nephron: there are only small and medium-size renal corpuscles, as a result, the average area of a cut of the renal corpuscles in the choroid glomus and the space between the visceral and parietal layers of the capsule is smaller than in the controls (Table 1). Excess of the parameters in volume and quantitative capillary density over the control figures against the background of a significant decrease in renal corpuscle sizes, is only possible with the proportional reduction of the volume and the number of capillaries themselves. The area of the capillaries is also statistically lower (Table 1).

Table 1

Morphometric parameters that characterize the changes in the glomerular apparatus of the kidneys of rats 100 days after thyroidectomy (I) and single-drug therapy with L- thyroxine (II)

	Area of the RC * 10^2 micron^2	Area of the CG, * 10^2 micron^2	Area of the space, * 10^2 micron^2	Volume density of capillaries in the RC, %	Quantitive density of capillaries in the RC* 10^{-6} micron^3	Area of capillaries * 10^2micron^2	Shape factor in capillaries
control	598,56±38,28	524,03±41,14	74,52±6,8	16,9±0,90	13,69±1,66	3,15±0,17	0,73±0,01
I	367,20±42,42 *	325,19±39,37 *	42,00±5,8 *	22,02±3,06*	39,35±2,95*	1,84±0,16 *	0,63±0,01
II	432,59±37,00*	329,22±32,46*	103,37±11,4*	22,37±1,22*	23,61±3,55*	2,45±0,43*	0,68±0,01

The worn areas of endothelial cells with diaphragmed and non-diaphragmed fenestra alternate with the protrusions of the cytoplasm, which frequently take the form of microclasmatose appendixes. The electron-dark material is stored in the the electron-dense cytoplasm. The basal membrane generally maintains a uniform thickness, and in places of capillary bifurcation where the mesangial cells are located in the control group, it is thickened. The cytoplasm of the mesangial cells is electron-dense and the nuclei are rare.

The shape and contents of the podocytes processes change. Most cytotrabecula acquire an increased electron density, the number of their organelles reduces; mitochondria are usually swollen, with visualised matrix and crista. Myosin-positive deposits increase in the cytoplasm, which is typical for cytopodies. The quantitative parameters indicate substantial restructuring in podocytes too: the average area of cytotrabecula and cytopodies reduces in comparison with the control, they become more elongated, as evidenced by the shape factor (Table 2).

The number of cytotrabecula and the volume that they occupy in the volume unit of the glomerulus, are lower than in the control group (Table 2). The number of cytopodies and their volume in the volume unit of the glomerulus are not different from the control values (Table 2).

But considering the fact that the average glomerular area values decrease in this period of observation, we can talk about a significant decrease in the total number of cytotrabecula and cytopodies. The prevalence of degenerated podocytes and changing their quantitative parameters are indicative of the death of some of these cells. It is confirmed by the fact that the number of glomerular capillaries decreases in the same period of observation.

Most of the proximal tubules have fragmented and desquamated microvilli. Almost all the epithelial cells that line the tubules, have lost their inherent form; basal labyrinths do not have a parallel orientation; mitochondria are randomly located in the cytoplasm and their number, as well as that of the biosynthetic

Table 2

Changes in quantitative indices of rats' podocytes 100 days after thyroidectomy (I) and single-drug therapy with L-thyroxine (II)

	Volume density of cytotrabecula in the RC, %	Quantitative density of cytotrabecula in the RC, * 10^{-2}/microns^3	Area of cytotrabecula microns^2	Shape factor in cytotrabecula
control	10,21±1,22	1,14±0,19	6,29±1,05	0,48±0,01
I	7,66±0,87	0,58±0,20*	4,73±1,02*	0,35±0,01*
II	10,39±2,42	1,06±0,57	5,70±1,03	0,55±0,01*
	Volume density of cytopodies in the RC, %	Quantitative density of cytopodies in the RC, * 10^{-2}/ microns^3	Area of cytopodies, microns^2	Shape factor in cytopodies
control	3,40±0,23	24,3±5,6	0,15±0,01	0,47±0,01
I	3, 26±0,16	25,22±2,90	0,11±0,006*	0,42±0,01
II	3,47±0,53	25,59±5,04	0,15±0,008	0,53±0,01

organelles reduce. Dark and cleared lysosomes are common in the cells, whereas other structures involved in the transcellular reabsorption of proteins are observed in a small number. There are some tubules, where epithelial cells are in different stages of apoptosis up to the formation of apoptotic bodies. The basement membrane varies in thickness: thinned sections alternate with sections where it is thickened and sometimes destructively changed.

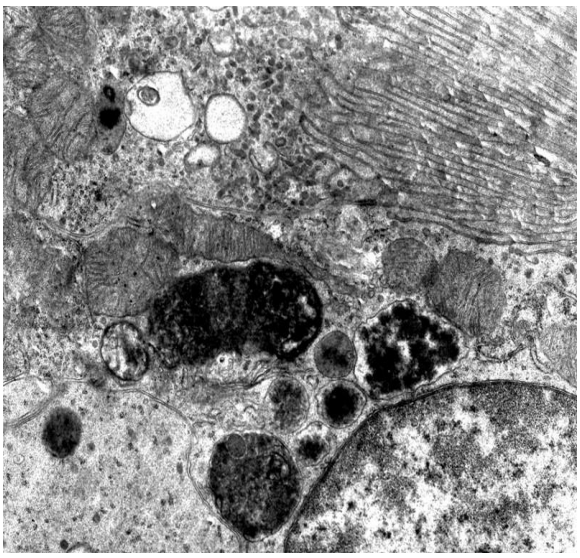
In the distal tubules the epithelial cells also change the ultrastructure, but the severity and extent of these changes is less pronounced than in the proximal ones. The changes are manifested primarily by vacuolization of cytoplasm, by penetration of basal plasmalemma invaginations deep into the cytoplasm and expansion of these invaginations so that the cell seems to be split into pieces. It should be noted that the damaged cells both of the distal tubules and the proximal ones are often located in a cluster way.

100 days after thyroidectomy the rats receiving L-replacement therapy with thyroxine had an average area of the renal corpuscles statistically higher than in the animals without corrective therapy, but it did not reach the control values (Table 1). The choroid glomus area remains statistically the same type with the value in rats treated with L-replacement therapy with thyroxine, whereas the space area exceeds the same figure in the controls (Table 1). Thus, an increase in the area of the renal corpuscles is due to the increase in the size of the space between the parietal and visceral layers of the capsule. Like the area of the renal corpuscle, the average capillary area value did not differ in the animals either (Table 1). An excess in the value of quantitive density of capillaries in the animals treated with replacement therapy with L-thyroxine, was most likely due to differences in renal corpuscles areas in the two groups, but not with changes in the total number of capillaries.

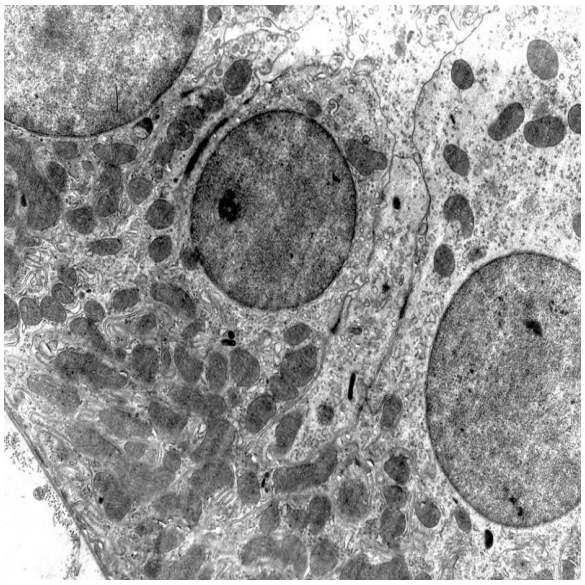
The lumen of most glomerular capillaries is open, the coagulated plasma is practically not seen. There is a cytoplasm protrusion in the worn peripheral areas of endothelial cells. The fenestra are unevenly placed in the cells: continuous endothelium is alternated with areas with a large number of fenestrae. The changes in the glomerular basement membrane are not visualized. Mesangial cells do not have any significant damage. The cytoplasm is of a moderate electron density, preservation of organelles is more pronounced, and the number of myosin-positive matter is less in comparison with the previous experimental group in the body of the podocytes and their cytotrabecula. The size and the quantitative density of cytotrabecula do not differ from the control values, whereas the form factor indicates their differences both from the control animals, and from those with hypothyroidism (Table 2). They are not only less flattened than in the latter ones but more oval than in the control group. It is due to the fact that some cytotrabecula with clear cytoplasm protrude into the urinary space and seem to contain some edematous fluid.

It should also be noted that adjacent podocytes are located rather close to each other and, as a result, the urinary space is narrowed. There are no epithelial cells in the proximal tubules in the final stages of apoptosis. The cells contact closely between each other via the slit and tight contacts. The microvilli which are in contact with endocytic structures of various shape, size and density are well preserved in them. Further, in the basal direction lysosomes are located. Basal labyrinths do not keep the parallelism in all the cells, in some places they are oriented along the basement membrane, so are the mitochondria. The latter are located throughout the cytoplasm, they vary in size, have a moderate electron density matrix, clearly structured outer membrane and cristae. The number of the latter is slightly decreased compared to the control (Figure). In the distal tubules the epithelial cells have signs of active biosynthetic processes: they contain a rounded nucleus with evenly distributed chromatin, a significant number of mitochondria, Golgi tubules, secretory granules. The latter ones, unlike those in the hypothyroid rats are not increased in size (Figure B). Dense contacts are mostly located close to the apical surface and have a great extent in some places. The difference between these cells and those in the control is thei small amount and sometimes complete disappearance of microvilli as well as a disorientation of basal protrusions.

Some areas of thickening, destruction, edema of the basal membrane in the tubules of the nephron, as well as cellular detritus in the interstitial space are indicative of an incomplete



A



B

Figure. The kidney of a thyroidectomized rat, treated with L-thyroxine. A – A fragment of the proximal tubule. B – A frafment of the distal tubule. Electron microscopy micrographs. Magn.: A. – 24000, B. – 6000.

resumption of the ultrastructure and the presence of dystrophic and destructive processes in the kidney after monotherapy.

Conclusions. 100 days after thyroidectomy in rats which received replacement therapy with L-thyroxine, in the kidney there is an incomplete resumption of the ultrastructure and the presence of dystrophic and destructive processes. The results reveal some of the mechanisms of functional disorders in the kidney with hypothyroidism even against replacement monotherapy and dictate the need for new treatment regimens.

Prospects for further research. In the future, it is advisable to study the ultrastructure of the kidney nephron after total thyroidectomy in rats which received integrated treatment with L-thyroxine and calcitonin.

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