Quantitative morphological evaluation of structural reconstruction in endothelial cells of arteries and veins of testes in the conditions at postresection pulmonary arterial hypertension

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Removal of the right or left lung can lead to postresection pulmonary arterial hypertension, venous stasis in the organs of the great circle of blood circulation and structural and functional changes in them. Morphological reconstruction of endotheliocytes of arteries and veins of testes at postresection pulmonary arterial hypertension are insufficiently studied. The purpose of our study was to conduct a quantitative morphological evaluation of structural changes of endothelial cells of the arteries and veins of the testes at postresection pulmonary arterial hypertension. The testes of 46 white male rats, which were divided into 3 groups, were morphological examined. The 1st group consisted of 14 intact animals, 2nd - 22 rats with postresection pulmonary arterial hypertension and compensated cor pulmonale, 3rd - 10 animals with postresection pulmonary hypertension and decompensated cor pulmonale. Postresection pulmonary arterial hypertension and cor pulmonale were modeled by performing right pulmonectomy in rats. Euthanasia of rats was performed by bloodletting under conditions of thiopental anesthesia one month after the start of the experiment. From the testes were made micropreparations, which morphometrically determined the height of endothelial cells of arteries and veins, the diameter of their nuclei, nuclear-cytoplasmic ratios in these cells, the relative volumes of damaged endotheliocytes. Quantitative indicators were processed statistically. In the conditions of postresection pulmonary arterial hypertension and decompensated pulmonary heart, the studied morphometric parameters changed most markedly. The height of endothelial cells of the arteries of the left testis was reduced by 2.9 % (p<0.05), and the right - by 2.3 %. Nuclear-cytoplasmic ratios in the endotheliocytes of these vessels increased by 7.6 % and 4.7 %, respectively (p<0.05), and the relative volume of damaged endotheliocytes increased by 11.7 and 9.3 times (p<0.001). The studied morphometric parameters in the testicular veins increased to a greater extent compared with the arteries. Thus, the height of endothelial cells of the left testis in the 3rd group of observations was statistically significant (p<0.001) decreased by 5.8 %, and in the right - by 5.4 %. Nuclear-cytoplasmic ratios in the studied structures increased by 8.2 % (p<0.001) and 6.5 % (p<0.01), respectively, which indicated a pronounced violation of structural cellular homeostasis. The relative volume of damaged endotheliocytes in the veins of the left testis in postresection pulmonary arterial hypertension combined with decompensated heart increased by 17.6, in the right - by 13.0 times (p<0.001). Optical on histological preparations of testes showed plethora and dilation of venous vessels, which was complicated by hypoxia, dystrophy, necrobiosis of cells and tissues of the studied organ, and in the long term - infiltrative and sclerotic processes. Venous vessels of the microhemocirculatory bed are unevenly dilated, tortuous, full-blooded, with numerous varicose veins and sacculations. Stasis, thrombosis, diapedetic hemorrhages, plasmorrhagia of the walls of venous vessels and paravasal tissues were found in these vessels. Elastic structures in vessels with the phenomena of multiplication, fragmentation and destruction. Endotheliocytes with signs of edema, dystrophically and necrobiotically altered, sometimes desquamated. Analysis of the obtained data revealed that postresection pulmonary arterial hypertension leads to violations of cellular structural homeostasis, an increase in the relative volumes...
Introduction

Lung resections are often performed today in modern surgical clinics for benign and malignant tumors, metastases, injuries, tuberculosis, alveolar echinococcosis, lung transplantation [10, 13, 15]. Removal of the lung can lead to various post-resection complications: hypertension in the small circle of blood circulation, pulmonary heart, cardiovascular failure, arrhythmias, postpneumonectomy edema of the single lung, thromboembolism of the branches of the pulmonary artery, postpneumonectomy syndrome bronchopleural fistulas, pleural empyema and others [15, 16, 19]. Postresection pulmonary arterial hypertension leads to structural reorganization of the circulatory system, as well as remodeling of their structures. The testis belongs to the organs whose hemodynamic disorders are complicated by various morphological changes in the vascular bed and their structures. It should be noted that the features of remodeling of testicular structures in postresection pulmonary arterial hypertension have not been studied enough [3, 9, 14, 16].

In recent years, morphologists are increasingly using morphometric research methods that allow to quantify and most objectively assess various physiological and pathological processes in organs and systems and logically interpret them [3, 4, 17].

The aim of the study was to conduct a quantitative morphological assessment of structural changes in endothelial cells of testicular arteries and veins in the conditions of postresection pulmonary arterial hypertension.

Materials and methods

The testes of 46 laboratory adult white male rats, which were divided into 3 groups, were studied by a complex of morphological methods. The 1st group consisted of 14 intact animals, the 2nd - 22 rats with postresection pulmonary arterial hypertension and compensated pulmonary heart, the 3rd - 10 animals with postresection pulmonary arterial hypertension and decompensated pulmonary heart.

All manipulations and euthanasia of rats were carried out in accordance with the basic principles of work with experimental animals in the provisions of the "European Convention for the protection of vertebrate animals used for experimental and other purposes" (Strasbourg, 1986), "General ethical principles of animal experiments" adopted by the first national Congress on Bioethics (Kyiv, 2001), as well as the Law of Ukraine "About protection of animals from cruel treatment" (from 21.02.2006) [1], the order of the Ministry of Education and Science, Youth and Sports of Ukraine № 249 from 01.03.2012. animals "Procedures for scientific institutions to conduct experiments on animals".

Postresection pulmonary arterial hypertension and pulmonary heart were modeled by performing right-sided pneumonectomy in rats [5, 16, 17]. In 10 animals after this operation developed decompensated pulmonary heart. The latter was confirmed in rats by shortness of breath, cyanosis of the mucous membranes, hydrothorax, hydropericardium, congestion in the circulatory system, peripheral edema. Euthanasia of rats was performed by bloodletting under conditions of thiopental-sodium anesthesia (40 mg/kg intraperitoneally) one month after the start of the experiment. Separate weighing and planimetry of the heart chambers were performed. Pieces were cut from the testes, which were fixed in a 10 % solution of formalin, passed through ethyl alcohols of increasing concentration and placed in paraffin. Microtome sections after dewaxing were stained with hematoxylin-eosin, according to the method of Weigert, Van Gieson's, Mallory, toluidine blue [1, 6].

Morphometrically in the left (LT) and right (RT) testicles measured the height of endothelial cells of arteries (HEA) and veins (HEV), the diameter of endothelial cells of arteries (DEA) and veins (DEV), nuclear-cytoplasmic ratios in these cells (NCRA, NCRV), as well as the relative volumes of damaged endothelial cells of arteries (RVDEA) and veins (RVDEV) [4, 5].

Quantitative morphological parameters were processed statistically. Processing of the latter was performed in the Department of System Statistical Surveys of Ternopil National Medical University named by I. Ya. Horbachevsky of the Ministry of Health of Ukraine using the software "STATISTICA" ("Statsoft", USA). The difference between the comparative morphometric parameters was determined by the criteria of Student and Mann-Whitney [7, 11].

Results

Separate weighing and planimetry of the heart revealed that a month after right-sided pneumonectomy in all observations revealed hypertrophy and dilatation of the heart chambers with a predominance of weight gain and expansion of the right ventricle, ie the development of the pulmonary heart.

The obtained morphometric parameters of vascular endothelial cells of the left and right testes of experimental animals are presented in Table 1.

A comprehensive analysis of the data in the table shows that most of them have changed markedly. Thus, in postresection arterial pulmonary hypertension and compensated pulmonary heart, the studied morphometric parameters of the arteries of the left testis changed slightly.
Under these experimental conditions, the relative volume of damaged endothelial cells of the left testicular arteries increased 2.05 times, in the right - 1.88 times (p<0.001), and in the veins - 2.99 times and 2.88 times, respectively (p<0.001).

The studied morphometric parameters of the veins of the left and right testicles in postresection pulmonary arterial hypertension were more pronounced compared with the arteries, which confirmed the nuclear-cytoplasmic ratio in the endotheliocytes of the veins, which increased by 5.8 % in the left testicle (p<0.001) - and in right by 5.4 % (p<0.01). In the conditions of postresection pulmonary arterial hypertension and decompensated pulmonary hypertension, the studied morphometric parameters changed more significantly compared with the data of the 2nd group of observations. The height of endothelial cells of the arteries of the left testis was reduced by 2.8 % (p<0.05), and the right - by 2.3 %. The nuclear-cytoplasmic ratios in the endotheliocytes of these vessels increased by 7.6 % and 4.7 %, respectively (p<0.05), and the relative volume of damaged endotheliocytes increased by 11.7 and 9.3 times (p<0.001).

The studied morphometric parameters in the testicular veins varied to a greater extent compared with the arteries. Thus, the height of endothelial cells of the left testis in the 3rd group of observations was statistically significant (p<0.001) decreased by 6.25 %, and in the right - by 5.6 %. Nuclear-cytoplasmic ratios in the studied structures increased by 8.2 % (p<0.001) and 6.5 % (p<0.01), respectively, which indicated a pronounced violation of structural cellular homeostasis [4, 5]. The relative volume of damaged endotheliocytes in the veins of the left testis in postresection pulmonary arterial hypertension combined with decompensated heart increased by 17.6 (p<0.001), in the right - by 13.0 times (p<0.001).

Histologically was observed plethora and dilation of venous vessels, which was complicated by hypoxia, dystrophy, necrobiosis of cells and tissues of the studied organ, and in the long term - infiltrative and sclerotic processes. Venous vessels of the microhemocirculatory tract are unevenly dilated, tortuous, full-blooded, with various varicose veins and sacculations. Stasis, thrombosis, diapedetic hemorrhages, plasmorrhagia of the walls of venous vessels and paravascular tissues were found in these vessels. Elastic structures in vessels with the phenomena of multiplication, fragmentation and destruction. Endothelial cells with signs of edema, dystrophically and necrobiotically altered, sometimes desquamated. The revealed structural changes dominated in the left testis and in the conditions of postresection pulmonary arterial hypertension with decompensated heart. The predominance of morphological and morphometric changes in the vessels of the left testis can be explained by the peculiarities of venous outflow from this organ [2, 8].

### Discussion

The conducted researches and the received results testify that changes of hemodynamics which take place at a postresection pulmonary arterial hypertension lead to structural reorganization of endothelial cells of arterial and venous channels of testicles. Changes in venous vessels, ie in the drainage systems of the testes [2, 8], were more pronounced compared with arteries. The combination of postresection pulmonary arterial hypertension with decompensated pulmonary heart led to more intense morphological changes in the studied testicular structures, which was confirmed by a significant violation of nuclear-cytoplasmic relations in endothelial cells of arteries and veins, as well as a significant increase in relative volume of damaged endotheliocytes [4].

In morphological- and morphometric studies of arterial and venous vessels of the testes in postresection pulmonary arterial hypertension, damage to endothelial cells in the above structures was observed. It is believed that the endothelium, which covers the inner surface of blood vessels, is an important auto-, para- and endocrine organ with numerous regulatory functions [18, 20]. It is known that normally functioning endothelial cells produce nitric oxide (NO), which regulates vascular tone, affects vascular wall remodeling processes, determines the system of antioxidant defense and peroxide aggression, inhibits platelet aggregation and adhesion, macrophages, and proliferative processes in the wall of the vessel [14, 18]. Damage to a significant number of endothelial cells in the studied vessels can lead to endothelial dysfunction.
which plays a leading role in vascular remodeling and morphogenesis of the studied pathology.

Impaired blood supply, hypoxia lead to a cascade of pathological reactions and the release of pro-inflammatory cytokines, inflammatory mediators, highly active free radicals that adversely affect the intercellular structures surrounding cell membranes, as well as the vascular wall [3, 4, 18]. This damages the basement vascular membrane, interendothelial contacts and endotheliocytes. Under these conditions, the concentration of endothelin-1 in the blood increases, which enhances vasoconstriction of arteries by activating Ca$^{2+}$-channels, proliferation of endothelial cells, smooth myocytes and vascular wall fibroblasts, apoptosis, causes the expression of adhesive molecules. When endotheliocytes are damaged, intercellular connections are disrupted, which leads to increased permeability of endotheliocytes and violation of their barrier function. Vascular permeability and endothelial barrier dysfunction are complicated by tissue edema and contribute to increased extravasation of inflammatory elements into the paravasal tissues and contribute to the chronicity of the pathological process. This reduces the relaxation function of the endothelium, which is associated with hyperproduction of endothelin-1, decreased synthesis of prostacyclin and deficiency of endogenous NO [12, 20].

Hypoxia is one of the leading components of the damaging effect on the cell. In conditions of oxygen deficiency, the transport of fatty acids across cell membranes increases, the content of free carnitine and the activation of carnitine-dependent oxidation of fatty acids increases. As a result of the described phenomena, the activation of free radical oxidation is enhanced, which leads to damage primarily to mitochondrial membranes, their destruction and degeneration, which significantly reduces the energy supply of cells. It is known that tissue hypoxia contributes to the deprivation of compensatory mechanisms of adaptation and reduces the resistance of cellular structures to the damaging effects of negative metabolic factors [18, 20].

**Conclusions**

The data obtained indicate that right-sided pulmonectomy leads to postsension pulmonary arterial hypertension, pulmonary heart and is complicated by violations of cellular structural homeostasis, an increase in the relative volumes of damaged endothelial cells in the vascular bed of the testes. The detected processes dominate in the venous bed of the left testis and in the decompensation of the pulmonary heart.

References


КІЛЬКИСНА МОРФОЛОГІЧНА ОЦІНКА СТРУКТУРНОЇ ПЕРЕБУДОВИ ЕНДОТЕЛІОЦІТІВ АРТЕРІЙ ТА ВЕН СІМ'ЯНИКІВ ПРИ ПОСТРЕЗЕКЦІЙНОЙ ЛЕГОЧНОЇ АРТЕРИАЛЬНОЇ ГІПЕРТЕНЗІНІ

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Видалення лівого або правого лівого може призводити до пострезекційної легеневої артеріальної гіпертензії, яка впливає на структури органів серцево-судинної системи. Морфологічні зміни в ендотеліоцитах артерій та вен сім'яників при пострезекційній легеневій артеріальній гіпертензії вивчені недостатньо. Ці зміни впливають на вегетативну регуляцію та структурно-функціональні зміни у них, що впливає на розвиток і патологічних процесів.

Метою нашого дослідження було проведе́ння колі́гографічної оце́нки структурних змін в ендотеліоцитах артерій та вен сім'яників при пострезекційній легеневій артеріальній гіпертензії. Морфологічно досліджені сім'яники 46 білих щурів-самців, які були розділені на 3 групи. У першій групі вивчалися в ендотеліоцитах артерій та вен сім'яників при пострезекційній легеневій артеріальній гіпертензії.

Кількісні показники обробляли статистично. Умови пострезекційної легеневої артеріальної гіпертензії та декомпенсованого легеневого серця обумовлювали найбільш виражені зміни в ендотеліоцитах артерій та вен сім'яників при пострезекційній легеневій артеріальній гіпертензії.

Ключові слова: пострезекційна легенева гіпертензія, ендотеліоцити, артерії, вен, морфометрія.
3-й группе наблюдений статистически достоверно (р<0,001) уменьшилась на 6,2 %, а в правом - на 6,0 %. Ядерно-цитоплазматические отношения в исследуемых структурах соответственно увеличились на 8,2 % (р<0,001) и 7,3 % (р<0,01), что свидетельствовало о выраженном нарушении структурного клеточного гомеостаза. Относительный объем поврежденных эндотелиоцитов в венах левого семенника при пострезекционной легочной артериальной гипертензии сочетанной с декомпенсированным легочным сердцем увеличился в 17,6, в правом - в 13 раз (р<0,001). Светооптически на гистологических препаратах семенников наблюдалось полнокровие и расширение венозных сосудов, которое осложнялось гипоксией, дистрофией, некробиозом клеток и тканей исследуемого органа, а в отдаленном периоде - инфильтративными и склеротическими процессами. Венозные сосуды микроциркуляторного русла неравномерно расширились, извилистые, полнокровные, с многочисленными варикозными расширениями и саккуляциями. В указанных сосудах выпячивались стазы, тромбы, дилатационные кровоизлияния, геморрагии стенки венозных сосудов и паравазальных тканей. Эластичные структуры в сосудах с явлениями мультипликации, фрагментации и разрушения. Эндотелиоциты с признаками отека, дистрофически и некробиотически изменены, местами десквамированы. В результате анализа полученных результатов установлено, что пострезекционная легочная артериальная гипертензия приводит к нарушениям клеточного структурного гомеостаза, росту относительных объемов поврежденных эндотелиоцитов в сосудистом русле семенников. Обнаруженные процессы доминируют в венозном русле левого семенника и при декомпенсированном легочном сердце.

Ключевые слова: пострезекционная легочная гипертензия, семенники, эндотелиоциты, артерии, вены, морфометрия.