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MORPHOFUNCTIONAL STATE AND MICROSTRUCTURE OF REGIONAL LYMPH NODES IN EXPERIMENTAL HYPOTHYROIDISM AND UNDER CORRECTION

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ABSTRACT

Lymph nodes play an important role in the life of humans and animals. In the modern concept, the lymph nodes are the main homeostatic organs of the internal environment of the body, the lymph nodes are part of the lymphatic bed, on the other hand, they are the organs of the lymphoid (immune) system.

Purpose: The work presents data on the morphofunctional state and microstructure of regional lymph nodes in experimental hypothyroidism and under correction.

Materials and Methods: Experimental hypothyroidism in animals was induced by *per os* administration of mercazolil at a dose of 20 mg per 100 g of body weight with drinking water for 21 days. To correct thyroid disorders, starting from the 22nd day of the experiment, the animals received for 30 days an iodine-containing dietary supplement "Renaissance Plus" balm in powder at the rate of 2 μg/100 g of body weight and a decoction of the roots of the white cinquefoil (*Potentilla alba L.)* 50 ml per 100 g per day with drinking water.

Results: The results of the study showed that in conditions of hypothyroidism, the lymph node becomes compacted with a decrease in the main structural and functional zones against the background of reduced lymphopoietic function. In the lymphoid nodule, there is a decrease of 1.43 times in the number of lymphoblasts, 1.56 times in the number of medium lymphocytes, 1.50 times in the number of small lymphocytes due to an increase of 1.29 times in the number of macrophages per unit area of the lymphoid nodule in the lymph node. There was a static decrease in the number of small lymphocytes in all structural and functional areas; the number of blasts decreases in the lymphoid nodule and increases in the paracortex and Billroth's strand; the number of macrophages increases in the lymphoid nodule, cerebral sinus, the number of mature plasma cells decreases in the paracortex and Billroth's strand, and there is also inhibition of cellular reactions in the lymph node, which reflects a decrease in the lymphopoietic function of immune activity and as a result of the immunosuppressive action of mercazolil.

Conclusions: After an administration of corrigents, the area of the medulla and paracortex was restored to

the control level and the ratio of cortical and medulla in the overall structure of the lymph node was leveled.

Keywords: hypothyroidism, correction, lymph nodes, cerebral sinus, paracortex.

INTRODUCTION

Lymph nodes play an important role in the life of humans and animals; they are specialized multifunctional lymphoid organs located along the lymphatic vessels and representing a common link between the two systems - lymphatic and lymphoid. On the one hand, the lymph nodes are part of the lymphatic bed, on the other hand, they are organs of the lymphoid (immune) system. The body has parietal and visceral lymph nodes. Parietal nodes are located along the course of the blood vessels. The visceral nodes of the parenchymal organs are located in the region of the portal, the lymph nodes of the tubular organs are located in their mesentery. Lymph nodes of white rats are located along the branches of the celiac artery and a number of veins: hepatic - near the portal vein of the liver, gastro-splenic, pancreatic and splenic - near the splenic vein [1-2].

Lymph nodes are the main homeostatic organs of the body's internal environment. In the modern concept of the lymphatic region, to determine the state of the organ drained by it, a great prognostic value is given to the structural and functional state of the lymph node. Lymph nodes are small organs that are distributed throughout the body and drain lymph fluid from tissues. Immune cells reside in the lymph nodes and examine the lymph for signs of infection. If the tissue is infected, the immune cells in the draining lymph node are activated and multiply. Lymph nodes are strategically located throughout the lymphatic system at key drainage points [3–4]. Lymph nodes are located in the loose connective tissue between the skin and muscles, often near large blood vessels. They have a connective tissue capsule with an admixture of smooth muscle fibers, which allows the node to contract and move the lymphatic fluid. The parenchyma of the lymph node is divided into cortex and medulla. The lymph nodes through which lymph flows from the internal organs are called visceral, the nodes located on the wall of the cavities are called parietal or somatic. Lymph nodes with a general type of structural organization have a dynamic change in structural and functional zones, which determines the features of immunomorphological reactions under the action of pathogenic and sanogenic environmental factors. In this case, there is a need for a morphological and functional assessment of the lymph nodes, since they are one of the elements of the overall protective system of the body. Lymph nodes, as an organ of the peripheral immune system, are the meeting point of antigens with immunocompetent cells, and are indicators of the state of the internal environment [5-7].

The purpose of the work: to study the morphofunctional picture of the regional lymph node in experimental hypothyroidism and under correction.

MATERIALS AND RESEARCH METHODS

The experiments were carried out on 80 linear Spraque Dawley (SD) male rats weighing 250 ± 3 g. The work was performed in accordance with ethical standards and recommendations for the humanist attitude of laboratory animals. All experiments were carried out in strict accordance with the rules developed and approved by the local ethical commission of the Kazakh National Medical University. S.D. Asfendiyarov (Minutes No. 7 (71) dated June 11, 2019), as well as with the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986). Feeding, the water schedule of rats was carried out according to the standard diet of the vivarium.

Animals were divided into three groups: 1) intact (control - 15 rats); rats of the 2nd (n=25) and 3rd (n=30) groups were modeled experimental hypothyroidism. For the development of experimental hypothyroidism, animals received mercazolil at a dose of 20 mg per 100 g of body weight with drinking water for 21 days [8]. To correct thyroid disorders, starting from the 22nd day of the experiment, the animals of the 3rd group received an iodine-containing biologically active additive "Renaissance Plus" balm in powder for 30 days at the rate of 2 μg/100 g of body weight and a decoction of the roots of cinquefoil (*Potentilla alba L.*) 50 ml per 100 g per day together with drinking water [9, 10].

Morphometric analysis was carried out using a morphometric grid, which was applied to a section of a lymph node. The intersections of the grid were counted for the entire section of the lymph node, as a whole and separately, for each of its structures - the capsule, cortical plateau, lymphoid nodules, paracortex, Billroth's strand and sinuses, recalculated as a percentage [11]. During cytoanalysis of the structure of lymph nodes, the number of cells in a standard area of 1600 μm^2 was counted with their differentiation into blasts, medium and small lymphocytes, plasma cells, macrophages, and others. [12]. The following structural and functional parameters were determined: volumetric density of the interfollicular space (stroma), including volumetric densities of the interstitium, blood and lymphatic vessels; bulk density of the follicular epithelium; bulk density of interfollicular epithelium; the bulk density of the colloid in the cavity of the follicles [13, 14]. Taking the cut area as 100%, we found the relative area of the capsule, marginal sinuses, lymphoid nodules with germinal centers, internodular zone, deep zone of the cortex, Billroth's strand, and cerebral sinuses. Cells were counted on sections in 5 visual fields: in the internodular zone, germinal

centers, Billroth's strand, and cerebral sinuses. Reference values for cells were used for some structural and functional zones. The ratio of the specific area of the cortex to the specific area of the medulla (cortical/medullary ratio - C/M) was calculated for the lymph nodes in each experimental group. Isolation of structural components and differentiation of cellular forms in the lymph nodes was carried out taking into account the international histological nomenclature [15].

RESEARCH RESULTS

After the administration of Mercazolil, we observed a decrease in the total area of the lymph node by 13.1% compared to the control group of animals. Experiments have shown that in hypothyroidism the cortical/medullary ratio decreases to 0.72 ± 0.01, which indicates the predominance of the medulla in the structure of the lymph node. In the medulla of the lymph nodes after the administration of Mercazolil, a statistically significant increase in the area of the cerebral sinus by 2.15 times was observed. An increase in the area of the cerebral sinus is associated with a decrease in the lymphoid parenchyma of the node, caused by taking Mercazolil.

The obtained data shows that hypothyroidism reduces the number of small lymphocytes in all structural and functional zones; the number of blasts decreases in the lymphoid nodule and increases in the paracortex and Billroth's strand; the number of macrophages increases in the lymphoid nodule, cerebral sinus, the number of mature plasma cells decreases in the paracortex and Billroth's strand, which reflects a decrease in immune activity and lymphopoietic function, and there is also inhibition of cellular reactions in the lymph node, as a result of the immunosuppressive action of Mercazolil.

After applying the correction, the total area of the lymph node changes, and it is 26.51±2.42% (in the control - 22.39±1.62%; Mercazolil course - 18.57±2.13%). The cortical/medullary ratio is 0.82±0.09, as evidence of an increase in the proportion of medulla in the overall structure of the lymph node. The subcapsular sinus remains narrowed with accumulation of lymphoid cells. After the abolition of Mercazolil, in the lymph nodes there is an increase of 1.76 times the area of the cerebral sinus, 1.44 times the Billroth's strand in comparison with the indicator that occurs when taking Mercazolil. Billroth's strand of small size are widely represented in the sinus system of the lymph node. The cortical plateau is reduced by 1.8 times in comparison with the indicator that occurs when taking Mercazolil. At the same time, low indicators of the area of the structures of the cortical substance remain, both in comparison with the control and with the use of Mercazolil. The ratio of lymphoid nodules with and without a germinal center is 2.1, which is close to the control value and indicates the predominance of lymphoid nodules with a germinal center. During rehabilitation, among the indicated structural and functional zones, the percentage areas of lymphoid nodules with a germinal center, paracortex decrease, and the area of Billroth's strand increases in comparison with control indicators (Table 1).

Table 1 - The structure of the cervical lymph node and normal indices, with hypothyroidism and against the background of correction

Structures of the mesenteric lymph node	Control group	Hypothyroidism	After correction
Capsule	1,28±0,05	0,97±0,03	1,12±0,02
Subcapsular sinus	0,71±0,09	0,28±0,08*	0,73±0,05*
Cortical plateau	2,41±0,14	1,23±0,02	2,24±0,09*
Lymphoid nodule with germinal center (F_2)	1,56±0,06	0,96±0,13*	1,24±0,11*
Lymphoid nodule without a germinal center (F_1)	3,22±0,13	1,32±0,39*	2,2±0,65*
Paracortex	5,35±0,14	3,52±0,42*	4,46±0,03*
Pulp strands	8,99±0,56	6,48±0,09	7,13±0,12*
Cerebral sinus	1,92±0,05	3,02±0,07**	2,89±0,01**
Total cross-sectional area of the lymph node	22,39±1,62	18,57±2,13	26,51±2,42

Cortical-brain index	1,26±0,05	0,41±0,03*	0,78±0,06*
Index F_1/ F_2	1,42	1,37	1,77

Note: P <0.05, P <0.01** - reliability of differences between indicators*

After correction, in comparison with hypothyroidism, the percentage areas of the cortical plateau, lymphoid nodules with a germinal center decrease, and the percentage areas of the structures of the medulla increase. In the general structure of the lymph node, the abolition of Mercazolil leads to an increase in the proportion of those intranodular zones that had low rates when taking Mercazolil, but they do not always reach the control values.

The use of corrigents during the rehabilitation period increases the immune and drainage-detoxification potential of the lymph node. First of all, this is expressed in an increase in the total area of the lymph node up to 27.7±1.59% (the course of Mercazolil is 21.6±1.23%). Administration after corrigents restores the ratio of cortical and medulla in the lymph node. The cortical/medullary ratio is 1.05±0.13 and indicates the formation of the morphotype of the lymph node, as in the control. The change in the total area under the influence of corrigents is accompanied by the transformation of intranodular zones. The size of the subcapsular sinus varies along its length.

The use of corrigents for a month affects the total area of the lymph node and the size of its separate structural and functional zones. Within a month, there is an increase in the area of the lymph node, the increase was 12-14% (Table 2). At the same time, the values of the area of the structures of the lymph node change statistically insignificant with the initial level. In comparison with the control parameters, the area of the paracortex decreases by 37.4%, and the area of the cerebral sinus increases by 115.4%, the other structural indicators did not have significant differences. Perhaps this can be attributed to the accumulation of lymphocytes in the parocortex during their migration through the wall of postcapillary venules into the lymph node. The development of the deep cortex leads to compaction of the lymph node, which is accompanied by an increase in the cortical-brain ratio.

LYMPH NODULE

After the use of corrigents in the lymphoid nodule, the number of blasts increases by 27.6% compared with the index in hypothyroidism, while their value remains within the control limits. During the period of correction in the lymphoid nodule, the number of medium lymphocytes increases by 22.4% compared with the hypothyroid status, and their number remains reduced by 1.3 times compared to the control group. After the use of corrigents in the lymphoid nodule the number of small lymphocytes increases but does not reach the control level (9.05±0.89 in control, 5.87±0.78* in hypothyroidism, 7.02±0.71* after correction), at the same time, with hypothyroidism, it was reduced by 1.4 times compared with the control (Table 2).

With correction in the lymphoid nodule, a 1.2-fold increase in the number of macrophages is observed in comparison with hypothyroidism. At the same time, in comparison with the hypothyroidism group, the number of macrophages after correction decreases by 8%, but is higher compared to the control values.

Table 2 - Structural and functional zones of the lymph node in experimental hypothyroidism and after correction

Cells, the number of cells per unit area (1600 μm^2)	Control group	Hypothyroidism	Hypothyroidism + correction
Lymphoid nodules			
Lymphoblasts	4,19±0,18	2,88±0,07*	4,0±0,03
Average lymphocytes	13,33±0,71	8,50±0,88**	10,4±0,92*
Small lymphocytes	9,05±0,89	5,87±0,28*	7,02±0,72*
Macrophages	4,86±0,03	6,21±0,16*	5,32±0,51*
Paracortex			
Lymphoblasts	3,19±0,36	3,91±0,38	3,54±0,41

Average lymphocytes	13,17±0,69	9,50±0,76**	11,3±0,81*
Small lymphocytes	7,52±0,51	6,39±0,43*	7,22±0,51
Reticular cells	1,01±0,12	–	0,91±0,06
Macrophages	5,96±0,47	3,41±0,31*	4,08±0,76*
Plasmocytes	0,17±0,03	–	0,11±0,01
Eosinophilic granulocytes	–	–	0,15±0,002*
Brain cords			
Plasmoblasts	2,52±0,39	4,96±0,43*	3,6±0,39
Plasmocytes	2,43±0,23	1,98±0,28*	2,32±0,19
Small lymphocytes	5,55±0,42	4,87±0,48	4,9±0,41
Average lymphocytes	8,67±0,53	4,50±0,41*	6,8±0,62*
Macrophages	4,69±0,32	4,62±0,58	4,09±0,39*
Reticular cells	1,50±0,09	0,83±0,10*	1,11±0,54
Eosinophilic granulocytes	0,33±0,07	0,53±0,09	0,61±0,09*
Cerebral sinus			
Small lymphocytes	8,44±0,76	6,84±0,55*	6,41±0,52*
Macrophages	4,05±0,39	6,11±0,47*	4,54±0,39
Reticular cells	1,67±0,13	1,0±0,14	1,41±0,09
Plasmocytes	0,42±0,14	0,52±0,19	0,61±0,12
Eosinophilic granulocytes	0,33±0,07	–	0,58±0,07

Note: P <0.05- reliability of differences between indicators*

Paracortex. After the use of corrigents in the paracortex, the number of blasts decreases by 8% compared to hypothyroidism (in the control group 3,19±0,36; with hypothyroidism 3,91±0,38; after correction 3,54±0,41). At the same time, the number of blasts per unit area of the paracortex is maintained within the boundaries of the control at all times after taking corrigents.

In comparison with hypothyroidism, during the period of correction in the paracortex, the number of medium lymphocytes increases, but is lower than the control value by 14.2%. It can be noted that the number of macrophages in the paracortex after correction increases by 42%, but remains quite low, it is 1.27 times less than the control indicator. During the period of correction in the paracortex, the number of plasmocytes per unit area changes little and remains within the limits of the control (table 2).

Brain cords. In hypothyroidism, the number of plasmablasts in the Billroth's strand increases by 136%, i.e. by 2.36 times, and after the use of corrigents by 1.48 times, respectively, in comparison with hypothyroidism. After applying the "Renaissance Plus" balm and white cinquefoil in the Billroth's strand, the number of plasmablasts remains within the limits of the control, tending to increase by the 30th day of correction.

The number of plasmocytes in the Billroth's strand in experimental hypothyroidism is reduced by 31%; in other periods after correction, there is no statistically significant difference in comparison with hypothyroidism. In the Billroth's strand, the number of small lymphocytes in control and in hypothyroidism is very close in size to each other. No statistically significant difference was found during the correction

period. After the use of corrigents in the Billroth's strand, the number of medium lymphocytes was increased by 1.51 times in comparison with hypothyroidism. In comparison with the control, there is a progressive decrease in the number of medium lymphocytes with recovery after correction. In experiments, it was shown that after corrigents in the Billroth's strand, a decrease in the number of macrophages by 1.16 times occurs in comparison with the control group and the hypothyroidism group. The number of reticular cells is reduced by 55% in hypothyroidism compared to the control group, and the use of corrigents increased by 33% (table 2). The number of reticular cells varies within the control, not showing a statistically significant difference. During the period of correction in the Billroth's strand, there is a tendency to increase the number of eosinophilic granulocytes in comparison with hypothyroidism, and their number is steadily increased in comparison with the control.

Cerebral sinus. During the correction period in the cerebral sinus, the number of small lymphocytes statistically significantly decreases by 28% and in hypothyroidism by 19.2% compared to the control group. In comparison with the control in hypothyroidism, the number of small lymphocytes in the cerebral sinus remains low and ranges from 5.2±0.21 to 7.0±0.35 (Table 2).

During the period of phytorehabilitation in the cerebral sinus, the number of macrophages decreases by 1.4-1.7 times in comparison with hypothyroidism. At the same time, the number of macrophages in the cerebral sinus remains within the initial background during the period of use of corrigents.

After the use of corrigents in the cerebral sinus, a progressive increase in the number of reticular cells occurs, reaching a statistically significant in comparison with hypothyroidism. In comparison with the control, a low content of reticular cells by 60% was noted, in the subsequent periods after correction, their number reaches the control value (table 2).

During the period of application of correction in the cerebral sinus, the number of plasmocytes changes little, tending to increase in comparison with hypothyroidism, but the difference becomes statistically significant by the end of taking corrigents, in comparison with the control group it increases by 84% (in control 0,33±0,07; with hypothyroidism 0,52±0,19; after correction 0,58±0,07). After correction in the cerebral sinus, the number of eosinophilic granulocytes tends to increase compared with the control (table 2).

DISCUSSION

Thus, it can be seen from the obtained data that the structural and functional zones of the lymph node are characterized by a certain quantitative and qualitative composition of cells, which depends on the thyroid status. The administration of Mercazolil and the formation of hypothyroidism are reflected in the cytoarchitectonics of the lymph node. The authors found that in hypothyroidism, there are not only changes in the ratio of the follicular epithelium, colloid and stroma of the thyroid gland, also there is an accumulation of tissue fluid, an increase in the proportions of the interstitium, as well as a decrease in the volume density of the blood and lymphatic bed [16]. In our experiment, taking Mercazolil blocks the production of thyroid hormones, which is manifested by a change in the morphology of the lymph node. Abolition of Mercazolil leads to an increase in the structural and functional zones of the lymph node, with the exception of the total area and the size of the paracortex area, which decreases. A decrease in the paracortex can be seen as its depletion. According to the literature, the lymph node is a reflection of the changes that occur in the lymphatic region of the organ in our case under conditions of hypothyroidism and further at the stage of correction. It is known that thyroid hormones stimulate the function of lymphoid organs [17], and hypothyroidism inhibits the activity of the immune system [18], but in this case it concerns a certain structure of the lymph node.

At the same time, the area occupied by the medulla increases, mainly due to the Billroth's strand in comparison with the experimental group. After applying the correction, there was an increase in the size of the total section of the lymph node, and among the intranodular structures - the area of the paracortex; the rest of the structures did not differ statistically significantly among themselves, probably, the mechanism of inhibition of the response of structural and functional zones after the abolition of mercazolil in comparison with the control. However, when corrected, all indicators of the area of the main intranodular structures are statistically significantly less than the control indicators, with the exception of the paracortex and Billroth's strand.

The ratio of lymphoid nodules with and without a germinal center is low and equal to 1.12, which indicates a decrease in the activity of lymphoid nodules. The results of the studies showed that when corrected, in comparison with hypothyroidism, the area of lymphoid nodules with a germinal center, the cerebral sinus decreases and the area of the paracortex and Billroth's strand increases; in comparison with the use of corrigents, there is an increase in the paracortex and a decrease in lymphoid nodules with a germinal center, cerebral sinus; in comparison with hypothyroidism, there is a decrease in the capsule, cortical plateau, lymphoid nodules with a germinal center and an increase in the paracortex and Billroth's strand. The positive effect of the "Renaissance Plus" balm and tincture of white cinquefoil on the immunoactive

zones of the lymph node was noted, which contributes to a more rapid restoration of its immune and drainage-detoxification potential at the stage of hypothyroidism rehabilitation.

After the use of corrigents in animals of the consequences of hypothyroidism, the number of blasts stabilizes at the control level, the number of lymphocytes gradually increases to control in the lymphoid nodules, increased in the paracortex and decreased in the cerebral sinus, macrophages at the control level, and by the end of the study may increase in the lymphoid nodule and decrease in the paracortex and Billroth's strand, the number of plasma cells increases in the Billroth's strand and cerebral sinus. There is a rapid recovery of the immune potential of the lymph node. Cytological reactions, along with dynamic stereotypy of changes in the structure of the lymph node, reveal morphological originality, which reflects the functional state of the thyroid gland in hypothyroidism and under correction. After the use of corrigents with the help of the "Renaissance Plus" balm and white cinquefoil, the cellular composition of the structural and functional zones of the lymph node is restored more quickly.

CONCLUSION

The results of the studies showed that under conditions of hypothyroidism, the lymph node becomes compacted with a decrease in the main structural and functional zones against the background of reduced lymphopoietic function. The number of macrophages increases in the lymphoid nodule, cerebral sinus, the number of mature plasma cells decreases in the paracortex and Billroth's strand, which reflects a decrease in immune activity. The dependence of the structure of the lymph node on changes in the functional state of the thyroid gland caused by hypothyroidism is shown. In turn, the use of corrigents after the abolition of Mercazolil, as a rule, equalizes the ratio of the cortical and medulla in the overall structure of the lymph node and leads to the restoration of the area of the medulla and paracortex to the control level. Corrigents, the combination of "Renaissance Plus" balm and tincture of white cinquefoil are considered as peculiar modifiers of the structure and function of the lymph node during the correction period, which justifies their use in hypothyroidism.

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