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MORPHOFUNCTIONAL CHARACTERISTICS OF MESENTERIC LYMPH NODES IN THE COMBINED EFFECT OF HYPOXIA AND INFECTION

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The article presents information on the study conducted, the purpose of which was a comprehensive study of morphofunctional changes in the mesenteric lymph nodes under the combined effects of hypoxia and infection, as well as compensatory-adaptive processes formed in the lymphoid tissue. The study was conducted on mesenteric lymph nodes of white rats weighing 180–200 grams, using histological, electron microscopic, and morphometric methods. On the 15th day of the study, acute destruction and necrosis of cells, a significant decrease in morphometric parameters, vacuolization of most organelles were observed. At a later stage of the study (day 30), depletion and atrophy of follicles, as well as, conversely, an increase in connective tissue were revealed. These findings suggest pathological remodeling of the lymph nodes, reflecting both a structural response and an adaptive reaction to the combined effects of hypoxia and infection.
Key words: hypoxia, infection, mesenteric lymph nodes.

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МОРФОФУНКЦІОНАЛЬНА ХАРАКТЕРИСТИКА БРИЖОВИХ ЛІМФАТИЧНИХ ВУЗЛІВ ПРИ ПОЄДНАНОМУ ВПЛИВІ ГІПОКСІЇ ТА ІНФЕКЦІЇ

У статті подано інформацію про проведене дослідження, метою якого було комплексне вивчення морфофункціональних змін у мезентеріальних лімфатичних вузлах під спільним впливом гіпоксії та інфекції, а також компенсаторно-адаптаційних процесів, що формуються в лімфоїдній тканині. Об'єктом дослідження стали брижові лімфатичні вузли білих щурів вагою 180–200 грам. У ході дослідження використовувалися гістологічні, електронно-мікроскопічні та морфометричні методи. На 15-й день дослідження спостерігалися гостра деструкція та некроз клітин, значне зниження морфометричних показників, вакуолізація більшості органел. На більш пізньому етапі дослідження (30-й день) виявлено виснаження та атрофію фолікулів, а також, навпаки, збільшення сполучної тканини. Отримані результати можуть бути розцінені як патологічна реконструкція лімфатичних вузлів, а також як загальна реакція на адаптацію до нового стану та спільного впливу гіпоксії та інфекції.
Ключові слова: гіпоксія, інфекція, брижові лімфатичні вузли.

Hypoxia and infection as an irritating factor have a damaging effect on the tissues and cells of the immune system, along with the respiratory, cardiovascular, and endocrine systems, and accelerate the development of inflammation in the body. Basically, the combined effect of hypoxia and infection causes tissue alteration, inflammation, or exposure to ischemia, as well as the progression of diseases and the emergence of autoimmunity in the body. At the same time, as a result of the combined effect of vascular pathology and microthrombosis, chronic hypoxia caused by a long-term infection leads to disruption of the blood supply of tissues and cells, exacerbation of existing pathological processes in the body [2, 3, 5, 8].

Any hypoxia and infection-causing irritating factor on the body is accompanied by the occurrence of adaptation processes aimed at preventing or eliminating hypoxia and infection, as well as maintaining homeostasis [4, 6]. The interaction of various mechanisms in different directions carries out the adaptation processes to the combined effect of hypoxia and infection. In this regard, it is important to evaluate the body's response to hypoxia and infection in order to predict and prevent the severity of both factors in time [7, 9].

The purpose of the study was to comprehensively determine the pathogenetic and morphogenetic mechanisms of structural changes in mesenteric lymph nodes in the combined effect of hypoxia and infection, as well as the basic principles of compensatory-adaptive processes in lymphoid tissue.

Materials and methods. The object of the study were 60 adult male white rats weighing 180–200 grams, bred in special conditions, healthy, mobile, and with rhythmic heartbeats. Experimental animals were divided into two groups: control (20) and main (40). The parameters of the animals included in the control group were taken as normal. The experimental animals included in the main group were placed in a barochamber after being infected with S. aureus culture and subjected to the combined effects of hypobaric hypoxia and staphylococcal infection. On the 15th and 30th days of the study, the mesenteric lymph nodes of the animals were taken in a complex and studied by histological, electron-microscopic, and morphometric methods.

Research on animals was conducted in the Department of Pharmacology and Experimental Surgery of the Scientific Research Center of AMU on the basis of ethical rules specified in Protocol No. 31 of the Ethics Rules Commission and bioethics committee under the Ministry of health of the Republic of Azerbaijan on 21.04.2008.

Results of the study and their discussion. In histological preparations, the connective tissue capsule surrounding the lymph nodes from the outside and the trabeculae from the capsule to the inside of the node are observed, and numerous anastomoses exist between the trabeculae. Under the capsule, the dark cortical substance consisting of follicles, the light-colored medullary substance in the center of the nodule, and the paracortical zone between the cortical and the medullary substances are clearly visible (Fig.1).

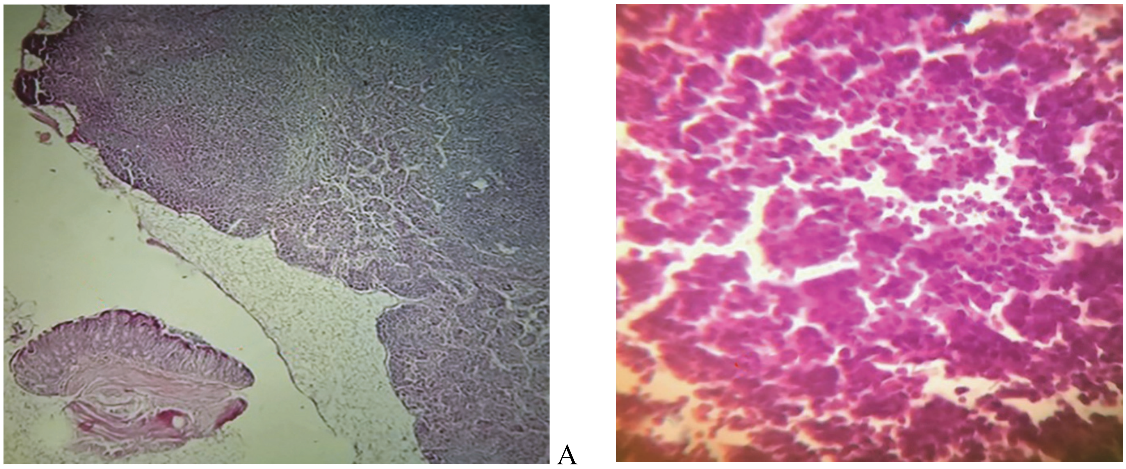


Fig. 1. Control group. Normal histotopography of mesenteric lymph nodes. A – stain: hematoxylin and eosin. X10; B – stain: PAS method. X40.

In the preparations, the follicles of the cortical substance, rich in blood vessels, appear in the form of nodules. In the central part of the nodule is the germinal center, and in the periphery, the location of small-sized lymphocytes in the form of a crown is noted. In histological preparations, medullary trabeculae located in the form of beams entering the medullary substance from the paracortical zone, and nodes are clearly visible.

58.3 % of the lymph node is the cortical substance, 38.3 % is the medullary substance, and 3.4 % is the paracortical zone. It is noted that their ratio is 58:38:4 (Table 1).

Table 1

Morphometric parameters of mesenteric lymph nodes in the norm, and combined effect of hypoxia and infection

Parameters		Control group	Duration of the experiment	
			Day 15	Day 30
n		10	16	16
Relative volume of cortical substance	M±m	58.2±0.6	52.2±0.7	47.7±0.7
	min-max	55.4–60.5	49.8–54.2	45.5–49.6
	Me	58.3	52.6	48.1
	Q_1-Q_3	56.7–59.8	50.6–53.5	46.1–48,8
	P		0.001	0.001
Relative volume of medullary substance	M±m	38.5±0.5	44.5±0.7	49.2±0.7
	min-max	36.3–41.2	42.5–46.7	47.1–51.4
	Me	38.3	44.2	48.7
	Q_1-Q_3	36.9–39.7	43.4–46.3	47.9–51.2
	P		0.001	0.001
Relative volume of paracortical zone	M±m	3.35±0.04	3.26±0.09	3.15±0.13
	min-max	3.20–3.60	2.90–3.50	2.70–3.50
	Me	3.30	3.33	3.20
	Q_1-Q_3	3.30–3.40	3.10–3.40	2.90–3.40
	P		0.619	0.246
Cortical/ medullary substances ratio	M±m	1.51±0.04	1.18±0.03	0.97±0.03
	min-max	1.34–1.66	1.07–1.28	0.88–1.05
	Me	1.52	1.19	0.99
	Q_1-Q_3	1.43–1.62	1.09–1.23	0.90–1.02
	P		0.001	0.001

Note: n – number of animals; M – average parameter of variation, m – standard error; min (max) – the smallest (biggest) indicator of the range of variation; Me – average structural parameter; Q_1 – I quartile, Q_3 – III quartile; statistical integrity of the difference between the parameters of the groups: p – with the parameters of the control group (by the U-Mann-Whitney criterion).

On microscopic examination, it is notable that the lymph node's parenchyma is pierced by a thick network of lymphatic sinuses, in which the afferent lymphatic vessels open. It is possible to visualize the subcapsular sinus, the intermediate sinuses between the nodule and the trabeculae, the medullary sinus between the medullary trabeculae, and the portal sinus at the hylus of the node.

On the 15th day of the study, most of the lymph nodes appear in the form of islets in histological preparations. Microscopically, the connective tissue capsule and parenchyma of the lymph nodes, follicles subjected to atrophic processes, become denser as a result of the proliferation of connective tissue elements in the stroma. The structure of the lymph nodes is disturbed, the cortical and the medullary substances are hardly separated from each other, the paracortical zone, trabeculae, as well as medullary trabeculae are poorly observed.

In addition to dystrophic changes in the lymphoid tissue, there is a violation of the relationship between the cortical and the medullary substance, mainly a decrease in the volume of the cortical substance. Statistically reliable parameters of atrophy of nodes are revealed in morphometric studies. Thus, the relative volume of the cortical substance decreases by 9.9 % compared to intact parameters and makes up 52.6 % of the lymph nodes ($p=0.001$). 44.2 % of the lymph nodes consisted of medullary substance, which was 15.5 % higher than the control group ($p=0.001$). The ratio of the cortical and the medullary substance to each other is 1.19 % ($p=0.001$) with the increase in the volume of the medullary substance.

In histological preparations, deformation of follicles, sharp reduction in size and lysis of small-sized follicles are clearly visible. Necrobiotic changes are noted in relatively large follicles. A large number of necrotic cells and stromal elements are found between the lymphoid follicles. In some preparations, signs of inflammation and the collection of exudate in the lymphoid tissue of the cortical substance drow attention (fig. 2, A). More acute dystrophic changes are noticeable in the medullary substance, in this zone, the depletion of lymphoid cells is noted. The cytoplasm of cells becomes vacuolated, their nuclei undergo karyopyknosis and karyolysis. The decrease in the number of lymphoid cells and the lysis of lymphocytes in the local area leads to the disruption of the structure of the medullary substance.

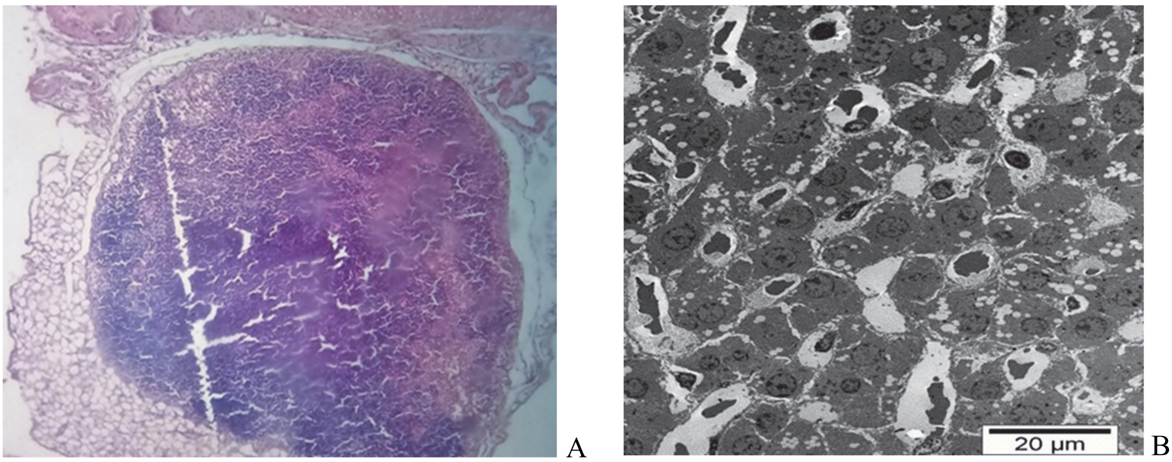


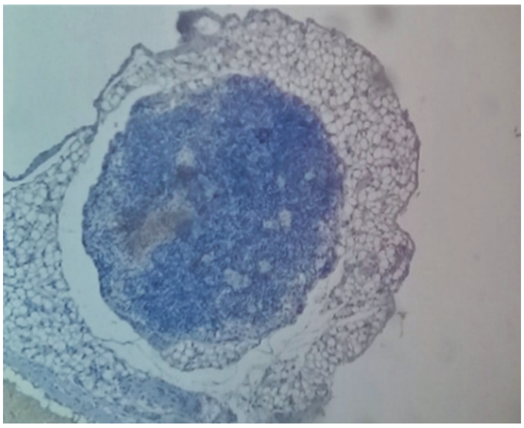
Fig. 2. Main group: day 15 of the study. A – Histotopography of mesenteric lymph nodes. Stain: Hematoxylin and eosin. X20; B – Ultrastructure of mesenteric lymph nodes. TEM. Stain: uranyl acetate and pure lead citrate. Scale – 20 μm.

Stress factors cause intensification of the extravascular insudation of plasma and increase of intra-organ progressive vascular changes. In some preparations, although the integrity of the capillar walls is not disturbed, the narrowing of the lumen is clearly noticeable. The histological examination reveals the lymphatic sinuses have expanded, include individual lymphocytes, macrophages, and plasmatic cells, and have dystrophic alterations in their walls.The destruction of macrophages in the medullary sinuses is clearly visible. Other lymphatic sinuses, especially the portal sinus, are notable for being filled with a necrotic mass.

Under the combined effect of hypobaric hypoxia and staphylococcal infection, focal damage of the plasmalemma and dystrophic changes of the organelles are observed in ultrathin sections prepared from lymph nodes (fig. 2, B). Vacuolation of the cytoplasm, mainly in the medullary substance, the eccentric location of the nuclei, and the fact that most of the nuclei are shrunken are clearly visible. Heterochromatin is located along the karyolemma in the form of large balls. Destruction of mitochondria, disintegration of cristae and fragmentation into separate fragments, degranulation of the Golgi complex, and sharp expansion of the cisterns of the endoplasmic reticulum are noted; lysosomes are not observed in most cells. Parts of degranulated organelles are unevenly distributed in the cytoplasm.

The reduction of exudative processes leads to an increase in the number of fibroblasts and the development of new connective tissue. This, by squeezing the lymphatic follicles, leads to a decrease in their size, and the lymph nodes acquire a microfollicular appearance. Despite the reduction in the size of the follicles, the processes of intrafollicular regeneration and follicular hyperplasia occur in the lymph nodes as a result of absorption of inflammation in the peritoneal cavity. Along with this, on the background of hypoxia, newly formed lymphocytes in the lymphoid tissue in quantitative and qualitative terms in response to infection are noted (Fig. 3, A).

The change in the relationship between the cortical and the medullary substance leads to a sharp decrease (17.5 %) in the volume of the cortical substance compared to the control group, which leads to the replacement of the lymphatic parenchyma by the stroma. At the same time, a significant (27.2 %) increase in the volume of medullary substance of a compensatory nature causes a change in the ratio between them, and this ratio is 0.99 % (p=0.001). On the other hand, compensatory stromal proliferation is the result of the next pathological process developing in the parenchyma of the follicles. This process has been characteristic of both the 15th and subsequent stages of study and continues to grow. Proliferation of connective tissue not only compensates for the loss of parenchyma, but also creates a certain stability locally for the remaining follicles and prevents complete atrophy of the follicular epithelium. This is the result of the combined effect of stress factors hypoxia and infection on lymphoid tissue.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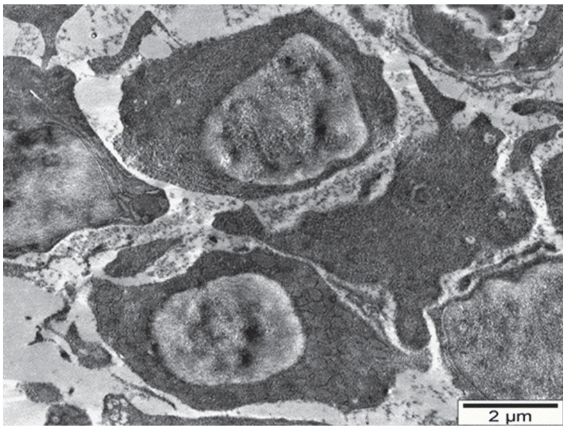

B

Fig. 3. Main group: day 30 of the study. A – Histotopography of mesenteric lymph nodes. Stain: Hematoxylin and eosin, X20; B – Ultrastructure of mesenteric lymph nodes. TEM. Stain: uranyl acetate and pure lead citrate. Scale – 2 μm.

At this stage of the study, vascular pathologies are non-specific. Vascular changes are mainly manifested in the form of stasis, micro-thrombosis and fibrinoid necrosis. Relative expansion of lymphatic sinuses, collection and aggregation of individual mono- (macrophages and lymphocytes) and polynuclear cells are observed in their walls, lumen, especially in the lumen of the subcapsular sinus.

At the end of the experiment, electron-microscopic changes in the lymph nodes reach a maximum. Ultrastructural manifestations of stress effects are characterized by depletion of parenchyma, disruption of intercellular contacts, decrease in the number of secretory vesicles, and strengthening of autophagocytosis (Fig. 3, B).

Depletion of parenchyma is manifested by necrosis and atrophy of cells. Electrograms show a clear expansion of the endoplasmic reticulum, which occupies almost the entire intracellular area. Reduction in the number of mitochondria, smoothing of their cristae, complete change of shape in some cells is clearly noticeable.

Conducted studies show that hypoxia and infection, which are stress factors, have a significant effect on the organs of the immune system, including the structure and functional activity of the lymph nodes, and serious pathomorphological changes related to each other are found in all zones of the lymph nodes [1, 10, 12]. These changes have a dual character. So, at this time, along with proliferative processes, the exhaustion and atrophy of follicles is observed. During the combined effect of hypoxia and infection, the changes in the lymph nodes lead to pathological reconstruction of the tissue, as well as adaptation to the new environment [2, 3, 4, 7, 8].

Conclusion

The obtained results can be considered as a pathological remodeling of the lymph nodes, as well as their adaptation to a new condition and a general response to the combined effects of hypoxia and infection. The response of lymphoid tissue to hypoxia and staphylococcal infection was not homogeneous, the nature of these reactions mainly depends on the type and pathogenicity of the microflora in the abdominal cavity in the experimental peritonitis, as well as the nature and development of the destructive processes occurring in the peritoneum, as well as the duration of hypoxia.

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