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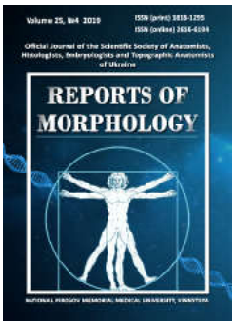

Evaluation of protective effect of Thiocetam drug by morphological changes in the heart and vessels after administration of lead nanoparticles of various sizes (experimental study)

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Introduction of nanotechnologies to the modern industry gave rise to new challenges. The issue of development and implementation of recommendations regarding the prevention of potential negative impact of lead nanoparticles on population health is of particular importance. The locally manufactured drug Thiocetam which possesses nootropic, antiischemic, antioxidative and membrane stabilizing properties has drawn our attention. The research aimed at studying the protective effect of Thiocetam in Wistar rats with simulated subchronic toxic effect of lead compounds of various sizes (by morphological changes in the heart and vessels). The experiments were conducted on Wistar rats (mean body weight of 160-180 g). Colloidal solutions of lead sulphide obtained by chemical synthesis with the use of sodium polyphosphate stabilizer, (PbS) with the mean size of 26-34 nm (1-PbS) and 50-80 nm (2-PbS), and lead nitrate $Pb(NO_3)_2$ (3-Pb) in the ionic form were used in simulating the toxic effect, while normal saline solution was administered to the control group. The studied substances were injected (intraperitoneally daily 5 times a week) in a dose of 0.94 mg/kg (in lead equivalent). The toxic effects were evaluated after 60 injections (three months) and one month after the discontinuation of exposure with and without Thiocetam. The drug Thiocetam in the dose of 250 mg/kg had been administered to rats intragastrically on an empty stomach daily for one month. Histological slides of the rats' myocardium and aortal wall were studied and morphometric analysis and statistical processing performed. In the postexposure recovery period period a lower degree of interstitial swelling and myocardial blood vessel filling was observed, which was considered to be a regression of damage. After the administration of Thiocetam a pronounced transverse striation of cardiomyocytes, the density of collagen fibers around cardiomyocytes and microvessels were revealed, which indicated the protective effect of pharmacological correction. However, leukocyte infiltration was also found in the myocardial or aortic microvessels in the experimental groups. Aortic morphometric data revealed no differences between the PbS NPs groups and $Pb(NO_3)_2$, although the aortic wall morphology was quite preserved. The use of Thiocetam prevented dystrophic changes in the atrial epicardium and the aortic adventitia, which indicate cytoprotective and connective tissue effects. In the postexposure period without pharmacological correction a tendency to spontaneous recovery of morphological changes of the heart and aortic walls under the influence of PbS NPs and lead nitrate was observed. However, morphometric parameters demonstrate the absence of complete recovery be it with or without Thiocetam.

Keywords: *lead, nanoparticles, morphological changes, aorta, myocardium, prevention of toxicities, Thiocetam.*

Introduction

The introduction of nanotechnology into modern production has opened wide prospects for technological development and significantly improves the consumer properties of products. In particular, synthesized nanocrystals of lead compounds, the so-called "quantum dots" 4-10 nm in size, have been successfully used in the

manufacturing of semiconductors, solar cells, biosensors, polymer composites, paints, electronic systems, including LEDs, and flat light emitting panels [8, 12, 19, 23, 29]. Today, the production and use of metal nanoparticles has become commercially available, which enables their penetration into the production areas and the environment and raises a number of questions about the potential risks of nanomaterials for human health [9, 11, 13, 27].

It is known that toxic effects of lead on human body are characterized by the presence of cardiovasotoxic, neurotoxic, hepatotoxic and nephrotoxic effects [3, 7, 20, 25, 26, 28]. In the current state of nanotechnology development researchers are faced with a new problem - the study of the effect of lead in the form of nanoparticles on the body, i.e. how the size of lead particles affects the body and is there any proportionality between their size and toxic effect [4, 15].

Of particular importance is the development and implementation of recommendations on preventing the potential negative impact of lead nanoparticles on population health. To date, a large number of pharmacological agents have been proposed for the purpose of clearing heavy metals from the body, reducing the manifestations of their toxic effects and increasing the general biological resistance of the body. The search for new drugs to prevent the development of intoxication is currently in progress [24].

For instance, the neuroprotective effect of Thiotriazoline, Mildronate and Magnesium B6 in mercury toxication was found [21, 22]; it was shown that under subchronic mercury exposure and combined pharmacological protection with Unithiol and Quercitin, myocardial structures restored due to the positive effect on the hemomicrocirculatory bed [10].

Experimental studies have shown high hepatoprotective efficacy of the drug Thiocetam in toxication with lead sulfide NPs: normalization of AST and ALT enzymes activity in blood serum was observed, as well as reduction of dystrophic changes in hepatocytes, normalization of blood filling of hemomicrocirculatory bed and structure of hepatic plates [2].

Our attention was also drawn to the locally manufactured drug Thiocetam which has nootropic, anti-ischemic, antioxidative and membrane-stabilizing properties.

The research aimed at studying the protective effect of Thiocetam in Wistar rats with simulated subchronic toxic effect of lead compounds of various sizes (by morphological changes in the heart and vessels).

Materials and methods

The experiments were conducted on Wistar rats (mean weight of 160-180 g). Animals were kept in the vivarium on a standardized diet with free access to drinking tap water. In simulating intoxication colloidal solutions of lead sulfide (PbS in sodium polyphosphate) with an average size of 26-34 nm (1-PbS) and 50-80 nm (2-PbS), and lead nitrate $Pb(NO_3)_2$ (3-Pb) in ionic form which is well soluble in water were used. The control group was injected with normal saline.

The studied substances were injected (intraperitoneally daily 5 times a week) (modelling of a working week) in a dose of 0.94 mg/kg (in lead equivalent). The toxic effects were evaluated after 60 injections (3 months) and one month after the discontinuation of exposure (postexposure recovery period - PEP).

Thus, 10 groups of animals were included into the study: 1 - control; 2 - (1-PbS); 3 - (2-PbS); 4 - (3-Pb); 5 - (1-PbS + Thiocetam); 6 - (2-PbS + Thiocetam); 7 - (3-Pb + Thiocetam); 8 - (1-PbS + PEP); 9 - (2-PbS + PEP); 10 - (3-Pb + PEP).

The drug Thiocetam had been administered to rats in the postexposure period (after 60 injections of the studied compounds of lead) daily on an empty stomach intragastrically at a dose of 250 mg/kg for 1 month. Thioacetam (manufactured by PJSC "Galichpharm" Lviv, Ukraine) is a fixed combination of Thiotriazoline (0.05 g in dry weight) and Piracetam (0.2 g).

The animals were withdrawn from the experiments by decapitation under mild ether anesthesia and their internal organs were harvested. All manipulations with animals were performed in accordance with the provisions of the "European Convention for the Protection of Vertebrate Animals, Used for Experimental and Other Scientific Purposes" (Strasbourg, 1985) and approved by the Bioethics Committee of the NAS of Ukraine.

The heart with aorta were fixed in 10% neutral formalin, dehydrated in isopropanol and embedded in paraffin (Leica Surgipath Paraplast Regular). Paraffin sections were made on a Thermo Microm HM 360 microtome. The sections were deparaffinized and stained with H&E following the picro-Mallory technique. The slides were studied using Olympus BX51 microscope. Morphometric analysis was performed using software Carl Zeiss (AxioVision SE64 Rel.4.9.1), magnification x200, x400.

Aorta wall thickness (mkm), adventitia of aorta thickness (mkm), comparative amount of collagen fibers in tunica adventitia (%), number of elastic membranes in tunica media (conventional units) were examined. The statistical study was performed in Origin Lab version 8.0 using the non-parametric Kruskal-Wallis test, because normal distribution of data was not proven. Data are presented as medians with smaller and larger quartiles (M [Q1-Q3]). The difference was considered statistically significant at $p<0.05$.

The studies were performed within the framework of research of State Institution "Kundiiev Institute of Occupational Health of the National Academy of Medical Sciences of Ukraine": "Comparative toxicity of micro- and nanoparticles of lead in experiments in vitro and in vivo (to the problem of improving the principles and methods of toxicological and hygienic studies of heavy metals)" (State registry number 0110U000299), "Investigation of the toxic effects of heavy metal nanoparticles, search and substantiation of preventive measures" (State registry

number 0116U000497) and Department of Histology and Embryology of Bogomolets National Medical University "Changes in internal organs and regulatory systems under conditions of experimental damage and historical aspects of the development of histology, cytology and embryology in Ukraine" (State registry number 0116U000121).

Results

Histological signs of toxic effect of lead on the heart have been studied. The increase in the interstitial space between the fibers of cardiomyocytes and their dystrophic changes in the atria were morphologically confirmed (in the ventricles there was only stasis of blood vessels) in the groups with PbS1 and PbS2. In the Pb3 group no dystrophic changes of the myocardium were detected, but in the atrial epicardium a focal accumulation of mast cells was found, which may indicate their infiltration/migration, and initiation of a proinflammatory response. In slides stained by the picro-Mallory technique for the detection of collagen fibers, a lower density of collagen around the arterioles and venules of the myocardium was found, which was considered to be a manifestation of the toxic effect of lead, and inhibition of the connective tissue elements morphogenesis. This was further indicated by the reduction in the number of cell nuclei in the wall of some myocardial vessels. In the aorta it was reflected by the decreased density of collagen fibers of the adventitia after 1-PbS and 2-PbS administration, impairment of the media (dissection, swelling, reduction of cell nuclei between the elastic membranes) (Fig. 1 A, B, C).

After Thiocetam administration cytological manifestations of the protective effect of pharmacological correction were revealed, such as a more pronounced transverse striation of cardiomyocytes, the density of collagen fibers around cardiomyocytes and microvessels. In group 1-PbS+Thiocetam local dystrophic changes of muscle fibers (loss of nuclei, acidophilia, loss of transverse striation, intercalated disks), focal accumulation of leukocytes in the ventricular epicardium were found.

Signs of stromal reorganization and increased fibroblast density were observed in the damage area. No differences in the morphofunctional changes of the myocardium in groups of different lead sizes (groups 2, 3, 5 and 6) were found. Infiltration of neutrophils, eosinophils, lymphocytes and macrophages around the aorta was also found in aorta of 1-PbS+Thiocetam and 2-PbS+Thiocetam groups. No pronounced structural disturbances were observed in the 3-PbS+Thiocetam, but by all morphometric

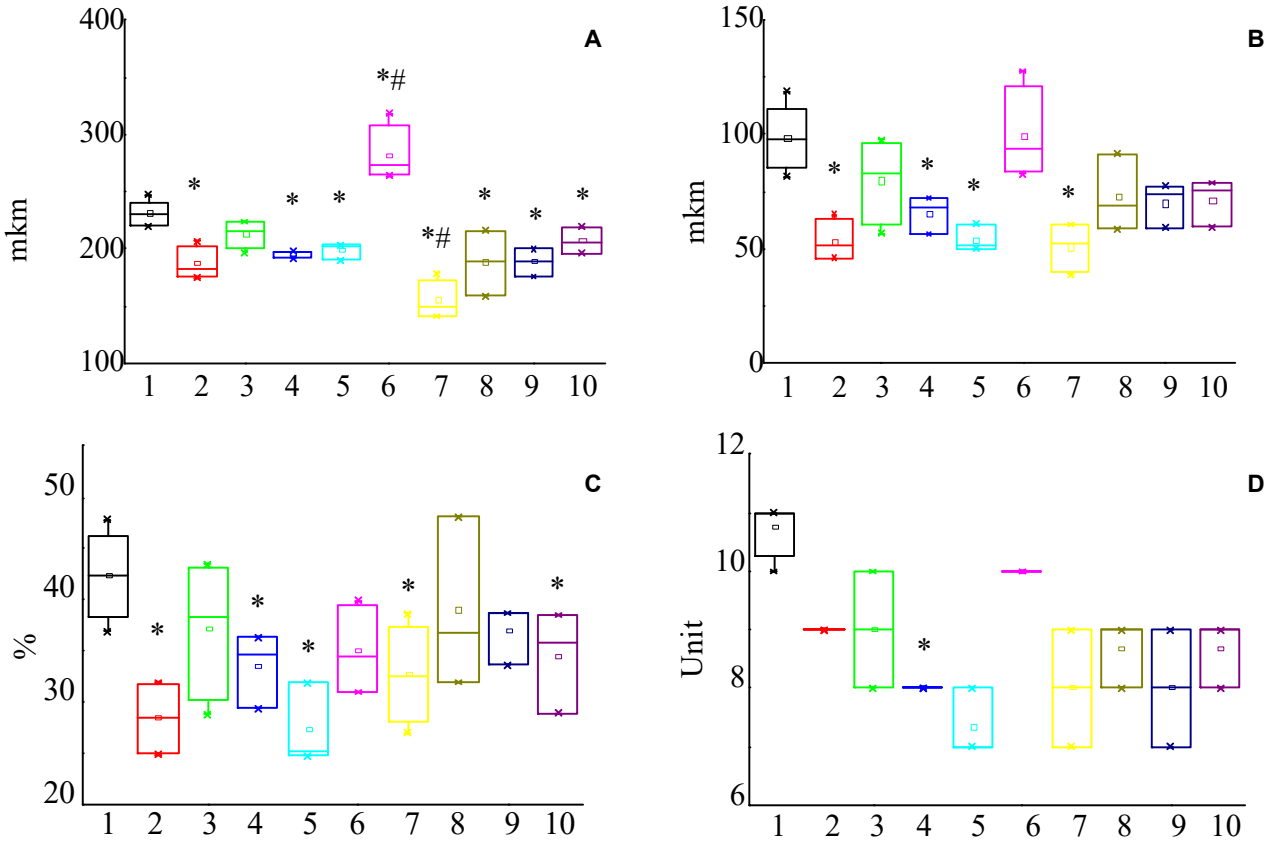

Fig. 1. Results of the aorta wall morphometry (M [Q1-Q3]). Note: A - aorta wall thickness; B - t. adv. thickness; C - relative amount of t. adv. in the aorta; D - number of elastic membranes in t. media; * - significant difference to control ($p<0.05$); # - significant difference to PEP period without Thiocetam ($p<0.05$).

indicators the wall thickness was less than the control values. The structural organization of the adventitia did not differ significantly in the comparison groups.

In the PEP period there was a lower degree of interstitial swelling and moderate blood filling of the myocardial vessels, with the accumulation of leukocytes in single ventricular capillaries detected in the 1-PbS+PEP group. Histological changes of the aortic wall were also reflected in the reduction of its thickness, however, no morphological signs of the media impairment, and the density of elastic membranes were observed. According to the results of morphometric evaluation no statistically significant difference between groups of subchronic experiment (groups 2, 3, 4) and PEP period (groups 8, 9, 10) was found, this was also the case with comparator Thiocetam groups.

The results of the studies indicate that administration of Thiocetam had a partial protective effect, but this was not reflected in the morphometric parameters.

Discussion

The task of this experimental study was to investigate the cardio- and vasotoxic effects of lead compounds and the possibility of their correction by Thiocetam. As described above, inhaled administration of the lead sulfide NPs to the body of experimental animals caused inflammatory changes in the lungs, which were accompanied by the development of oxidative stress and decreased activity of antioxidant enzymes. Moreover, the toxicity of PbS NPs was closely related to their size [14]. Prolonged intraperitoneal administration of lead sulfide NPs revealed their hepatotoxic effect with impaired protein metabolism [1, 16, 18]. In vivo experiments have shown high neurotoxicity of lead sulfide NPs and demonstrated the pathogenetic role of calcium homeostasis disorders; also found was significant reproductive toxicity of lead compounds in nanoforms [5, 6]. To date, there are only few works on the toxic effect of lead NPs on the cardiovascular system and there is virtually no data on their long-term effects.

The results of our own studies lead to the conclusion that PbS NPs have cardiotoxic effects and negatively affect myocardial microcirculation. Morphological signs of cytotoxic action in the myocardium include decreased density of the cardiomyocytes cytoplasm, loss of transverse striation which is more pronounced in the inner layers of the myocardium muscle fibers, with the outer layers being more compact. In this case no pronounced difference in toxic effect between lead nanoparticles of 26-34 nm (1-PbS) and 50-80 nm (2-PbS) was detected, except for single areas of necrosis after administration of 2-PbS. There were no specific differences in structural changes of the aorta wall when exposed to lead nanoparticles of different sizes. The main morphological manifestation of the disorders is the reduction of connective tissue content in adventitia in the NP 1-PbS, 2-PbS and disorganization of the elastic membranes of the media caused by the interstitial swelling, which result in a statistically significantly smaller aortic wall thickness by 14.3 %. However, no statistically significant difference was observed between the effects of PbS NPs and lead nitrate $Pb(NO_3)_2$.

Analysis of the effect of Thiocetam on the development of toxic damage to the heart and aorta showed cytoprotective effects in exposure to lead nitrate. The morphological structure of the aortic wall was more preserved despite its smaller thickness, whereas after the introduction of 2-PbS an increase in the thickness of the aorta was observed due to structural disorders, dissection of elastic membranes of t.media fibers in particular. Increased fibroblast density in t.adventitia in the NPs 1-PbS, 2-PbS and lead nitrate groups was considered to be a protective effect on aortic connective tissue morphogenesis. The effect of Thiocetam can be explained by its effect on the processes of peroxidation, the antioxidant system, especially superoxide dismutase, which reduces the cytotoxic effect of lead in vitro and in vivo [17].

Conclusions

The use of Thiocetam prevented dystrophic changes in the atrial epicardium and the adventitia of the aorta, indicating cytoprotective effect and influence on the connective tissue. In the post-exposure period without pharmacological correction, a tendency to spontaneous recovery of morphological changes of the heart and aortic walls under the influence of PbS NPs and lead nitrate was detected. However, as evidenced by morphometric parameters complete recovery does not occur neither with nor without Tiocetam.

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ОЦІНКА ПРОТЕКТОРНОЇ ДІЇ ПРЕПАРАТУ "ТІОЦЕТАМ" ЗА МОРФОЛОГІЧНИМИ ЗМІНАМИ В СЕРЦІ ТА СУДИНАХ ПРИ ВВЕДЕННІ НАНОЧАСТИНОК СВИНЦЮ РІЗНИХ РОЗМІРІВ (ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ)

Губар І.В., Сокуренко Л.М., Савосько С.І., Апихтіна О.Л., Яворовський О.П., Чайковський Ю.Б.

Впровадження нанотехнологій в сучасне виробництво призвело до нових викликів. Особливо важливим є питання розробки та впровадження в практику рекомендацій щодо попередження можливого негативного впливу наночасток свинцю на здоров'я населення. Нашу увагу привернув вітчизняний фармакологічний препарат "Тіоцетам", котрий має ноотропні, протиішемічні, антиоксидантні та мембраностабілізуючі властивості. Метою дослідження було вивчення протекторної дії фармакологічного препарату "Тіоцетам" при моделюванні у щурів лінії Вістар субхронічної інтоксикації сполуками свинцю різного розміру (за морфологічними змінами в серці та судинах). Експерименти проведено на щурах лінії Wistar (середня вага 160-180 г). При моделюванні інтоксикації були використані колоїдні розчини сульфіду свинцю, отримані методом хімічного синтезу, з використанням стабілізатора поліфосфату натрію, (PbS) з середнім розміром 26-34 нм (1-PbS) і 50-80 нм (2-PbS), та в іонній формі - нітрат свинцю $Pb(NO_3)_2$ (Pb3). Контрольній групі вводили фізіологічний розчин. Досліджувані речовини вводили внутрішньоочеревинно щоденно 5 разів на тиждень у дозі 0,94 мг/кг (у перерахунку на свинець). Токсичні

ефекти оцінювали після 60 введень (3 місяці) та через 1 місяць після припинення експозиції. Препарат "Тіоцетам" вводили щурам у постекспозиційному періоді щоденно натще внутрішньошлунково у дозі 250 мг/кг протягом 1 місяця. Вивчали гістологічні зрізи міокарда щурів та стінки аорти з наступним морфометричним аналізом та статистичною обробкою. У постекспозиційному відновлювальному періоді відмічено менший ступінь інтерстиційного набряку і кровонаповнення судин міокарда, що оцінено як регрес ушкодження. Після введення "Тіоцетаму" виявлено виражену поперечну посмугованість кардіоміоцитів, щільність колагенових волокон навколо кардіоміоцитів і мікросудин, що вказувало на захисний вплив фармакокорекції. Але у мікросудинах міокарда або аорти в експериментальних групах встановлено інфільтрацію лейкоцитів, також не виявлено різниці за морфометричними даними аорти між групами PbS NPs і $Pb(NO_3)_2$, хоча морфологія стінки аорти була досить збереженою. Застосування "Тіоцетаму" запобігало дистрофічним змінам у епікарді передсердь та адвентиційній оболонці аорти, що свідчить про цитопротекторну дію і вплив щодо сполучної тканини. У постекспозиційному періоді без фармакологічної корекції виявлено тенденцію до спонтанного відновлення морфологічних змін стінок серця та аорти під впливом PbS NPs і нітрату свинцю. Однак повноцінного відновлення ні при застосуванні "Тіоцетаму", ні без нього, не відбувається, про що свідчать морфометричні показники.

Ключові слова: *свинець, наночастинки, морфологічні зміни, аорта, міокард, профілактика інтоксикації, "Тіоцетам".*

ОЦЕНКА ПРОТЕКТОРНОГО ДЕЙСТВИЯ ПРЕПАРАТА "ТИОЦЕТАМ" ПО МОРФОЛОГИЧЕСКИМ ИЗМЕНЕНИЯМ В СЕРДЦЕ И СОСУДАХ ПРИ ВВЕДЕНИИ НАНОЧАСТИЦ СВИНЦА РАЗЛИЧНЫХ РАЗМЕРОВ (ЭКСПЕРИМЕНТАЛЬНОЕ ИССЛЕДОВАНИЕ)

Губар И.В., Сокуренко Л.М., Савосько С.И., Апыхтина Е.Л., Яворовский А.П., Чайковский Ю.Б.

Внедрение нанотехнологий в современное производство привело к новым вызовам. Особенно важным является вопрос разработки и внедрения в практику рекомендаций по предупреждению возможного негативного влияния наночастиц свинца на здоровье населения. Наше внимание привлек отечественный фармакологический препарат "Тиоцетам", который имеет ноотропные, противоишемические, антиоксидантные и мембраностабилизирующие свойства. Целью исследования было изучение протекторного действия фармакологического препарата "Тиоцетам" при моделировании у крыс линии Вистар субхронической интоксикации соединениями свинца разного размера (по морфологическим изменениям в сердце и сосудах). Эксперименты проведены на крысах линии Wistar (средний вес 160-180 г). При моделировании интоксикации были использованы коллоидные растворы сульфида свинца, полученные методом химического синтеза с использованием стабилизатора полифосфата натрия, (PbS) со средним размером 26-34 нм (1-PbS) и 50-80 нм (2-PbS), и в ионной форме - нитрат свинца $Pb(NO_3)_2$ (Pb3). Контрольной группе вводили физиологический раствор. Исследуемые вещества вводили внутрибрюшинно ежедневно 5 раз в неделю в дозе 0,94 мг/кг (в пересчете на свинец). Токсические эффекты оценивали после 60 введений (3 месяца) и через 1 месяц после прекращения экспозиции. Препарат "Тиоцетам" вводили крысам в постэкспозиционном периоде ежедневно натощак внутрижелудочно в дозе 250 мг/кг в течение 1 месяца. Изучали гистологические срезы миокарда крыс и стенки аорты с последующим морфометрическим анализом и статистической обработкой. В постэкспозиционном восстановительном периоде отмечены меньшая степень интерстициального отека и кровенаполнения сосудов миокарда, что оценено как регресс повреждения. После введения "Тиоцетама" выявлено выраженную поперечную исчерченность кардиомиоцитов, плотность коллагеновых волокон вокруг кардиомиоцитов и микрососудов, что указывало на защитное влияние фармакокоррекции. Но в микрососудах миокарда или аорты в экспериментальных группах установлено инфильтрацию лейкоцитов, также не выявлено разницы по морфометрическими данным аорты между группами PbS NPs и $Pb(NO_3)_2$, хотя морфология стенки аорты была достаточно сохранной. Применение "Тиоцетама" предотвращало дистрофические изменения в эпикарде предсердий и адвентициальной оболочке аорты, что свидетельствует о цитопротекторном действии и влиянии на соединительную ткань. В постэкспозиционном периоде без фармакологической коррекции выявлена тенденция к спонтанному восстановлению морфологических изменений стенок сердца и аорты под влиянием PbS NPs и нитрата свинца. Однако полноценного восстановления ни при применении "Тиоцетама", ни без него, не происходит, о чем свидетельствуют морфометрические показатели.

Ключевые слова: *свинец, наночастицы, морфологические изменения, аорта, миокард, профилактика интоксикации, "Тиоцетам".*

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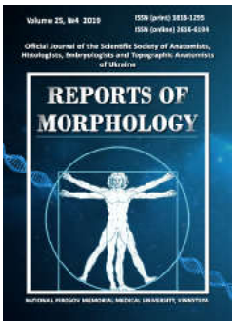

Structural features of the lower limb deep vein remodeling as a morphologic component in the pathogenesis of pulmonary thromboembolism in cancer patients

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Oncological patients are at high risk of developing thromboembolic complications, which is a manifestation of a complex set of symptoms - cancer. At the same time, the analysis of the literature shows that the question of the involvement of structural changes of the vascular wall in the pathogenesis of possible primary thrombus formation in cancer patients remains open. The aim of the study - to study the structural features of remodeling of the deep vein of the lower extremity as a morphological link of pathogenesis of pulmonary embolism in cancer patients. Retrospective analysis of 54 protocols of autopsy of deaths from cardiopulmonary shock caused by pulmonary embolism in 2014-2018 was performed. In parallel, all patients were determined the number of free-circulating endothelial cells in the citrate blood by Hladovez J. method, in modification of Sivak V.V. (2007). Statistical processing of digital data was performed using the software "Excel" and "STATISTICA" 6.0. In retrospective analysis of autopsy protocols, the highest proportion of pulmonary embolism was report in patients with cancer of the uterus and colon. Morphological changes of the deep vein of the lower extremities in cancer patients were manifested by endothelium desquamation and circular and focal muscular-fibrous hyperplasia of the intima, which caused disturbances of laminar flow of blood; muscular-fibrous atrophy with neovascularization of the middle membrane and sclerosis of vasa vasorum vessels of adventitia. The process of remodeling was also manifest by the inflammatory transformation of the vascular wall, the formation of obstructing and floating blood clots with their subsequent organization, vascularization and recanalization. The cause of intimal thickening, atrophy, and sclerosis with midbrain neovascularization is most likely a hypoxic mechanism of activation of transforming connective tissue growth factors that stimulate collagenogenesis and neoangiogenesis. Desquamation of endothelial cells can also be considered as a significant contributor to thrombus formation. Endothelial cells have a protective function aimed at eliminating damage to the vascular wall by thrombus formation and the development of fibrous intima hyperplasia. In addition, tumor cells are themselves capable of producing excess platelet growth factor, which causes intima proliferation. So, a component of pathomorphogenesis of pulmonary artery thromboembolism in cancer patients is a complex structural reconstruction of the wall of the deep vein of the lower extremity, which causes the development of its thrombosis. Deep vein remodeling in cancer patients is characterized by endothelial cell desquamation, intima and middle-membrane thickening and sclerosis in combination with vasa vasorum fibrous degeneration and perforant vein thrombosis. In response to hemodynamic disorders, compensatory remodeling develops: the combination of leiomyocyte atrophy with their hypertrophy and neovascularization of the middle membrane.

Keywords: *deep thigh vein, cancer, pulmonary embolism.*

Introduction

Oncological diseases are one of the causes of lower extremity vein thrombosis [8]. According to statistical studies, pulmonary embolism, as a complication of thrombosis of the lower extremities and pelvis, is recorded

in 35-40 people per 100 thousand population and is one of the leading causes of death in 15% of patients and in 43% of patients - a background for other fatal complications [6, 8, 10]. Among these patients, a significant percentage have patients with cancer, who are detected in 50% of cancer patients, which determines the course of the disease, requires dynamic monitoring and timely appointment of both pharmacological and mechanical means of preventing thrombus formation [7, 8, 13]. The high number of thromboembolic complications necessitates optimization of anticoagulant therapy and diagnostic methods [15, 16]. In most cancer patients, pulmonary embolism occurs through deep vein thrombosis of the lower extremities, or pelvis [4, 5, 11]. Among its most probable causes are: prolonged immobilization of patients, which causes impaired blood flow from the lower extremities, hypercoagulation caused by the procoagulative activity of tumor cells and the release of inflammatory cytokines and clotting factors; chemotherapy, hormone therapy, surgery and use of central venous catheter [1, 2, 5, 12]. At the same time, its morphogenesis at the specified pathology is not sufficiently elucidated. The analysis of the literature regarding remodeling of the deep vein of the lower extremities shows that there are no studies of the structural changes of the vascular wall in the pathogenesis of pulmonary artery thromboembolism in oncological patients, and the elucidation of its morphogenesis is of paramount importance in phlebology as a morphological basis of understanding.

The aim of this work is to find out structural features of remodeling of the deep vein of the lower extremity, as a morphological link of pathogenesis of pulmonary embolism in cancer patients.

Materials and methods

A retrospective analysis of 54 protocols of autopsy of deaths from cardiopulmonary shock caused by pulmonary embolism in 2014-2018 was conducted in the Ternopil Regional Clinical Oncology Center. In addition to necropsy data, morphological study of deep vein biopsies obtained from different topographic areas of the lower extremity was performed in 12 operations for acute ascending thrombophlebitis in cancer patients.

For the preparation of micropreparations standard protocols of sealing and dehydration of previously fixed in 10% solution of neutral formalin tissues were used, followed by pouring into paraffin and preparation of sections [3]. Deparaffin sections were stained with hematoxylin and eosin, Malory trichrome, resorcinol-fuchsine according to Weigert, alcian blue, and PAS reaction.

Submicroscopic examination was performed on biopsy material only. The biopsies of the veins were pre-fixed in a 2.5% solution of glutaraldehyde with an active reaction of medium pH 7.2-7.4 prepared on Millonig phosphate buffer. Postfixation was carried out with a 1% solution of osmium tetroxide on Millonig buffer for 60 minutes, after which the material was dehydrated in alcohols and acetone and poured into epoxy resins according to conventional methods [3]. Ultra-thin sections made on a UMTP-7 ultramicrotome were stained with a 1% aqueous solution of uranyl acetate, counterstained with lead citrate according to the Reynolds method, and studied in a PEM-125K electron microscope. Semi-thin sections were stained with methylene blue [3].

In all patients, the number of free-circulating endothelial cells in the citrate blood was determined according to the method of Hladovez J., in modification by Sivak V.V. [14].

In the work with histological preparations and semi-thin sections used microscopes SEOSCAN, Lumam R-8, MBI-15. Images from microscopes were displayed on a computer monitor using a VISION Color CCD Camera and Inter VideoWinDVR program.

Statistical processing of digital data was carried out using the software "Excel" ("Microsoft", USA) and "STATISTICA" 6.0 ("Statsof", USA).

Results

In retrospective analysis of autopsy protocols, the highest proportion of pulmonary artery thromboembolism was reported in women (61.1% of observations versus 38.9% in men). The mean age of deceased women was 61.93±1.51 years and 62.44±2.61 for men. It is noteworthy that in all 54 deaths pulmonary embolism complicated 5-6 days postoperatively. It was most frequently observed in patients with malignant epithelial neoplasms of the uterus (22.2%), large intestine: 13.0% - rectal, 7.4% - colon, 5.6% - rectosigmoid and urinary bladder (9.3%). Patients with gastric cancer (7.4%), ovaries (7.4%), prostate (5.6%), thyroid (1.8%), and pancreas (1.8%) were slightly less likely. It is worth noting that a high percentage (18.5%) of thromboembolism is also reported in patients with bronchial cancer and lung cancer. This can be explained by the presence of chronic right ventricular heart failure with varicose veins of the lower extremities. In all these cases (n=54), autopsy revealed signs of phlebotrombosis of the deep veins of the lower leg and thigh with impaired blood flow caused by occlusion of blood clots of various manifestations of structural organization. Of these, in five cases, pathomorphologically, they corresponded to fresh red blood clots that closely connected with the intimate filaments of fibrin, and in five cases the red blood clots were freely placed in the lumen of the vein (floating blood clots). In these cases, all layers of the vein are swollen, with the branching of all its structures and diffuse neutrophilic infiltration (Fig. 1). The thrombotic masses were dominated by platelets, erythrocytes, fibrin filaments and leukocytes. We interpret the presence of neutrophils as the cause of possible lysis of the thrombus, as well as thromboembolic complications. Red blood clots were also detected in the lumen of the perforated veins (Fig. 2).

In the remaining 44 cases, occlusion of the lumen of the vein with organized blood clots with signs of fibrosis, recanalization and revascularization was registered.

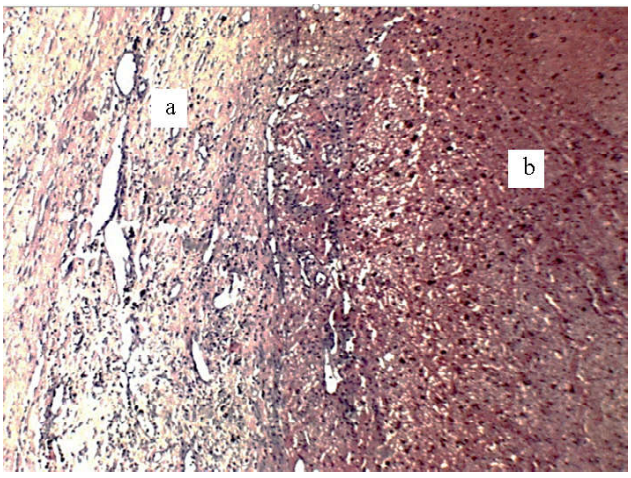

Fig. 1. Edema and diffuse neutrophilic infiltration of the vein wall (a), red blood clot (b). Histological section of deep vein of thigh. Staining with hematoxylin and eosin. x100.

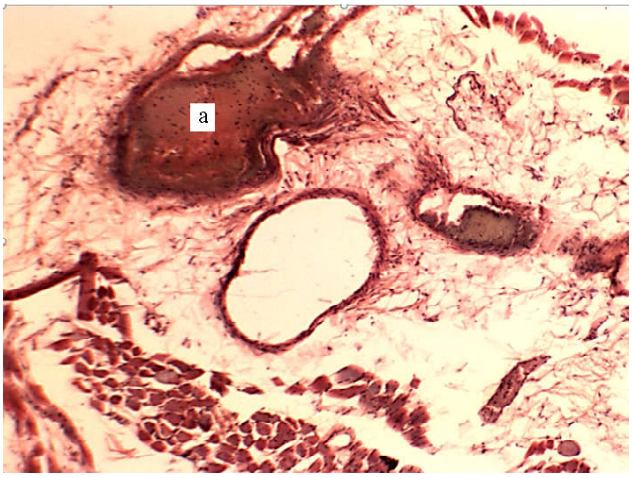

Fig. 2. Red blood clot (a) in the lumen of the perforated vein. Histological section of thigh soft tissue. Staining with hematoxylin and eosin. x100.

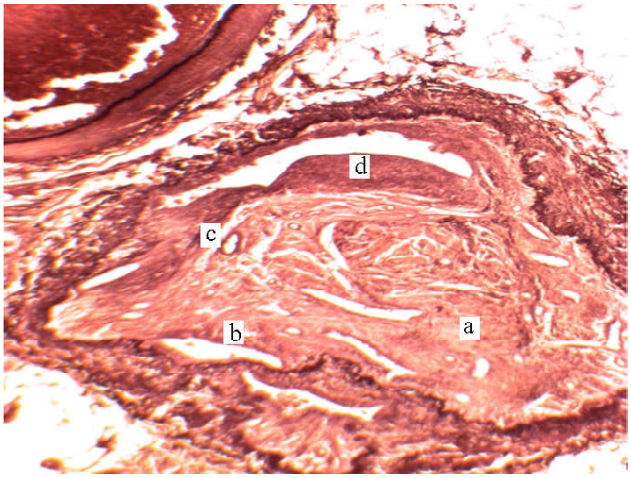

Fig. 3. Fibrosis (a), recanalization (b), revascularization (c), and secondary thrombosis (d). Histological section of deep vein of thigh. Weigert stain. x100.

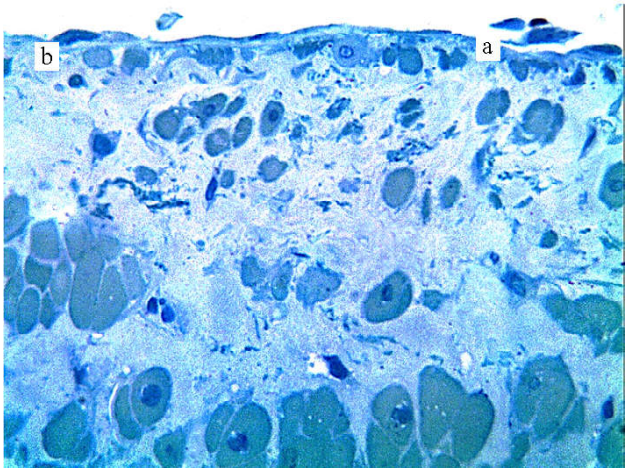

Fig. 4. Desquamation of the endothelial cell (a) and pyknosis of its nucleus (b). Semi-thin section of deep vein of thigh. Methylene blue stain. x200.

The lumens of the newly formed canals are enlarged with the near wall layer of fibrin, which can be interpreted as a morphological manifestation of secondary thrombosis (Fig. 3). The lumps of newly formed blood clots have slit-like outlines and chaotic placement. The wall of the deep vein of the hip is sclerosed. Inner elastic membrane wavy, thickened, soldered to the structural components of the thrombus. The thickness of the inner layer of such veins was 14.93±1.82 μm, the average 65.74±14.31 μm and the outer 25.82±8.43 μm, which indicates the thickening of all its layers.

The endothelial cells had elongated outlines with a large hyperchromic nucleus. Their desquamation was registered histologically (Fig. 4).

Quantitative analysis of free-circulating blood endothelial cells revealed that in acute thrombosis their level was 9.42±0.51 $x10^4$/l, and in cases of organized thrombus - 6.53±0.20 $x10^4$/l ($p<0.001$). Microscopically desquamated endothelial cells are polymorphic. Cells with pyknosis and rexis of the nucleus, as well as with karyolysis, cytoplasm edema and partial fragmentation were identified.

Electron microscopy revealed that the elongated endothelial cells were adjacent to a wide, swollen, area of collagen fiber accumulation. In the swollen, enlightened cytoplasm, there are numerous organelles that are destructively altered. The tubules of the granular endoplasmic reticulum are expanded and deformed, with the formation of irregular cavities. Damage to the mitochondria is accompanied by significant matrix enlightenment and destruction of the cristae. Primary and secondary lysosomes are presented and are freely located both in the cytoplasm and near the Golgi complex. The nuclei are star-shaped, with irregular outlines, due to the deep torsion of the karyolemma. In the center of the nucleoplasm there is an electron-transparent karyoplasm. Wide areas of condensed osmiophilic karyoplasm located below the karyoplasm (Fig. 5).

We recorded fibrotic remodeling of the intima in all cases of observation, both in the prethrombotic and

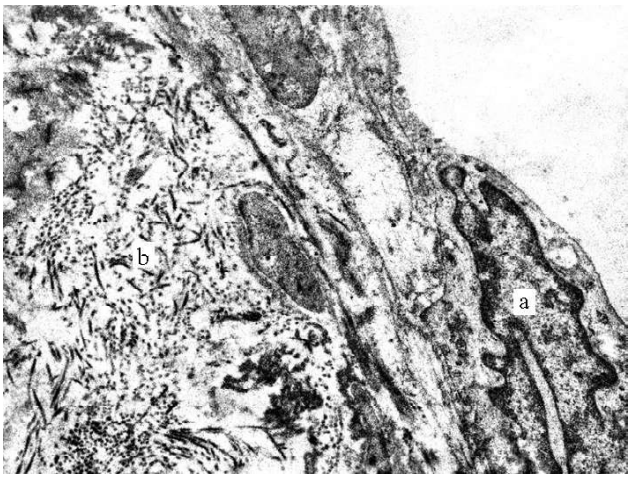

Fig. 5. Submicroscopic organization of the intima of the deep vein of a patient with colon cancer. Destructively altered endothelial cell (a), sclerosis (b). x9000.

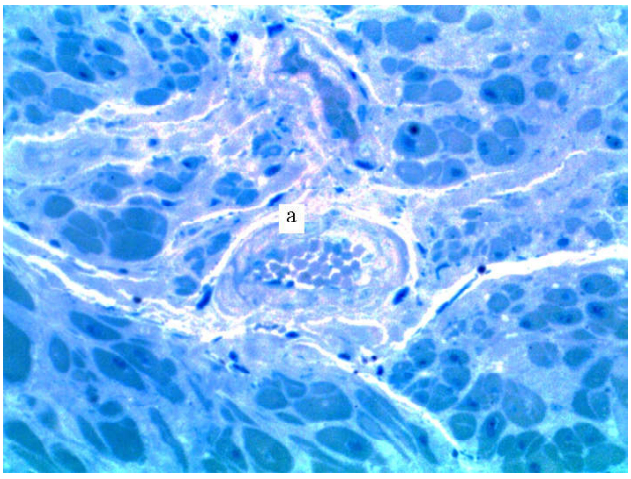

Fig. 6. Intramural capillaries in the middle layer of the deep vein of the thigh (a). Semi-thin section. Methylene blue stain. x200.

postthrombotic segment of the investigated vein, which indicates the diffuse manifestation of the pathological process. Preferably, the thickening of the fibrous intima was circular or local in nature and combined with a thickening at the border of the middle layer and subendothelial space, in which noted a large number of activated fibroblasts with elongated hyperchromic nuclei that produce excessive amount of extracellular matrix, as evidenced by the increased positive response to the presence of non-sulfated and sulfated glycosaminoglycans when stained with alcian blue and PAS reaction.

Medium layer remodeling in pre- and post-thrombotic vein segments was manifested by severe sclerosis with leiomyocyte atrophy, in combination with their secondary hypertrophy. A characteristic feature of the middle layer is the presence of a neovascularization process (Fig. 6). The lumps of the arterioles are full-blooded, the endothelium with an enlarged, hyperchromic nucleus.

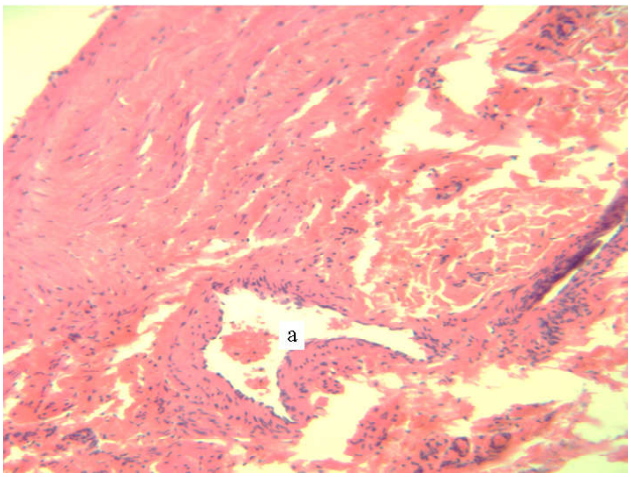

Fig. 7. Dilatation of lumen and wall sclerosis of vasa vasorum (a). Histological section of deep vein. Staining with hematoxylin and eosin. x120.

Changes in the outer layer of the venous wall were manifested by severe sclerosis. Particular attention is paid in all cases the presence of dilatation lumen vasa vasorum, plethora and walls sclerosis (Fig. 7).

Discussion

Pulmonary embolism, as a complication of thrombosis of the lower extremities and pelvis, is recorded in 35-40 people per 100 thousand population and is one of the main causes of death in 15% of patients and in 43% of patients - a background for other fatal complications [6, 8, 10]. The analysis of the literature regarding remodeling of the deep vein of the lower extremities shows that there are no studies of the structural changes of the vascular wall in the pathogenesis of pulmonary embolism in oncological patients.

We have found that the degree of endothelial cell desquamation in the process of postthrombophlebitic syndrome depended on the process prescription. Damaged endotheliocytes underwent conjugation, exposed intima, circulated with blood flow, which can be considered the structural basis of thrombus formation. According to Okhotnikova O.M. et al. [9], it is the endothelial cells that have a protective function aimed at eliminating damage to the vascular wall by thrombus formation and the development of fibrous hyperplasia of the intima, and Rozanov I.D. et al. [11] state that tumor cells are themselves capable of producing excess platelet-derived growth factor in excess, which causes intima proliferation.

We do not exclude that the structural changes of the deep vein wall outside the thrombus segment have a significant role in the development of postthrombophlebitic syndrome. This is evidenced by the remodeling of all layers of the vascular wall in the pre- and post-thrombotic segments of the vein: internal, middle and external.

The morphological changes of the proximal and distal segments with respect to deep vein thrombosis in patients

with cancer are manifested by circular and focal muscular-fibrous intima hyperplasia with neovascularization of the middle layer. Morphogenetically, it can be assumed that the process of vein remodeling begins with thrombophlebitis with the subsequent development of thrombus organization and recanalization and vascularization.

The absence of dystrophic-inflammatory processes in the inner and middle layers of the peritrombotic segments of the vein allows us to consider intima thickening and neovascularization as independent processes. Basically, their fibrotic degeneration have probably a hypoxic mechanism, which is confirmed by sclerosis vasa vasorum, and neovascularization should be considered a compensatory process.

Conclusions

1. The component of pathomorphogenesis of pulmonary artery thromboembolism in cancer patients is a complex structural reconstruction of the wall of the deep vein of the lower extremity, which causes the development of its thrombosis.

2. Deep vein remodeling in cancer patients is characterized by endothelial cell desquamation, intima and middle layer thickening and sclerosis in combination with vasa vasorum fibrous degeneration and perforant vein thrombosis.

3. In response to hemodynamic disorders, compensatory remodeling develops: the combination of leiomyocyte atrophy with their hypertrophy and neovascularization of the middle layer.

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СТРУКТУРНІ ОСОБЛИВОСТІ РЕМОДЕЛЮВАННЯ ГЛИБОКОЇ ВЕНИ НИЖНЬОЇ КІНЦІВКИ, ЯК МОРФОЛОГІЧНОЇ ЛАНКИ ПАТОГЕНЕЗУ ТРОМБОЕМБОЛІЇ ЛЕГЕНЕВОЇ АРТЕРІЇ ХВОРИХ НА РАК

Боднар П.Я.

Онкологічні хворі належать до групи високого ризику розвитку тромбоемболічних ускладнень, що є проявом складного симптомокомплексу - ракової хвороби. Водночас, аналіз літератури свідчить, що питання участі структурних змін судинної стінки в патогенезі можливого первинного тромбоутворення у хворих на рак залишається відкритим. Мета дослідження - з'ясувати структурні особливості ремоделювання глибокої вени нижньої кінцівки як морфологічної ланки патогенезу тромбоемболії легеневої артерії у хворих на рак. Проведено ретроспективний аналіз за 2014-2018 рр. 54 протоколів розтину померлих від кардіопульмонального шоку, зумовленого тромбоемболією легеневої артерії. Окрім аналізу некропсій проведено морфологічне дослідження біопсій глибокої вени, отриманих з різних топографічних ділянок нижньої кінцівки при 12 операціях з приводу гострого висхідного тромбофлебіту у хворих на рак. Паралельно усім пацієнтам визначали кількість вільноциркулюючих в цитратній крові ендотеліоцитів за методикою Hladovez J., у модифікації Сівак В.В. (2007). Статистичне опрацювання цифрових даних здійснювали за допомогою програмного забезпечення "Excel" та "STATISTICA" 6.0. При ретроспективному аналізі протоколів розтину встановлено, що найбільша частка тромбоемболії легеневої артерії була

зареєстрована у хворих на рак матки та ободової кишки. Морфологічні зміни глибокої вени нижніх кінцівок у хворих на рак проявлялися десквамацією ендотелію з циркулярною та вогнищевою м'язово-фіброзною гіперплазією інтими, що викликало порушення ламінарності току крові; м'язово-фіброзною атрофією з неоваскуляризацією середньої оболонки і склерозом судин vasa vasorum адвентиції. Процес ремоделювання проявлявся також запальною трансформацією судинної стінки, утворенням обтуруючих і флотуючих тромбів із подальшою їх організацією, васкуляризацією та реканалізацією. Причиною потовщення інтими, атрофії та склерозу з неоваскуляризацією середньої оболонки, найімовірніше, є гіпоксичний механізм активації трансформуючих факторів росту сполучної тканини, який стимулює колагено- та неоангіогенез. Десквамацію ендотеліоцитів теж можна вважати суттєвим фактором сприяння тромбоутворенню. Саме ендотеліоцитам належить захисна функція, спрямована на усунення пошкодження судинної стінки шляхом тромбоутворення та розвиток фіброзної гіперплазії інтими. Окрім цього, пухлинні клітини самі по собі здатні продукувати в надлишку тромбоцитарний фактор росту, що спричиняє проліферацію інтими. Таким чином, складовою ланкою патоморфогенезу тромбоемболії легеневої артерії у хворих на рак є складна структурна перебудова стінки глибокої вени нижньої кінцівки, що сприяє розвитку тромбозу. Ремоделювання глибокої вени у хворих на рак характеризується десквамацією ендотеліоцитів, потовщенням і склерозом інтими та середньої оболонки у поєднанні із фіброзною дегенерацією vasa vasorum та тромбозом перфорантних вен. У відповідь на гемодинамічні порушення розвивається компенсаторне ремоделювання: поєднання атрофії лейоміоцитів з їх гіпертрофією і неоваскуляризація середньої оболонки.

Ключові слова: *глибока вена стегна, рак, тромбоемболія легеневої артерії.*

СТРУКТУРНЫЕ ОСОБЕННОСТИ РЕМОДЕЛИРОВАНИЯ ГЛУБОКОЙ ВЕНЫ НИЖНЕЙ КОНЕЧНОСТИ, КАК МОРФОЛОГИЧЕСКОГО ЗВЕНА ПАТОГЕНЕЗА ТРОМБОЭМБОЛИИ ЛЕГОЧНОЙ АРТЕРИИ БОЛЬНЫХ РАКОМ

Боднар П.Я.

Онкологические больные относятся к группе высокого риска развития тромбоэмболических осложнений, которые являются проявлением сложного симптомокомплекса - раковой болезни. Вместе с тем, анализ литературы свидетельствует, что вопрос участия структурных изменений сосудистой стенки в патогенезе возможного первичного тромбообразования у больных раком остается открытым. Цель исследования - выяснить структурные особенности ремоделирования глубокой вены нижней конечности, как морфологического звена патогенеза тромбоэмболии легочной артерии у больных раком. Проведен ретроспективный анализ за период с 2014-2018 гг. 54 протоколов вскрытия умерших от кардиопульмонального шока, обусловленного тромбоэмболией легочной артерии. Кроме анализа некропсий проведено морфологическое исследование биопсий глубокой вены, полученных с разных топографических участков нижней конечности при 12 операциях по поводу острого восходящего тромбофлебита у больных раком. Параллельно всем пациентам определяли количество свободно циркулирующих в цитратной крови эндотелиоцитов по методике Hladovez J., в модификации Сивак В.В. (2007). Статистическую обработку цифровых данных осуществляли с помощью программного обеспечения "Excel" и "STATISTICA" 6.0. При ретроспективном анализе протоколов вскрытия установлено, что наибольшая доля тромбоэмболии легочной артерии зарегистрирована у больных раком матки и ободочной кишки. Морфологические изменения глубокой вены нижних конечностей у больных раком проявлялись десквамацией эндотелия с циркулярной и очаговой мышечно-фиброзной гиперплазией интимы, что вызывало нарушение ламинарности тока крови; мышечно-фиброзной атрофией с неоваскуляризацией средней оболочки и склерозом сосудов vasa vasorum адвентиции. Процесс ремоделирования проявлялся также воспалительной трансформацией сосудистой стенки, образованием обтурационных и флотирующих тромбов с последующей их организацией, васкуляризацией и реканализацией. Причиной утолщения интимы, атрофии и склероза с неоваскуляризацией средней оболочки, скорее всего, является гипоксический механизм активации трансформирующих факторов роста соединительной ткани, который стимулирует колагено- и неоангиогенез. Десквамацию эндотелиоцитов тоже можно считать существенным фактором содействия тромбообразованию. Именно эндотелиоцитам принадлежит защитная функция, направленная на устранение повреждения сосудистой стенки путем тромбообразования и развитие фиброзной гиперплазии интимы. Кроме этого, опухолевые клетки сами по себе способны продуцировать в избытке тромбоцитарный фактор роста, что вызывает пролиферацию интимы. Таким образом, составным звеном патоморфогенеза тромбоэмболии легочной артерии у больных раком является сложная структурная перестройка стенки глубокой вены нижней конечности, что вызывает развитие тромбоза. Ремоделирование глубокой вены у больных раком характеризуется десквамацией эндотелиоцитов, утолщением и склерозом интимы и средней оболочки в сочетании с фиброзной дегенерацией vasa vasorum и тромбозом перфорантных вен. В ответ на гемодинамические нарушения развивается компенсаторное ремоделирование: сочетание атрофии лейомиоцитов с их гипертрофией и неоваскуляризацией средней оболочки.

Ключевые слова: *глубокая вена бедра, рак, тромбоэмболия легочной артерии.*

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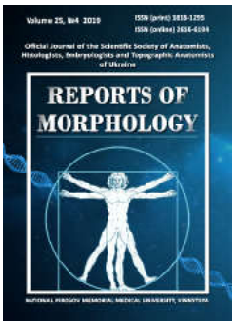

Histological picture in the skin of rats within a month after burn II-III degree against the background of the introduction for first 7 days 0.9% NaCl solution

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Burn disease is a complex of pathological changes that occur in the body due to the action of a thermal agent and are life-threatening. The problem of skin burns still remains relevant today. Insufficiently studied features of pathogenesis and methods of treatment of thermal trauma are the cause of considerable interest of scientists in this problem. The aim is to study the features of microscopic changes in the skin of rats during the month after grade II-III burn on the background of the introduction of the first 7 days of 0.9% NaCL solution. The studies were performed on 360 laboratory white male rats weighing 155-160 g. During the experiment, the animals were divided into 4 groups: 1st, 2nd groups - rats without thermal trauma infused with 0.9% NaCl solution and HAES-LX-5% at a dose of 10 ml/kg. In the 3rd, 4th groups, rats were infused with 0.9% NaCl solution and HAES-LX-5% at a dose of 10 ml/kg after skin burns. Burning skin damage was caused by applying to the lateral surfaces of the trunk of rats for 10 seconds four copper plates, heated in water at a constant temperature of 100°C. Histological preparations were prepared by standard procedure and examined using an OLYMPUS BH-2 light microscope. Conducted microscopic studies of the skin of animals after thermal trauma under the conditions of application of 0.9% NaCl solution found that in the early stages of the experiment (1, 3, 7 days) compensatory and adaptive changes of its structural components are combined with signs of destructive disorders. In the later periods of thermal trauma (14, 21 and 30 days), the destructive changes of the structural components of the skin in the lesion area deepen, and the process becomes irreversible.

Keywords: *skin burns, histological changes, necrosis, 0.9% NaCL solution.*

Introduction

Despite the significant advances in modern medicine and the improvement of methods of diagnosis and treatment, the problem of skin burns is still relevant today. Scientists have linked this fact to an increase in the frequency of burns in the home, at work, and in connection with military regional conflicts [13, 20, 21, 23]. In addition, the question remains regarding the features of the pathogenesis of burn disease and its treatment [3, 6, 12]. Special attention of scientists to thermal skin damage is also explained by the fact that it leads to considerable complications and mortality [9-11, 18, 22].

In recent years, the methods of treatment of thermal trauma have been significantly improved, but in most cases and today assistance to patients with severe burns consists in the direct treatment of local skin lesions, despite the well-known fact that the pathogenesis of the acute period of burn disease lies in systemic disorders [3, 4, 6, 12, 15-17, 19]. Therefore, the problem of thermal trauma is multifaceted, requires a comprehensive solution and a more detailed study of the features of its course.

Materials and methods

The studies were performed on 360 laboratory white male rats weighing 155-160 g. During the experiment, the animals were divided into 4 groups: 1st, 2nd groups - rats without thermal trauma infused with 0.9% NaCl solution and HAES-LX-5% at a dose of 10 ml/kg. In the 3rd, 4th groups, rats were infused with 0.9% NaCl solution and HAES-LX-5% at a dose of 10 ml/kg after skin burns. Burning skin damage was caused by applying to the pre-depilated lateral surfaces of the trunk of rats for 10 seconds four copper plates (two plates on each side, each with a surface

area of 13.86 cm^2), which were pre-heated for 6 minutes in water at a constant temperature of 100°C. [1, 7]. The total area of skin lesion in rats was 21-23%. All solutions were introduced into the inferior vena cava after catheterization under aseptic conditions through a femoral vein at a dose of 10 ml/kg body weight of the animal. Shaving of the lateral surfaces of rats' trunk, catheterization of veins, staging of skin burns, and decapitation of animals were performed under intravenous propofol anesthesia (calculated at 60 mg/kg body weight). For further in-depth study, we selected skin changes at 1, 3, and 7, 14 21, and 30 days from the start of the experiment. For histological examination, skin fragments were fixed in 10% neutral formalin solution, washed in running water, dehydrated in a battery of alcoholic solutions of increasing concentration, and enclosed in a steamer [8]. Sections 4-6 μm thick were made on a rotary microtome, placed on slides, stained with eosin hematoxylin after standard wiring, and poured into Canadian balm. Histological specimens were examined in an OLYMPUS BH-2 light microscope using x10 and x40 lenses and x10 eyepieces.

Results

In rats which, after thermal burns of the skin, a 0.9% solution of NaCl at a dose of 10 ml per kg was injected, after 1 day the structural signs of the condition of the burn wound indicate burn IIB degree. In the affected area of the skin revealed coagulation necrosis of the epidermis and dermis, and in the hypodermis, there were signs of edema.

In the epidermis, the layered organization of keratinocytes is impaired, necrobiotic changes are present, cell boundaries are not visualized. The intercellular spaces between the epidermocytes are enlarged. The basement membrane is loosened, the collagen fibers in it are destructed, there are areas of detachment of the epidermis from the dermis, which leads to disruption of its barrier function.

In this period of experiment, there is swelling of the papilla and mesh layers of the dermis and their infiltration by leukocytes. The papillary layer of the dermis in the burn area is smoothed, without well-defined papillae. The luminescence of the vessels of the hemomicrocirculatory bed within the burn wound of the skin is considerably enlarged, full-blooded, containing blood clots. There was pronounced interstitial swelling and hemorrhage around the hemocapillaries. The lymphatic capillary glands were enlarged, containing lymph and blood cells. Collagen fibers around the lymphatic capillaries are homogenized. Also revealed disorders of blood circulation, in the form of stasis, marginal standing and diapedesis of leukocytes through the walls of the blood vessels of the circulatory microcirculatory bed, as well as plasmorrhagia. The epitheliocytes of the hair follicles and sebaceous glands in the burn zone are necrobiotically and dystrophically altered (Figs. 1, 2).

In rats which, after thermal burns of the skin, 0.9% NaCl solution was injected after 1 day also showed dystrophic changes in skin areas located on the border with the burn zone. In the spinous layer of the epidermis, the interepithelial spaces are enlarged. The lymphocyte count is increased in all layers of the epidermis.

In the dermis of the marginal area of the wound there is no differentiation into papillary and mesh layers. Collagen fibers with signs of edema, blood vessels full-blooded (Fig. 3).

Histologically, for 3 days of the experiment in the group of animals with burns and the introduction of 0.9% NaCl solution in the central and peripheral areas of the wound, destructive changes associated with tissue necrosis increase. The keratinocytes of all layers of the epidermis are damaged, in many cells pyknosis and karyorrhexis of

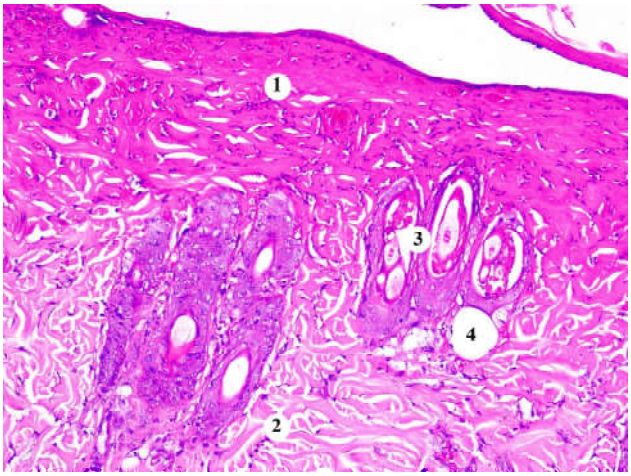

Fig. 1. Histological changes in the skin of the rats 1 day after burn, which was administered 0.9% NaCl solution: 1 - coagulation necrotizing epidermis and papillary layer of the dermis, 2 - mesh layer of the dermis, 3 - destructively altered skin appendages, 4 - enlarged lymphatic capillary. Staining with hematoxylin and eosin. x200.

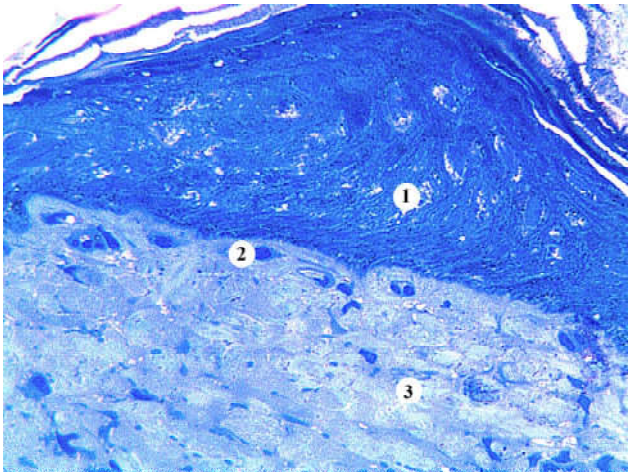

Fig. 2. Microscopic changes in the skin of the rat, 1 day after burn, which was administered 0.9% NaCl solution: 1 - necrotized epidermis, 2 - hemostasis in the capillary, 3 - coagulated collagen fibers of the dermis. Semi-thin section. Methylene blue staining. x400.

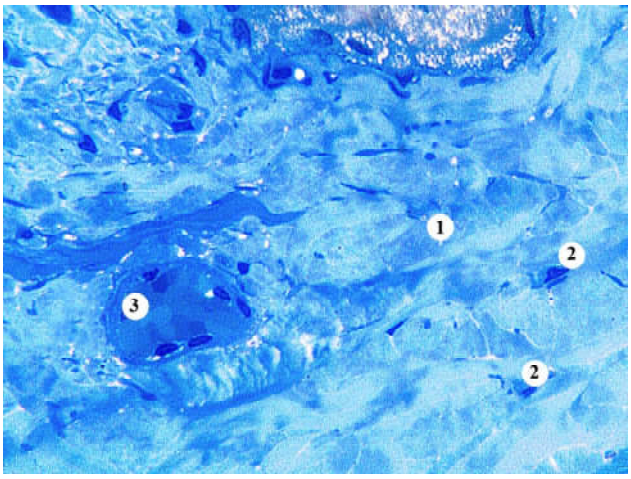

Fig. 3. Microscopic changes in the skin of the rat, 1 day after burn, which was administered 0.9% NaCl solution: 1 - swollen collagen fibers, 2 - fibrocytes, 3 - hemostasis in the enlarged capillary. Semi-thin section. Methylene blue staining. x400.

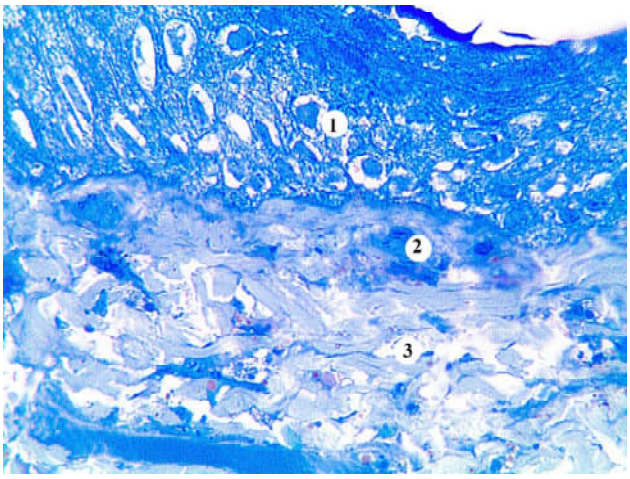

Fig. 4. Microscopic changes in the skin of the rat, 3 days after burn, which was administered 0.9% NaCl solution: 1 - necrotized keratinocytes of the epidermis, 2 - damaged and blood-filled capillaries, 3 - fragmented collagen fibers. Semi-thin section. Methylene blue staining. x400.

the nuclei are present (Fig. 4).

Fibroblasts, numerous rod-like neutrophils, eosinophils, macrophages, lymphocytes, and a considerable number of tissue basophils with phenomena of degranulation are present around and in the center of the damage zone among fibrous connective tissue structures. Most cells of the hair follicles and sebaceous glands are dystrophically altered. The blood vessels of the blood and lymphatic vessels are enlarged, sometimes with a stasis of shaped elements in their lumen.

Perifocal formation of the epidermal wedge is observed, in which most of the cells are in a state of dystrophy or destroyed. In numerous places, it is detached from connective tissue, and there are hemorrhages underneath (Fig. 5).

Microscopically for the 7th day of the experiment under the conditions of introduction of 0.9% NaCl solution, the burn area is covered by a scab, formed from the destroyed epidermocytes, fibrin, and blood cells. In many areas, the crust goes far beyond the damage and covers a somewhat thickened layer of the epithelium. In such places, the epidermis is deformed, sometimes in the form of an amorphous mass, densely infiltrated by cellular detritus, neutrophils, and macrophages.

In the papillary layer of the dermis, lesions of the collagen fibers are homogenized, eosinophilic, leukocyte infiltrated, among which rod-core neutrophils prevailed. Epithelial cells composed sheath of hair follicles are in a state of necrosis. All these altered structures form a rough solid crust that is closely soldered to the underlying mesh layer of the dermis. A demarcation shaft (Fig. 6) is located between the bark and the preserved part of the dermis. The luminescence of blood vessels contained blood clots, hemorrhages were found around the vessels.

At the periphery of the burn wound, the epidermis is hypertrophied, with the formation of single new capillaries due to the proliferation of endothelial cells in their walls. The gaps of most hemocapillaries are enlarged with dystrophic and necrobiotic altered endothelial cells. Hemorrhage,

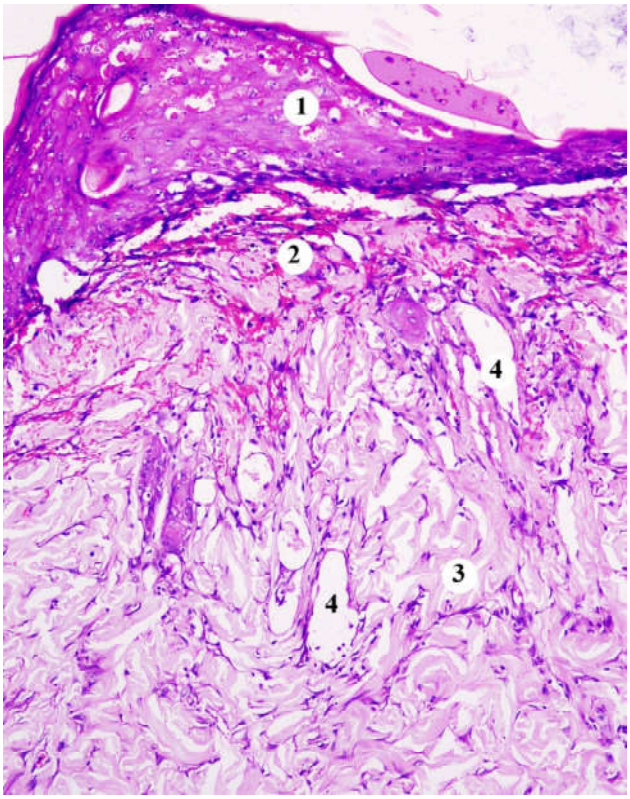

Fig. 5. Histological changes of the marginal area of the wound at 3 day after burn, with the introduction of 0.9% NaCl solution: 1 - epidermal wedge, 2 - hemorrhage in the papilla of the dermis, 3 - mesh layer of the dermis, 4 - enlarged lymphatic capillaries. Staining with hematoxylin and eosin. x100.

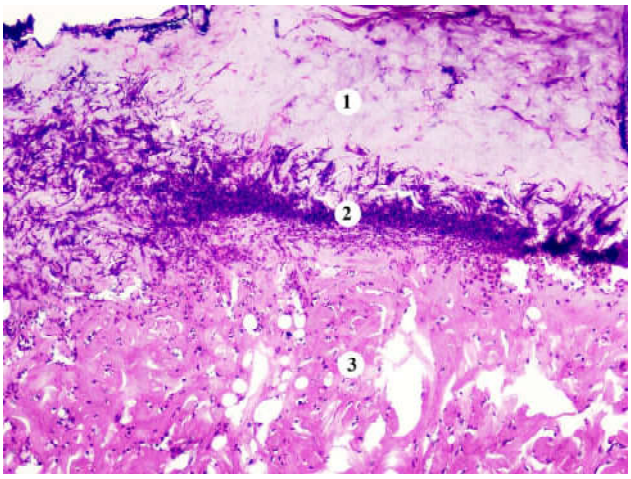

Fig. 6. Histological changes of the central area of the wound for 7 days after burn, with the introduction of 0.9% NaCl solution: 1 - scab, 2 - demarcation shaft, 3 - damaged and infiltrated leukocytes of the fiber of the mesh layer of the dermis. Staining with hematoxylin and eosin. x200.

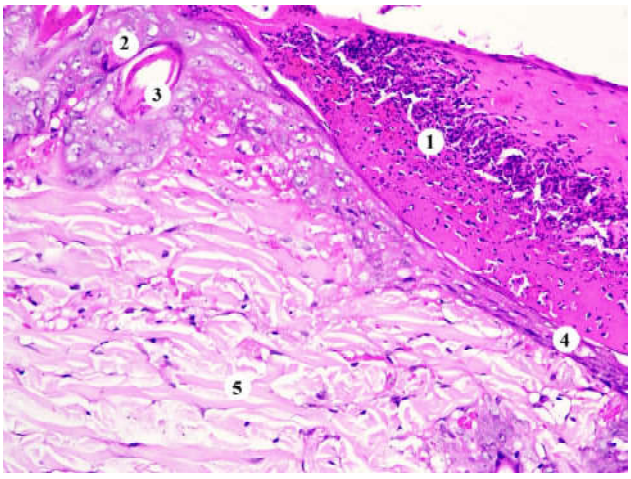

Fig. 7. Histological changes of the lesion site at 14 days after burn, with the introduction of 0.9% NaCl solution: 1 - scab, 2 - thickening of the epidermis, with the participation of 3 - hair roots, 4 - epithelial regenerate, 5 - dermal collagen fibers. Staining with hematoxylin and eosin. x200.

leukocyte infiltration and pronounced interstitial edema are found around the vessels.

Microscopically, for 14 days after thermal trauma under the conditions of introduction of 0.9% NaCl solution in the wound area, a dense scab with a clearly limited area of necrosis was observed. Under the scab revealed areas of suppuration and a thin layer of epidermal regenerate, in which there is no layered differentiation of keratinocytes. The bottom of the wound is filled with a dense connective tissue that contains parallel directed bundles of collagen fibers between which are isolated single fibroblasts. The number of blood capillaries was lower than in the previous study period.

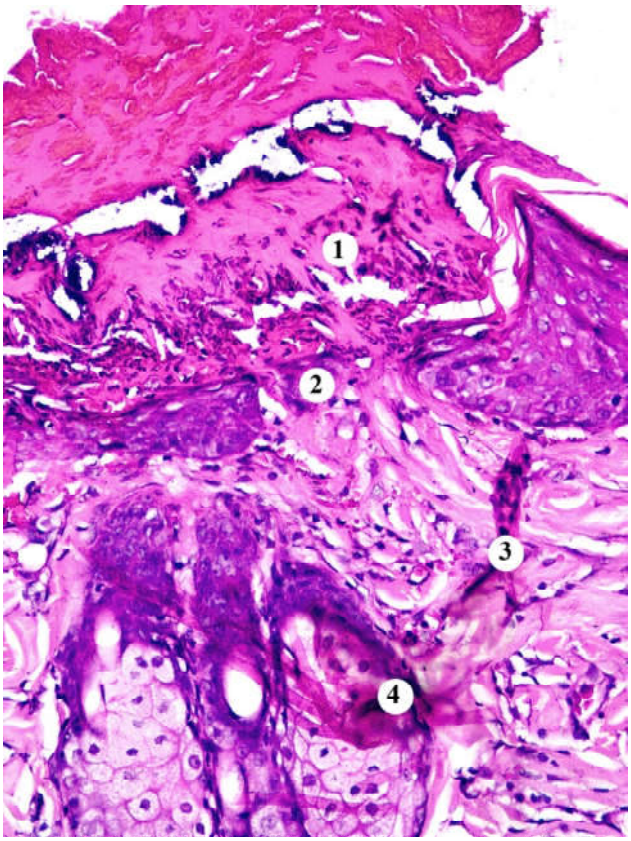

Fig. 8. Histological changes in the area of damage at 21 days after burn, with the introduction of 0.9% NaCl solution: 1 - scab, 2 - epithelial regenerate, 3 - dermis, 4 - skin appendages. Staining with hematoxylin and eosin. x200.

In the peripheral areas of the wound present expressed marginal epithelialization, epithelial heel in the form of a wedge grows under the scab. In the marginal areas, the wounds in the epidermis were differentiated between the basal, spinous, granular and stratum corneum layers. In the dermis of the skin, the papillary and mesh layers are poorly differentiated, the papillae are smoothed (Fig. 7).

On the 21st day after thermal trauma, the animals of this experimental group had a scab exfoliation. The epithelialization of the wound is more pronounced than in the previous study, but incomplete. In the center of the burn wound, areas of the epithelial layer were identified as a wedge, in which no differentiation into layers was evident (Fig. 8).

The formed connective tissue of the dermis lacks hair follicles and sebaceous glands. Leukocyte infiltration and slight interstitial edema are present around the vessels. In the newly formed connective tissue at the bottom of the wound, collagen fibrils have a parallel direction to the skin surface, and the blood capillaries are located along the fibers, indicating the maturation of the newly formed granulation tissue in the deep layers of the wound. The lumen of the lymphatic vessels is widened (Fig. 9).

In rats, which after the burn of the skin for 7 days was injected with 0.9% NaCl solution, for 30 days of the study the wound is covered with a layer of epidermis. The papillary

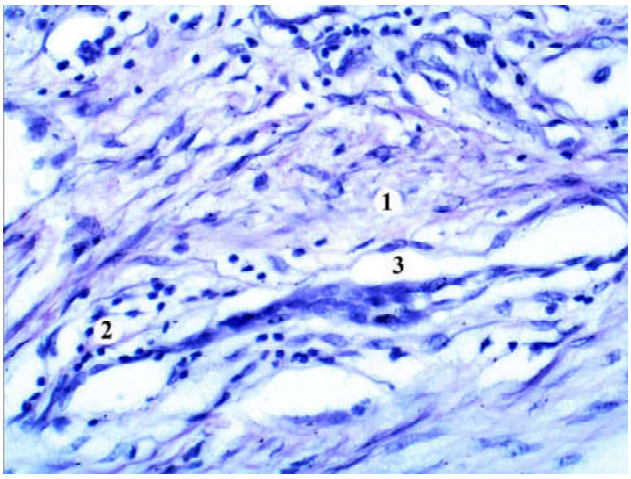

Fig. 9. Microscopic changes of rat skin dermis for 21 days after burn, with 0.9% NaCl solution administration: 1 - collagen fibrils, 2 - leukocyte infiltration, 3 - lymphatic capillary. Semi-thin section. Methylene blue. x200.

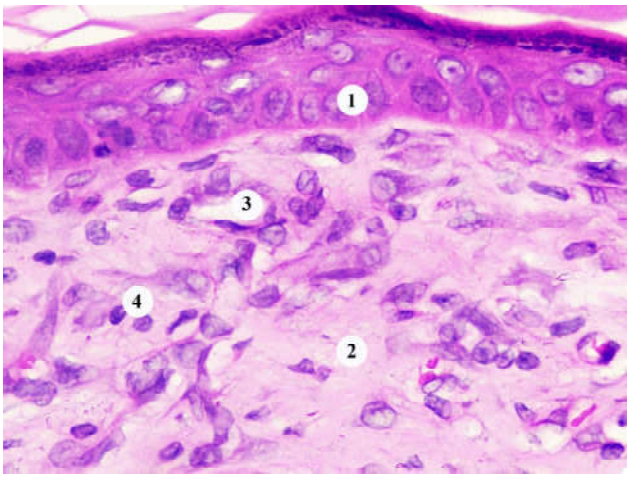

Fig. 10. Histological organization of the skin healing area of the rat for 30 days after burn, with the introduction of 0.9% NaCl solution: 1 - epidermis, 2 - dermis, 3 - capillary, 4 - lymphocytes. Staining with hematoxylin and eosin. x400.

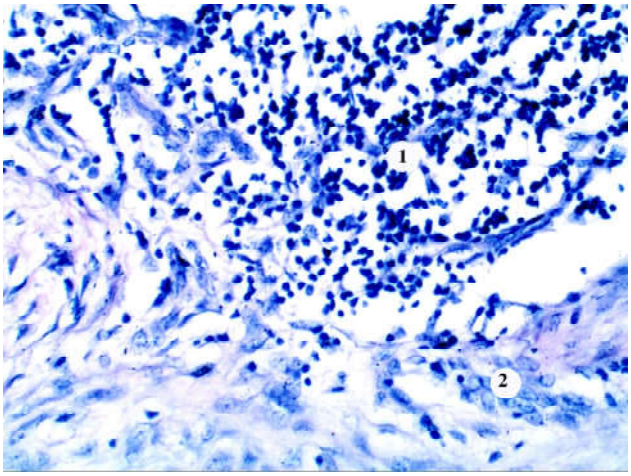

Fig. 11. Microscopic changes of the dermis of rat skin for 30 days after burn, with the introduction of 0.9% NaCl solution: 1 - leukocyte infiltration, 2 - fibroblasts. Semi-thin section. Methylene blue stain. x200.

dermis is smoothed, the papillae are not expressed. The formed connective tissue of the dermis lacks hair follicles and sebaceous glands, few fibroblasts and vessels (Fig. 10).

In single fields of view revealed foci of leukocyte infiltration. As a rule, such foci were located around the vessels of the circulatory microcirculatory bed (Fig. 11).

Discussion

Experiment data on the effect of 0.9% NaCl solution on the features of microscopic changes of organs and tissues in different terms of burn disease are associated with the results of our histological examination. In particular, it was found that the introduction of it to rats during the first 7 days after thermal injury of the skin did not prevent the development of dystrophic and destructive changes in the spleen. On the 1st day after burn revealed dystrophic processes in lymphocytes and macrophages, plethora of sinusoidal vessels of the spleen, swelling and infiltration of perivascular connective tissue. After 3 days, the process was deepened by the destruction of the periarterial zones, germination centers, and marginal areas of the white pulp [2].

In the adrenal cortex in the early periods after skin burns (1, 3, 7 days) with the introduction of 0.9% NaCl solution revealed a combination of processes of destruction and compensatory changes, while at 14, 21 and 30 days further development and deepening lesions of all structural components of the organ cause the development of irreversible changes [5].

Histological examination of the structure of the lungs at day 14 after skin burn under the conditions of the first 7 days of 0.9% NaCl solution revealed disorders of microcirculation in the respiratory department, thickening of the interalveolar septa due to infiltration by their lymphocytes and macrophages. After 30 days, there were signs of pneumosclerosis, destruction, and no compensation for pathological changes, indicating their irreversibility [14].

Available data on the effect of infusion of 0.9% NaCl solution under conditions of thermal injury on the transformation of the intercellular substance of the internal organs. It is established that on 1, 3, 7 and 14 days after skin burns, there is a development of cell alteration and intercellular edema. Under these conditions significant changes undergo the vessels of the microcirculatory bed, which are manifested in the form of progressive thinning of endothelial cells, their microclasmatosis and even necrosis [4].

Conclusions

1. Conducted microscopic studies of the skin of animals after thermal trauma under the conditions of application of 0.9% NaCl solution found that in the early stages of the experiment (1, 3, 7 days) compensatory and adaptive

changes of its structural components are combined with signs of destructive disorders. In the epidermis of the affected area of the skin there is destruction of the epitheliocytes of all its layers. Vascular disorders in the dermis are combined with changes in fibroblasts, homogenization and swelling of the components of the intercellular substance.

2. In the late stages of the experiment (14, 21 and 30 days), the destructive changes of the structural components of the skin in the lesion area deepen. This process is irreversible. As a consequence, granulation and maturation occur slowly. The marginal epithelialization of the wound with the formation of a complete, differentiated into layers regenerate is also slowed. At the 30th day of the experiment, with the use of 0.9% NaCl solution, no dermal papillae and skin appendages were present in the healing area.

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ГІСТОЛОГІЧНА КАРТИНА В ШКІРІ ЩУРІВ ПРОТЯГОМ МІСЯЦЯ ПІСЛЯ ОПІКУ II-III СТУПЕНЯ НА ФОНІ ВВЕДЕННЯ ПЕРШИХ 7 ДІБ 0,9% РОЗЧИНУ NACL

Міронов Є.В.

Опікова хвороба - це небезпечний для життя комплекс патологічних змін, що виникає в організмі внаслідок дії термічного

агенту. Проблема опіків шкіри досі залишається актуальною в наш час. Недостатньо вивчені особливості патогенезу та методи лікування термічної травми є причиною значної зацікавленості науковців даною проблемою. Метою роботи є вивчення особливостей мікроскопічних змін в шкірі щурів протягом місяця після опіку II-III ступеня на фоні введення перших 7 діб 0,9% розчину NaCL. Дослідження проведені на 360 лабораторних білих щурах-самцях масою 155-160 г. В ході експерименту тварин було розподілено на 4 групи: 1, 2 групи - щури без термічної травми, котрим проводили інфузію 0,9% розчину NaCl та HAES-LX-5% у дозі 10 мл/кг. У 3 та 4 групах щурам проводили інфузію 0,9% розчину NaCl та HAES-LX-5% у дозі 10 мл/кг після опіку шкіри. Опікове пошкодження шкіри викликали шляхом прикладання до бічних поверхонь тулуба щурів на 10 секунд чотирьох мідних пластинок, нагрітих у воді з постійною температурою 100°C. Гістологічні препарати готували за стандартною методикою та досліджували за допомогою світлового мікроскопа OLYMPUS BH-2. Проведені мікроскопічні дослідження шкіри тварин після термічної травми за умов застосування 0,9% розчину NaCl встановили, що в ранні терміни експерименту (1, 3, 7 доби) компенсаторно-пристосувальні зміни її структурних компонентів поєднуються з ознаками деструктивних порушень. У пізні періоди термічної травми (14, 21 та 30 доби) відбувається поглиблення деструктивних змін структурних компонентів шкіри у ділянці ураження, а процес набуває незворотного характеру.
Ключові слова: *опіки шкіри, гістологічні зміни, некроз, 0,9% розчин NaCL.*

ГИСТОЛОГИЧЕСКАЯ КАРТИНА В КОЖЕ КРЫС В ТЕЧЕНИЕ МЕСЯЦА ПОСЛЕ ОЖОГА II-III СТЕПЕНИ НА ФОНЕ ВВЕДЕНИЯ ПЕРВЫХ 7 ДНЕЙ 0,9% РАСТВОРА NACL

Миронов Е.В.

Ожоговая болезнь - это опасный для жизни комплекс патологических изменений, возникающих в организме в результате действия термического агента. Проблема ожогов кожи до сих пор остается актуальной в наше время. Недостаточно изученные особенности патогенеза и методы лечения термической травмы являются причиной значительного интереса ученых к данной проблеме. Целью работы является изучение особенностей микроскопических изменений в коже крыс в течение месяца после ожога II-III степени на фоне введения первых 7 дней 0,9% раствора NaCL. Исследования проведены на 360 лабораторных белых крысах-самцах массой 155-160 г. В ходе эксперимента животные были распределены на 4 группы: 1 и 2 группы - крысы без термической травмы, которым проводили инфузию 0,9% раствора NaCl и HAES-LX-5% в дозе 10 мл/кг. В 3 и 4 группах после ожога кожи крысам проводили инфузию 0,9% раствора NaCl и HAES-LX-5% в дозе 10 мл/кг. Ожоговое повреждение кожи вызвали путем приложения к боковым поверхностям туловища крыс на 10 секунд четырех медных пластинок, нагретых в воде с постоянной температурой 100°C. Гистологические препараты готовили по стандартной методике и исследовали с помощью светового микроскопа OLYMPUS BH-2. Проведенные микроскопические исследования кожи животных после термической травмы в условиях применения 0,9% раствора NaCl установили, что в ранние сроки эксперимента (1, 3, 7 суток) компенсаторно-приспособительные изменения ее структурных компонентов сочетаются с признаками деструктивных нарушений. В поздние периоды термической травмы (14, 21 и 30 суток) происходит углубление деструктивных изменений структурных компонентов кожи в области поражения, а процесс приобретает необратимый характер.
Ключевые слова: *ожоги кожи, гистологические изменения, некроз, 0,9% раствор NaCL.*

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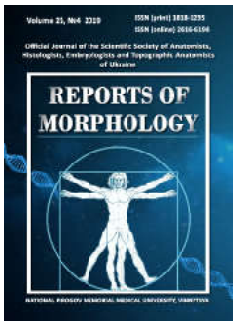

Pathophysiological model of indirect revascularization in rats with microangiopathy of limbs caused by experimental streptozocin diabetes

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Despite the large number of publications, the model of experimental diabetes after the introduction of streptozotocin remains a subject of lively scientific debate. The purpose of this study was to develop a pathophysiological model of indirect revascularization in rats with microangiopathy of limbs caused by experimental streptozotocin diabetes. Experimental studies were carried out in a chronic experiment on 100 sexually mature Wistar rats weighing 180-250 g. The streptozotocin diabetes model used. After culling animals from increased resistance to pancreatotropic toxicity by the criterion of the absence of hyperglycemia, three experimental groups were formed: Group I (control) - rats with streptozotocin-induced angiopathy without treatment (n=10); II group - rats with streptozotocin-induced angiopathy treated with pentoxifylline (100 mg/kg IP for 10 days) for therapeutic purposes (n=25); III group - rats with streptozotocin-induced angiopathy, which together with the treatment were injected with platelet-rich plasma (in the right hind limb, once, with a volume of 0.2 ml, linearly, retrogradely, from two points) and pentoxifylline (100 mg/kg IP for 10 days) (n=25). The duration of the experiment was 110 days. We studied the level of glycemia, the state of microcirculation, and the degree of pathomorphological changes in the various study groups. Statistical processing was performed by non-parametric methods using software Statistica 10.0. The developed pathophysiological model of indirect revascularization with the introduction of pentoxifylline and plasma enriched with platelets in diabetic angiopathy is adequate to the needs of clinical physiology. It has been shown that the isolated administration of pentoxifylline is inferior to combined therapy by revascularizing activity. The results of the work may be an experimental justification for the feasibility of clinical application of the combination of pentoxifylline and platelets rich plasma in the treatment of diabetic angiopathy, as well as its use in prophylactic purposes in patients with diabetes mellitus.
Keywords: *diabetes mellitus, diabetic angiopathy, pentoxifylline, platelets rich plasma, streptozotocin, experimental model.*

Introduction

According to WHO definition, diabetes mellitus (DM) is called a non-communicable epidemic of the XXI century. In economically developed countries, 4-6% of people suffer from diabetes [20, 23]. In Ukraine, the incidence of diabetes, as in other countries in the world, is increasing every year. In Ukraine, 1270929 cases of diabetes were registered in 2017, but the real spread of the disease is even higher - according to experts, almost 50% of cases of diabetes have not been diagnosed [9].

Insulin-dependent diabetes is a serious condition that usually occurs in children and young people. Disease is the same often affects both men and women. The cause of insulin-dependent diabetes is autoimmune damage to the insulin-producing pancreatic β-cells. The incidence of the disease varies widely [18], the highest incidence rates are reported in Finland and Sardinia (more than 20 cases per 100,000 population per year) and the lowest in Asia (less than 3 cases per 100,000 population per year). Insulin-independent diabetes often occurs in adulthood. Risk factors are overweight, abuse of refined carbohydrates. It is this clinical form that is most common [20].

DM is one of the priority global problems for humanity today. According to experts, there are more than 200 million patients with diabetes in the world, with half of them not

being diagnosed on time and manifesting complications, among which diabetic angiopathy is of great importance [17, 23].

The pathogenesis of diabetic angiopathy [19] remains the most pressing and complex problem in diabetology. The use of experimental models of diabetes is necessary both for the study of the pathophysiology of this disease and for the study of the anti-diabetic properties of new compounds [21], as well as the effect of transplantation of endocrine islets to diabetic animals, since it opens the possibility of studying clinical parameters before and after transplantation or treatment [1, 3, 4, 8, 10-12, 22].

Today, there are a large number of experimental models that allow to reproduce certain links of the pathogenesis of diabetes. These methods include, but are not limited to, DM models that are spontaneous or genetically derived, chemically induced, diet-induced, surgically, and more recently transgenic or knockout [3, 4, 8].

For over 50 years, the only model of experimental diabetes has been diabetes caused by the removal of the pancreas. However, pancreatectomy requires a high level of surgical skill and adequate technical equipment. The operation results in traumatization of the animals and a high mortality rate. The high risk of infection requires postoperative antibiotic treatment [11]. In order to prevent absorption disorders in the intestine, it is necessary to replace the excretory functions of the gland. Regarding genetic modeling tools [7], the cost of one genetically modified rat today exceeds 15000 UAH, which makes it practically impossible to use them in the current state of science funding in Ukraine.

Due to the length of time and considerable resources required for most of these methods, chemically induced experimental DM remains the fastest and most economically viable option. After first reporting its ability to cause diabetes in 1963 [8], researchers have begun to widely use streptozotocin on animal models to study both the pathology of diabetes and the possible complications associated with the disease. Streptozotocin has the added benefit of being able to select specific traits of interest, since streptozotocin can induce diabetes in most rodent strains. This may be important for the study of islet transplantation, as specific immune responses or lack thereof may be critical for experimental design [8, 15, 16].

For our experiments related to the study of the effects of different strategies of indirect revascularization in diabetic angiopathy, we decided to use the model with the introduction of streptozotocin.

Despite the large number of publications on this topic (over 18,000 titles for streptozotocin on PubMed as of 01/01/2019), the model of experimental diabetes after the introduction of streptozotocin remains a subject of lively scientific debate. Critics of the method indicate that streptozotocin-induced diabetes does not fully reflect the pathogenesis of the disease in humans. In addition, this model gives a highly variable glycemic profile [3, 8, 15]. There is no standard protocol for the drug, dose or administration of streptozotocin. Most of the literature to date contains very little detail of induction of diabetes in cases where target points are associated with its complications. This complicates the design process, does not allow you to correctly estimate the optimal sample sizes for experiments and set realistic graphics.

The purpose of this study was to develop a pathophysiological model of indirect revascularization in rats with microangiopathy of extremities on the background of experimental streptozotocin diabetes.

Materials and methods

Experimental studies were conducted under the conditions of a chronic experiment on 100 adult rats of the Wistar line weighing 180-250 g.

Following the administration of streptozotocin, animals with increased resistance to pancreatotropic toxicity were excluded from the experiment by the criterion for the absence of hyperglycemia, after which 2 animals were withdrawn for 6 weeks for morphological studies. At week 7, another 10 animals were withdrawn from the experiment to evaluate the effectiveness of the experimental model. Of the remaining animals (n=60), three experimental groups were formed.

Group I (control). Rats with streptozotocin-induced angiopathy without treatment (n=10).

Group II. Rats with streptozotocin-induced angiopathy treated with pentoxifylline for therapeutic purposes (100 mg/kg IP per day for 10 days) (n=25).

Group III. Rats with streptozotocin-induced angiopathy, which were co-administered with platelet-enriched plasma (in the right hind limb, one time, 0.2 ml in volume, linear, retrograde, two-point) and pentoxifylline (100 mg/kg IP per day for 10 days) (n=25).

4 weeks after the start of experimental therapy, 5 animals were removed from the experiment. The total duration of the experiment was 110 days.

Animal preparation, all interventions, anesthetics and withdrawal from the experiment were carried out in full compliance with the requirements of the Guidelines of the State Pharmacological Center of the Ministry of Health of Ukraine (Kyiv, 2001), as well as the GLP rules provided by the European Commission for the supervision of laboratory and other studies, in accordance with Code of Scientist of Ukraine. Animal euthanasia was carried out in accordance with the provisions regulated by Annex 8 of the "Rules for the humane treatment of laboratory animals", "Sanitary rules for equipment, equipment and maintenance of experimental biological clinics (vivarium)" No. 1045-73.

Laboratory rats were kept in individual boxes with 12 hours of light and dark, humidity of 60%, constant temperature of 22±1oC, with free access to water and food.

The experimental DM was reproduced in rats with intraperitoneal administration of streptozotocin (60 mg/kg; "Alfa Aesar", USA, [J61601, Lot: F30X011]), which was

dissolved in sodium citrate buffer (pH=4.5).

The body weight of rats was determined in grams on special mechanical laboratory scales VL-120 (Tomsk, Russia), the error of the method was ±100 mg.

Urine analysis was performed using Citolab 3GK diagnostic test strips, Pharmasco (Ukraine). Glucose and urine acetone were determined in mmol/l, and urine protein in g/l.

For the subsequent experiments, only those rats with blood glucose concentrations exceeding 12 mmol/l were selected. The simulation efficiency of the experimental DM was 92-95% (an average of only 1 rat out of 20 blood glucose concentrations at 2 days after streptozotocin administration was less than 10 mmol/l).

Blood glucose content was determined using the glucosidase method [5].

Quantitative determination of glucose in the blood and urine, as well as acetone and urine protein in the first week after injection of the drug was determined on the 1st, 3rd and 7th day, and then 1 time per week before the formation of experimental diabetic angiopathy (8 week).

The rats were observed for 8 weeks without treatment (this time interval was sufficient to form experimental diabetic angiopathy), after which treatment was started. Drugs for the treatment of rats with experimental diabetic angiopathy were administered starting at 53 days of the experiment.

The formation of experimental diabetic angiopathy was confirmed by morphological examination of the soft tissues of the hind paws of rats (to this end, 2 animals from each group were disposed of each week).

Platelet-enriched plasma was prepared according to the standard procedure [13], after preparation immediately injected into the right hind limb of rats of the third group with a volume of 0.2 ml linearly retrograde from two points.

Statistical processing was performed by non-parametric methods using Statistica 10.0 software (Dell StatSoft Inc., USA).

Results

The average weight of animals involved in the study was 190.0±0.6 g at c_v=2.9%. When analyzing the distribution of blood glucose content in animals after the administration of streptozotocin, it was found that at an amplitude of 9.9-17.0 mmol/l, this average was 14.23±0.21 mmol/l. Further analysis showed that the simulation efficiency of the experimental DM was 92%, which is acceptable for the subacute experiment [3].

Monitoring of the glycemic profile over 8 weeks showed a fairly stable glucose content with a tendency to increase (Fig. 1). This indicates a high risk of endothelial damage and the likelihood of developing specific complications for diabetes. On the other hand, these values indicate a low risk of hyperosmolar syndrome in experimental animals. Against the background of the experimental DM, the animals were lethargic, apathetic, with polydipsia and polyuria.

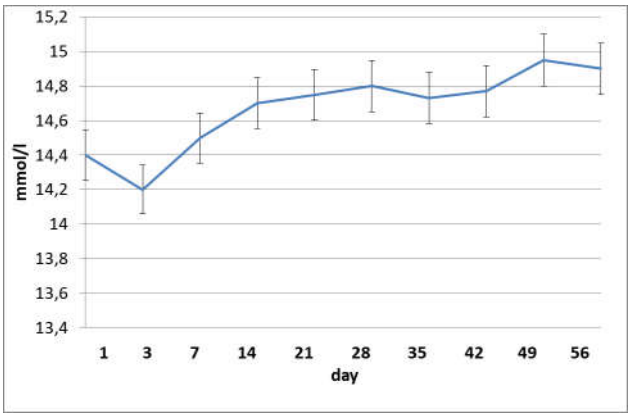

Fig. 1. The content of glucose in the blood of experimental animals within the first 8 weeks.

Table 1. State of microcirculation of distal segments of lower extremities of experimental animals, assigned to different groups (M±m).

Indicator	I group	II group	III group
Perivascular infiltrate, μm	7.932±0.812	5.938±0.613*	3.624±0.312**
The number of microvessels, mm^2	3.714±0.211	4.635±0.223**	4.828±0.214**
The diameter of the vessels, μm	7.736±0.723	11.53±0.94**	11.62±0.61**
Specific volume of vessels of the microcirculatory bed,%	3.224±0.222	3.941±0.312*	4.446±0.324**

Notes: * - differences with the control group are statistically significant, p<0.05; ** - differences with the control group are statistically significant, p<0.01.

The rapid analysis of glucose content in urine, as well as acetone and urine protein, showed rapid development of signs of diabetic nephropathy. By the end of the first week, urine protein excretion exceeded 25 mg per day, averaging 25,74±0,42 mg/day, and by the end of 2 months the observation had increased to 27,93±0,33 mg/day. In terms of glucose content, in all experimental animals it exceeded 2%, which corresponds to the average level (11,14±0,31 Mmol/l).

Quite high levels of proteinuria and glucosuria indicate impaired renal functional reserve resulting from dystrophy and partial necrosis of the distal tubule epithelium, mesangial cell proliferation, and moderate thickening of the vascular glomerular capillary membrane.

In 56.3% of cases, acetonuria, indicating the presence of ketoacidosis, was discovered by the qualitative method. However, the general condition of the animals suffered little, and the overall behavioral changes characteristic of the experimental DM predominated.

Diabetic angiopathy is characterized mainly by lesions of microcirculatory vessels, as evidenced by the results of our morphological studies of skin vessels. Thus, improving microcirculation can slow the development of diabetic angiopathy and, accordingly, improve the repair capacity of

damaged tissues.

Thus, the severity of perivascular infiltrate was the lowest (3.624±0.312 μm) in group III. This was 2.2 times less than that of rats with streptozotocin-induced diabetes ($p<0.01$) and 39% less than rats with streptozotocin-induced diabetes ($p<0.05$).

The counts of the number of microvessels, their diameter and the specific volume of microcirculatory vessels were comparable in groups of rats with streptozotocin-induced diabetes treated with pentoxifylline and platelet-enriched plasma (Table 1).

Discussion

Thus, the experimental model created adequately reflects the features of diabetes of moderate severity in the stage of decompensation, which allows us to consider further conclusions regarding the course of diabetic angiopathy sufficiently substantiated.

As shown by our studies, rats of group I underwent significant changes in all layers of the skin, which were partially offset by the use of experimental therapy aimed at indirect revascularization of ischemic tissues.

The results showed that in the condition of reproduction of diabetes, after 6 weeks, a typical picture of diabetic angiopathy develops in rats. In addition to dystrophic changes in the skin, the animals had phenomena of diabetic peripheral polyneuropathy, which was manifested by weakness of the extremities and diminished reflex response to tingling irritation [11]. There were also signs of temperature hyperalgesia.

We consider it worth mentioning that the positive effects of indirect revascularization can be explained by the influence and pathogenesis of diabetic angiopathy, such as inflammation (increase in fibrinogen, C-reactive and protein amyloid-1, cytokines) [1, 12], oxidative stress (increasing the content of reactive oxidants and simultaneously reducing the functional activity of the enzymatic and non-enzymatic units of the antioxidant system), the reduction of the activity of NO synthase and the activity of nitrergic mechanisms [7], disorders of the hemocoagulation [10-12], vascular dysfunction of the microcirculatory bed [3, 16].

The important question is what is the mechanism of realization of the protective effect of pentoxifylline and its combination with platelet-enriched plasma under model conditions? According to the literature [16], the following effects are indicative of pentoxifylline: inhibition of phosphodiesterase activity and cAMP accumulation in myocytes of the smooth muscle layer of blood vessels, blood cells, as well as in other tissues and organs; inhibition of erythrocyte and platelet aggregation; increasing the flexibility of erythrocytes and, accordingly, their ability to pass through capillaries; reduction of increased plasma fibrinogen concentration and increased fibrinolysis. In addition, pentoxifylline has a weak myotropic vasodilator effect, which leads to a decrease in overall peripheral vascular resistance. Pentoxifylline also has a positive inotropic effect, improves microcirculation and reduces the oxygen debt of peripheral tissues [4, 6, 16]. In addition, pentoxifylline has a potent anticytokine effect that allows a number of researchers to view this drug as a component of pathogenetic therapy for systemic inflammatory responses [6, 16, 17].

The immunomodulatory properties of pentoxifylline are mediated as a direct inhibition of the production of tumor necrosis factor (TNF-α) and a number of other cytokines (interleukins-1, -2, -6 and γ-interferon) [16], as well as by influencing chemotaxis and migration, leukemia adhesion of endothelial leukocytes, reduction of degranulation and excretion of superoxides in neutrophils, reduction of leukocyte sensitivity to interleukin and TNF-α inhibition of T and B lymphocyte activity, as well as NK cells [9, 16-18, 20, 23].

It should be noted that the endotheliumprotective effect of pentoxifylline is also partly explained by the decrease in the activity of TNF-α. In addition, pentoxifylline also protects endothelial cells from the harmful effects of neutrophil leukocytes by eliminating the effect of platelet activation factor on granulocytes [1, 16].

Pentoxifylline has various effects on the blood. Various in vitro and in vivo models have demonstrated its ability to suppress thrombus formation processes. Reduction of platelet aggregation under the influence of the drug can be associated with both direct inhibition of phosphodiesterase of the platelet membrane, which catalyzes the conversion of cAMP to AMP [16], and to stimulate the secretion of prostacyclin [1, 16].

Pentoxifylline and its metabolites affect blood clotting and fibrinolysis: stimulate the secretion of tissue plasminogen activator (tPA) and reduce the concentration in the blood of its inhibitors, slow the secretion of the Von Willebrand factor, increase the levels of plasmin and antithrombin-3 [6, 16]. However, despite the very wide range of effects, the effect on microcirculation in the application of pentoxifylline is due primarily to the effect on blood rheology and the precapillary element of the vascular system [16].

Instead, platelet-rich plasma has a slightly wider range of effects. Platelets contain granules that can secrete into the environment a significant number of biologically active compounds, both with procoagulative activity and those that affect cell proliferation and modulate inflammation processes. Even 30 years ago, platelets were considered mainly in terms of their participation in the coagulation system, but it is now known that platelets contain growth factors and a large number of cytokines that can affect inflammation, angiogenesis, cell migration and cell proliferation [2, 9, 14].

Platelet-rich plasma is a powerful natural source of signaling molecules. Upon activation of platelets contained in platelet-enriched plasma, a significant amount of biologically active raw material capable of modifying the pericellular microenvironment is secreted. Some of the

growth factors secreted by platelet-enriched plasma have a direct effect on cell proliferation processes. The most important growth factors are vascular endothelial growth factor, fibroblast growth factor, platelet growth factor itself, epidermal GF, hepatocyte growth factor, insulin-like growth factors type 1 and 2 (IGF-1, IGF-2). In addition, platelets secrete matrix metalloproteinases 2 and 9, interleukin-8 [2].

In fact, platelet-enriched plasma is a plasma fraction of autoblood with platelet concentrations above baseline (before centrifugation) [2, 17]. Thus, platelet-rich plasma contains not only a high platelet count but also a complete set of blood coagulation factors, as well as chemokines, cytokines and other plasma proteins [14]. Thus, the introduction of platelet-rich plasma not only stimulates cell proliferation, promoting repair and regeneration of damaged tissues, but also influences angiogenesis, enhancing the formation of new vessels and promoting the preservation of the existing vascular network. All this has a positive effect on microcirculation.

The introduction into the damaged tissues of platelet-enriched plasma allows to cause the aggregation of inflammatory cells through the release of chemotactic, angiogenic and mitogenic growth factors. Increasing macrophage migration plays a significant role. During the proliferative phase of healing, macrophages accumulate in the damaged site and realize either a pro-inflammatory or anti-inflammatory phenotype. M1 macrophages actively phagocytize, destroying damaged cells and microorganisms; they are capable of recruiting additional reparative cells (eg, myofibroblasts), and promote the release of proinflammatory cytokines. M2 macrophages promote angiogenesis, affect the restoration of the soft tissue connective tissue framework, affecting remodeling and ultimately, scar tissue formation. These macrophages mainly produce anti-inflammatory cytokines [9]. Both phenotypes play important roles in reparative processes, affecting angiogenesis, differentiation of fibroblasts and collagen formation [9, 17].

Conclusions

1. The developed pathophysiological model of indirect revascularization with the introduction of pentoxifylline and platelet-rich plasma in diabetic angiopathy is adequate to the needs of clinical physiology.

2. Isolated administration of pentoxifylline by revascularizing activity is inferior to combination therapy.

3. The results of the work may be an experimental justification for the clinical utility of the combination of pentoxifylline and platelet-enriched plasma in the treatment of diabetic angiopathy, as well as its use for prophylactic purposes in patients with diabetes.

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ПАТОФІЗІОЛОГІЧНА МОДЕЛЬ НЕПРЯМОЇ РЕВАСКУЛЯРИЗАЦІЇ У ЩУРІВ З МІКРОАНГІОПАТІЄЮ КІНЦІВОК НА ТЛІ ЕКСПЕРИМЕНТАЛЬНОГО СТРЕПТОЗОЦИНОВОГО ДІАБЕТУ

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Незважаючи на велику кількість публікацій, модель експериментального цукрового діабету після введення стрептозоцину залишається предметом жвавої наукової дискусії. Метою даного дослідження була розробка патофізіологічної моделі непрямої реваскуляризації у щурів з мікроангіопатією кінцівок на тлі експериментального стрептозоцинового діабету. Експериментальні дослідження були проведені за умов хронічного експерименту на 100 статевозрілих щурах лінії Вістар масою 180-250 г. Використали стрептозоцинову модель діабету. Після вибракування тварин із підвищеною резистентністю до панкреатотропної токсичної дії за критерієм відсутності гіперглікемії, сформовано три експериментальні групи: I група (контроль). Щури із стрептозотоцин-індукованою ангіопатією без лікування (n=10); II група - щури зі стрептозотоцин-індукованою ангіопатією, яким з лікувальною метою вводили пентоксифілін (100 мг/кг в/очеревинно щоденно протягом 10 днів) (n=25); III група - щури зі стрептозотоцин-індукованою ангіопатією, яким з лікувальною метою сумісно вводили збагачену тромбоцитами плазму (в праву задню кінцівку одноразово, об'ємом 0,2 мл, лінійно ретроградно з двох точок) та пентоксифілін (100 мг/кг в/очеревинно щоденно протягом 10 днів) (n=25). Загальна тривалість експерименту склала 110 днів. Досліджували рівень глікемії, стан мікроциркуляції та ступінь патоморфологічних змін у різних групах дослідження. Статистичну обробку проводили непараметричними методами за допомогою програмного забезпечення Statistica 10.0. Розроблена патофізіологічна модель непрямої реваскуляризації з введенням пентоксифіліну та плазми, збагаченої тромбоцитами при діабетичній ангіопатії є адекватною потребам клінічної фізіології. Показано, що ізольоване введення пентоксифіліну за реваскуляризуючою активністю поступається комбінованованій терапії. Результати роботи можуть бути експериментальним обґрунтуванням доцільності клінічного застосування комбінації пентоксифіліну та плазми збагаченої тромбоцитами при лікуванні діабетичної ангіопатії, а також їх застосування з профілактичною метою у хворих на цукровий діабет.

Ключові слова: *цукровий діабет, діабетична ангіопатія, пентоксифілін, плазма збагачена тромбоцитами, стрептозоцин, експериментальна модель.*

ПАТОФИЗИОЛОГИЧЕСКАЯ МОДЕЛЬ НЕПРЯМОЙ РЕВАСКУЛЯРИЗАЦИИ У КРЫС С МИКРОАНГИОПАТИЕЙ КОНЕЧНОСТЕЙ НА ФОНЕ ЭКСПЕРИМЕНТАЛЬНОГО СТРЕПТОЗОЦИНОВОГО ДИАБЕТА

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Несмотря на большое количество публикаций, модель экспериментального сахарного диабета после введения стрептозоцина остается предметом оживленной научной дискуссии. Целью данного исследования была разработка патофизиологической модели непрямой реваскуляризации у крыс с микроангиопатией конечностей на фоне экспериментального стрептозоцинового диабета. Экспериментальные исследования были проведены в условиях хронического эксперимента на 100 половозрелых крысах линии Вистар массой 180-250 г. Использована стрептозоциновая модель диабета. После выбраковки животных с повышенной резистентностью к панкреатотропному токсическому действию по критерию отсутствия гипергликемии, сформированы 3 экспериментальные группы: I группа (контроль), крысы со стрептозотоцин-индуцированной ангиопатией без лечения составили II группу (n=10); крысы со стрептозотоцин-индуцированной ангиопатией, которым с лечебной целью вводили пентоксифиллин (100 мг/кг в/брюшинно в течение 10 дней) (n=25) составили III группу. Крысы со стрептозотоцин-индуцированной ангиопатией, которым с лечебной целью совместно вводили обогащенную тромбоцитами плазму (в правую заднюю конечность однократно 0,2 мл, линейно, ретроградно, из двух точек) и пентоксифиллин (100 мг/кг в/брюшинно в течение 10 дней) (n=25). Длительность эксперимента составила 110 дней. Исследовали уровень гликемии, состояние микроциркуляции и степень патоморфологических изменений в различных группах исследования. Статистическую обработку проводили непараметрическими методами с помощью программного обеспечения Statistica 10.0. Разработанная патофизиологическая модель непрямой реваскуляризации с введением пентоксифиллина и плазмы, обогащенной тромбоцитами при диабетической ангиопатии является адекватной потребностям физиологии. Показано, что изолированное введение пентоксифиллина по реваскуляризирующей активности уступает комбинированованной терапии. Результаты работы могут быть экспериментальным обоснованием целесообразности клинического применения комбинации пентоксифиллина и плазмы, обогащенной тромбоцитами при лечении диабетической ангиопатии, а также их применения с профилактической целью у больных сахарным диабетом.

Ключевые слова: *сахарный диабет, диабетическая ангиопатия, пентоксифиллин, плазма обогащенная тромбоцитами, стрептозоцин, экспериментальная модель.*

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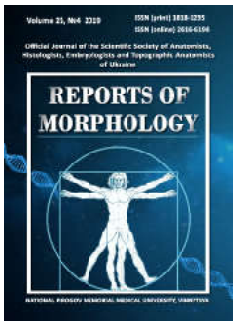

The influence of hydrogen sulfide on the structural characteristics of leukocytes mitochondrial apparatus in patients with arterial hypertension

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One of the leading causes of arterial hypertension (AH) is mitochondrial dysfunction (MD) - moreover, disorders in regulation of blood pressure occur on the background of progressive energy deficiency. At the same time, the cardioprotective effect of H_2S has been proven. In particular, the inhibition of mitochondrial pore opening by hydrogen sulfide plays an important role, and H_2S should affect the structural component of MD, namely, the ultrastructure of mitochondria. However, at present, the question of structural changes in the mitochondrial apparatus in patients with hypertension is extremely insufficiently studied. For the study of MD in healthy people and patients with pathology, almost the only objects (in the absence of surgical intervention) are blood cells, in particular leukocytes. Based on the above, the aim of the study was to investigate the effect of a hydrogen sulfide donor on the ultrastructure of the mitochondrial apparatus of leukocytes in patients with arterial hypertension. The effect of a hydrogen sulfide donor on some ultrastructural characteristics of the leukocyte mitochondrial apparatus in patients with hypertension was studied. The examination involved patients (men) with arterial hypertension aged 30-60 years, who were divided into 2 age groups: 30-40 and 40-60 years. Control groups (healthy men without signs of hypertension) were randomized by age. An electron microscopic and morphometric assessment of the structure of mitochondria revealed that under hypertension it undergoes significant changes that depend on the age of the patients. It has been established that the addition of a hydrogen sulfide donor (Full Spectrum Garlic phytopreparation (Swanson Health Products, USA) at a dose of 400 mg per day) to the traditional therapy of hypertension leads to positive changes in the mitochondrial ultrastructure of the studied cells aimed at increasing the energy capacity of the mitochondrial apparatus - the quantity reduction of structurally damaged mitochondria, and with an increase in the duration of treatment - the increase their total number in people of the younger age group (by 57.5%), and in the older age group - by 53.7%. Thus, the indicated effect of H_2S significantly depends both on the age of the patients (young people respond more intensively) and on the duration of the hydrogen sulfide donor using (long-term use is accompanied by a more pronounced positive dynamics of changes).

Keywords: *arterial hypertension, hydrogen sulfide, mitochondrial ultrastructure, leukocytes.*

Introduction

In the scientific literature of recent years, much attention has been paid to low molecular weight signaling molecules, which are generally referred to as NO and CO_2. Much later, it was found that H_2S also belongs to them [2, 6, 28]. Currently, it is believed that the main biological effects of H_2S include the regulation of vascular tone, contractile activity of the myocardium, participation in long-term synaptic potentiation, pro- and anti-inflammatory reactions, regulation of insulin secretion, etc. [3, 4, 29]. Hydrogen sulfide has also been shown to have antioxidant and anti-apoptotic properties [26].

The cardiovascular system is an important object of H_2S action. Like NO and CO, it relaxes the smooth muscles of the blood vessels, decreases blood pressure and heart rate [21, 22, 23]. There is a direct correlation between the severity of the disease and the level of hydrogen sulfide in

the blood: the more severe hypertension is, the greater is the deficit of hydrogen sulfide [2].

It is now considered established - one of the leading causes of cardiovascular pathology is mitochondrial dysfunction (MD) - especially since the violation of normal regulation of blood pressure occurs against the background of progressive energy deficit [10, 17]. In recent years, cardioprotective effect of H_2S, in particular in the case of arterial hypertension (AH), has been proven, and inhibition of mitochondrial pore opening by hydrogen sulfide plays an important role in the mechanism of the protective effect [9, 24, 25]. In addition, the development of MD leads to damage to the membranes of organelles, a decrease in ATP synthesis, which is accompanied by a decrease in contractile activity and functional reserves of the heart and, as a consequence, a decrease in its pumping function. Based on the available data on the cardioprotective effect of hydrogen sulfide associated to some extent with its effect on mitochondria, namely Ca2+-induced mitochondrial pore opening [24, 25], it can be assumed that H_2S should affect the structural component of MD, and namely, the ultrastructure of mitochondria. However, at present, the question of structural changes of the mitochondrial apparatus in patients with hypertension has not been sufficiently studied.

For the study of MD in healthy people and patients with pathology, virtually the only objects (in the absence of surgery) are blood cells, in particular leukocytes. They are involved in many processes in the body, both related and not directly related to hypertension: in the regeneration of tissues, the development of inflammatory and immune responses, the provision of primary homeostasis. Leukocytes contain enough mitochondria to study the structure and function of these organelles in the development of pathological conditions of different genesis, including hypertension [11, 12].

Based on the above, *the aim* of the study was to study the effect of hydrogen sulfide donor on the ultrastructure of the mitochondrial leukocyte apparatus in patients with hypertension.

Materials and methods

Investigation of the ultrastructure of the mitochondrial apparatus of leukocytes was performed in patients (men) with newly diagnosed hypertension aged 30-60 years, which were divided into 2 age groups: 1) 30-40 years (mean age was 33.9±1.1 years); 2) 40-60 years (mean age - 50.6±1.7 years). The average blood pressure in persons in group 1 was 140/90 mm Hg and in patients of group 2 - 160/100 mm Hg. Control groups (C1 and C2 were healthy men without signs of hypertension) were randomized by age.

The examination of each patient was conducted in 4 stages: before the study; after receiving the course of treatment of the underlying disease; after a 2-week course of specific treatment; after a month of specific treatment. The latter consisted of the exogenous (per os) addition of hydrogen sulfide donor to the therapy of 400 mg/day, which was contained in a herbal drug Full Spectrum Garlic (garlic in capsules) (Swanson Health Products, USA). The course of administration of the phytopreparation was 30 days, during which the patients were examined twice - after 2 weeks (relatively short course of treatment) and at the end of treatment (conditionally long course of treatment).

Blood plasma enriched with leukocytes was obtained by centrifugation of whole blood at room temperature for 15 minutes at 120 g on a laboratory T-30 centrifuge (Ukraine). The plasma was gently separated from the precipitated cells and centrifuged at 2000 g for 20 minutes using a Vortecs Combispin FVL-2400N mini-centrifuge (Latvia) [5].

Samples for electron microscopic examination were prepared according to conventional methods for blood cells with double fixation of glutaraldehyde and OsO_4, dehydration in alcohols of increasing concentration and pouring into Epon-Araldite (reagents of firm Fluka, Switzerland) [8]. Ultrathin sections 40-60 nm thick were counterstained with solutions of uranyl acetate and lead citrate (Sigma reagents, USA) and examined in a PEM-124c electron microscope (Ukraine).

Morphometric calculations were performed using the Image Tool (USA) computer program in 130-150 fields for each group of subjects.

The statistical processing of the obtained data was performed using the programs "Microsoft Ecxel" and "OriginPro" using the Student's t test, due to the coherence of the samples of the surveyed. Because, due to the considerable mass of digital material, according to the Shapiro-Wilk criterion, the data obtained were within the normal distribution law, the data are presented as mean ± error of mean (M±m) [14]. The differences between the averages were considered statistically significant at $p<0.05$.

Results

We found significant differences in the structural organization of leukocytes in individuals with hypertension in different age groups, examined before the start of the standard treatment process.

In the analysis of some structural features of the mitochondrial apparatus of leukocytes in patients with hypertension, it was found that they have a significant number of mitochondria was structurally altered (especially in the older age group), but their total number was not significantly changed relative to the control values (Table 1).

It should be noted that in addition to the swelling and vacuolation of organelles, which is a nonspecific response to most negative endo- or exogenous effects on cells, there was a marked activation of mitochondrial autophagy (Fig. 1).

In hypertension, mitochondrial swelling led to an increase in their average diameter (see Table 1). However, in patients of age group 1 such increase was 27.8%, in patients of group 2 it was 44.3%.

Conducting traditional 2-week therapy was more effective

Table 1. Characteristics of the ultrastructure of the mitochondrial apparatus of leukocytes in persons with hypertension.

Groups of surveyed	Number of mitochondria, un./10 μm^2	Number of structurally damaged mitochondria,%	The average diameter of mitochondria, μm
Control (the average age 30.40±2.80 years)	11.30±0.50	2.400±0.090	0.540±0.070
Control (the average age 54.20±1.90 years)	9.500±0.60	3.600±0.070	0.610±0.040
Patients with hypertension aged 33.90±1.10 years			
Before treatment	12.20±0.80	26.20±2.40**	0.690±0.050*
After 2 weeks of standard treatment	12.70±0.90	19.40±3.90**o	0.630±0.060*
After a 2-week course of specific treatment	13.10±0.60*	18.60±2.60**o#	0.580±0.030o
After a month of specific treatment	17.80±0.70*o#	12.80±4.20**o#	0.470±0.040o#
Patients with hypertension aged 50.60±1.70 years			
Before treatment	10.30±0.70	32.60±4.80**	0.880±0.060*
After 2 weeks of standard treatment	11.20±0.80	28.80±5.30**	0.790±0.050*
After a 2-week course of specific treatment	11.90±0.60	22.30±3.70**o	0.770±0.060*
After a month of specific treatment	14.60±0.50*o#	20.20±1.60**o#	0.700±0.030o#

Notes: * - p<0.05 relative to the control group; ** - p<0.01 relative to the control group; o - p<0.05 relative to the group before treatment; # - p<0.05 relative to the standard treatment group.

for younger patients. Its most important results include the fact that the number of structurally damaged mitochondria in leukocytes is reduced by 35.1%. These changes affect only 1 group of people with hypertension (see Table 1). It can be assumed that the absence of a pronounced response of the studied indicators to the traditional treatment is associated with both the duration of the disease and the short period of treatment (due to the absence in the persons of previous therapy with hypertension).

As a result of 2-week specific treatment, positive changes were observed in the mitochondrial apparatus of leukocytes (see Table 1). In both groups of subjects with hypertension, the number of structurally altered mitochondria decreased significantly, not only with respect to the condition before treatment, but also with respect to the determined number of such mitochondria after standard treatment (by 40.9% in the first and by 29.1% in the second group). Such changes

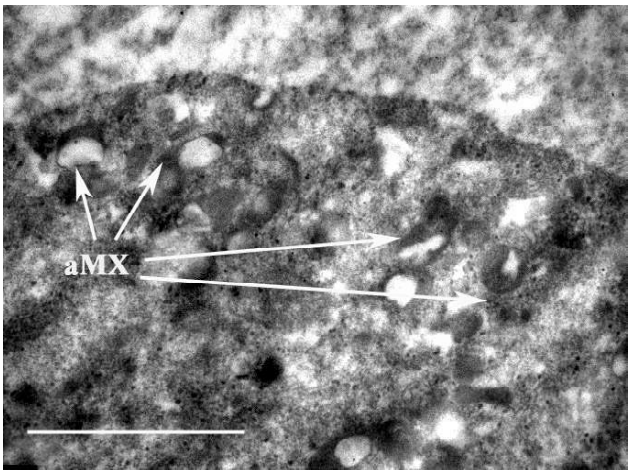

Fig. 1. Autophagy of mitochondria in the leukocyte of a patient with hypertension. aMX - autophagy. Scale - 1 micron.

should be seen as an opportunity to increase the capacity of energy metabolism. With regard to other leukocyte morphometric parameters, the addition of hydrogen sulfide donor to standard treatment increased (in group 1) or showed a tendency (in group 2) to approach the studied indicators to levels that are characteristic of healthy individuals of the appropriate age (see Table 1).

The most optimal, in view of the obtained results, was the treatment of patients with hypertension with a monthly course of specific treatment. Long-term addition to the traditional therapy of Full Spectrum Garlic herbal medicine improved all our investigated indicators of ultrastructure of leukocyte mitochondria in both groups of persons (see Table 1). Only with such a regimen was observed a significant increase in the total number of mitochondria (see Table 1): in persons of the younger age group - by 57.5%, and in the older age group - by 53.7%.

A further decrease in the number of structurally damaged mitochondria was also detected (see Table 1). In group 1 patients, the reduction was 2-fold relative to treatment status and 51.6% relative to traditional treatment; in group 2 patients, by 61.4% relative to the condition before treatment, and by 39.6% relative to traditional treatment. In younger patients, the changes were more pronounced, probably due to the greater adaptive capacity associated with age, the number of altered mitochondria (including in the state of autophagy) was significantly reduced, which, with the increase in their total, had to significantly optimize energy metabolism.

Discussion

As noted above, in recent decades, energy deficiency at the cellular level has been considered as the main cause of the primary increase in blood pressure. A source of impaired energy metabolism of tissues is considered to be a decrease in the energy-forming function of

mitochondria due to disruption of the structure of the mitochondrial apparatus [20]. At the same time, it has been shown experimentally and clinically in 2000 that hypertension decreases the level of H_2S in blood plasma, and the introduction of an exogenous hydrogen sulfide donor can cause a significant therapeutic effect [7, 27]. There is little data available today about the effect of H_2S on the total number of mitochondria in cells. However, H_2S is thought to exert different effects on the number, function and dynamics of mitochondria depending on the dose used [18]. Probably the concentration we use is effective for increasing the number of organelles.

One of the damaging factors for the myocardium and blood vessels is the increase in the amount of reactive oxygen species, which provokes the development of oxidative stress. At the same time, tissue respiration and ATP synthesis by mitochondria are impaired, which leads to a deterioration of cardiac contractile function due to negative structural changes in the mitochondria, which cause their functional energy failure. That is why the antioxidant properties of hydrogen sulfide improve the condition of ischemic myocardium and prevent its further damage [2]. The data obtained by us regarding the increase in the total number of mitochondria and the decrease in the percentage of structurally damaged organelles can be considered as evidence of such changes of the mitochondrial apparatus in leukocytes, which should contribute to the optimization of energy metabolism.

Positive changes in the morphometric characteristics of mitochondria include the decrease in the average diameter of organelles. This difference is important given that the increase in mitochondrial diameter within 25-30% is considered to be an adaptive response aimed at enhancing the energy capacity of organelles caused by the activation of the ATP-dependent K+ channel. More growth in the diameter of mitochondria indicates the possibility of their necrotic death and is often irreversible [16, 23]. The exact mechanism of action of H_2S on KATP-channels remains unclear, but it is suggested that hydrogen sulfide exerts its influence through the K+ conductivity feature, and specific KATP-channel inhibitors completely inhibit H_2S effects on it [1]. We are inclined to take this view, since it is precisely such dynamics of changes of the mitochondrial apparatus in different tissues of the body that promotes not only the ultrastructure but also the function of the mitochondria [16, 19].

Another of our findings is the activation of autophagy in hypertension. As autophagy is thought to be a process that contributes not only to the death of damaged cells but also to their preservation (by preventing apoptosis by mitochondrial pathways) [4, 19], it can be assumed that in hypertension regardless of patients' age, such a mechanism is activated at least in blood cells. Addition to the traditional therapy of AH donor H_2S did not lead to significant changes in the intensity of mitochondrial autophagy, although in the literature there are isolated data on the reduction of the process under the influence of endogenous H_2S [15, 22]. It can be assumed that under such conditions, the increase in the concentration of hydrogen sulfide does not significantly affect the process of autophagy, which we (given its physiological role) tend to consider as an additional positive fact in favor of the therapy of hypertension.

The results indicate a positive effect of hydrogen sulfide on the ultrastructure and, consequently, the function of mitochondria in leukocytes, which requires a further search for effective ways to increase the content of H_2S in patients with hypertension.

Conclusions

1. Hypertension is accompanied by significant changes in the ultrastructure of the mitochondrial apparatus of leukocytes, the severity of which depends on the age of the patients.

2. Addition to the traditional therapy of hypertension of a donor of hydrogen sulfide leads to positive changes in the ultrastructure of mitochondria of leukocytes aimed at increasing the energy capacity of the mitochondrial apparatus.

3. The established positive effect depends significantly on the age of the patients (more young people react more intensively) and the duration of use of the hydrogen sulfide donor (long-term use is accompanied by more pronounced positive dynamics of change).

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ВПЛИВ СІРКОВОДНЮ НА УЛЬТРАСТРУКТУРУ МІТОХОНДРІАЛЬНОГО АПАРАТА ЛЕЙКОЦИТІВ У ПАЦІЄНТІВ З АРТЕРІАЛЬНОЮ ГІПЕРТЕНЗІЄЮ

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Однією з провідних причин розвитку артеріальної гіпертензії (АГ) є мітохондріальна дисфункція (МД) - тим більше, що порушення нормальної регуляції артеріального тиску відбувається на тлі прогресування енергетичного дефіциту. Водночас, доведено кардіопротекторну дію H_2S. Зокрема, важливу роль відіграє гальмування сірководнем відкривання мітохондріальної пори, і H_2S повинен впливати на структурну складову МД, а саме - на ультраструктуру мітохондрій. Проте, на теперішній час питання про структурні зміни мітохондріального апарата у пацієнтів з АГ досліджені вкрай недостатньо. Для вивчення МД у здорових людей і пацієнтів з патологією практично єдиними об'єктами (при відсутності оперативного втручання) є клітини крові, зокрема, лейкоцити. Виходячи із зазначеного, метою дослідження було вивчення впливу донора сірководню на ультраструктуру мітохондріального апарата лейкоцитів у пацієнтів з артеріальною гіпертензією. Проведено вивчення впливу донора сірководню на деякі ультраструктурні характеристики мітохондріального апарата лейкоцитів у пацієнтів з АГ. В обстеженні приймали участь пацієнти (чоловіки) з артеріальною гіпертензією віком 30-60 років, котрі були розподілені на 2 вікові групи: 30-40 та 40-60 років. Контрольні групи (здорові чоловіки без ознак АГ) були рандомізовані за віком. Електронно-мікроскопічна та морфометрична оцінка структури мітохондрій виявили, що при АГ вона зазнає значних змін, котрі залежать від віку пацієнтів. Встановлено, що додавання до традиційної терапії АГ донора сірководню (фітопрепарат Full Spectrum Garlic (Swanson Health Products, США) в дозі 400 мг на добу) призводить до позитивних змін ультраструктури мітохондрій досліджуваних клітин, спрямованих на зростання енергетичної потужності мітохондріального апарата - зменшується кількість структурно пошкоджених мітохондрій, а при збільшенні тривалості лікування - їх загальної кількості в осіб молодшої вікової групи (на 57,5%), а в старшій віковій групі - на 53,7%. Отже, вказаний ефект H_2S суттєво залежить як від

віку пацієнтів (інтенсивніше реагують молоді особи), так і від тривалості використання донатора сірководню (тривале застосування супроводжується більш вираженою позитивною динамікою змін).
Ключові слова: *артеріальна гіпертензія, сірководень, ультраструктура мітохондрій, лейкоцити.*

ВЛИЯНИЕ СЕРОВОДОРОДА НА УЛЬТРАСТРУКТУРУ МИТОХОНДРИАЛЬНОГО АППАРАТА ЛЕЙКОЦИТОВ У ПАЦИЕНТОВ С АРТЕРИАЛЬНОЙ ГИПЕРТЕНЗИЕЙ

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Одной из ведущих причин развития артериальной гипертензии (АГ) является митохондриальная дисфункция (МД) - тем более, что нарушение нормальной регуляции артериального давления происходит на фоне прогрессирования энергетического дефицита. В то же время, доказано кардиопротекторное действие H_2S. В частности, важную роль играет торможение сероводородом открывания митохондриальной поры, и H_2S должен влиять на структурную составляющую МД, а именно - на ультраструктуру митохондрий. Однако, в настоящее время вопросы о структурных изменениях митохондриального аппарата у пациентов с АГ исследованы крайне недостаточно. Для изучения МД у здоровых людей и пациентов с патологией практически единственными объектами (при отсутствии оперативного вмешательства) являются клетки крови, в частности, лейкоциты. Исходя из указанного, целью исследования было изучение влияния донора сероводорода на ультраструктуру митохондриального аппарата лейкоцитов у пациентов с артериальной гипертензией. Проведено изучение влияния донора сероводорода на некоторые ультраструктурные характеристики митохондриального аппарата лейкоцитов у пациентов с АГ. В обследовании принимали участие пациенты (мужчины) с артериальной гипертензией в возрасте 30-60 лет, которые были разделены на 2 возрастные группы: 30-40 и 40-60 лет. Контрольные группы (здоровые мужчины без признаков АГ) были рандомизированы по возрасту. Электронно-микроскопическая и морфометрическая оценка структуры митохондрий выявили, что при АГ она претерпевает значительные изменения, которые зависят от возраста пациентов. Установлено, что добавление к традиционной терапии АГ донора сероводорода (фитопрепарат Full Spectrum Garlic (Swanson Health Products, США) в дозе 400 мг в сутки) приводит к позитивным изменениям ультраструктуры митохондрий исследуемых клеток, направленных на увеличение энергетической мощности митохондриального аппарата - уменьшается количество структурно поврежденных митохондрий, а при увеличении продолжительности лечения - их общего количества у лиц младшей возрастной группы (на 57,5%), а в старшей возрастной группе - на 53,7%. Таким образом, указанный эффект H_2S существенно зависит как от возраста пациентов (интенсивнее реагируют молодые люди), так и от продолжительности использования донора сероводорода (длительное применение сопровождается более выраженной положительной динамикой изменений).
Ключевые слова: *артериальная гипертензия, сероводород, ультраструктура митохондрий, лейкоциты.*

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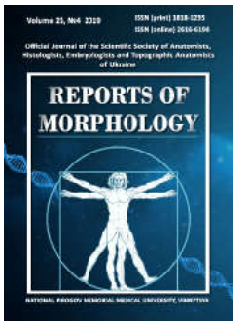

Structural and functional state of the liver in patients with extrahepatic cholestasis of non-tumor genesis

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To date, the diagnosis and treatment of extrahepatic cholestasis (EHC) at various stages of development remains one of the most pressing problems of modern biliary surgery. The purpose of the study is to determine the structural changes of the liver in patients with EHC of non-tumoral genesis according to shear wave elastometry and ultrasound in comparison with biochemical indicators of endotoxemia, inflammation and markers of fibrosis. Preoperatively, 121 patients with EHC of non-tumoral genesis were examined: standard general clinical studies, ultrasound examination of the liver and its shear wave elastometry were performed. Depending on the presence of jaundice and symptoms of hepatocyte damage, all the patients were divided into 4 groups. Serum content of medium molecular weight peptides, free hydroxyproline and glycosaminoglycans was determined. Statistical processing of the obtained data was performed using SPSS 16.0. In patients with EHC of non-tumoral genesis according to shear wave elastography, the stiffness of the liver varied depending on the increase in the intensity of jaundice. It was found that the serum bilirubin level in such patients was significantly different depending on the available jaundice and symptoms of hepatocyte damage both in comparison with the control group and between the study groups. In EHCs, structural changes in the liver depend on abnormalities in the biochemical composition of serum, which are significantly altered by prolonged extrahepatic cholestasis and manifested by severe liver failure. Thus, an increase in endotoxemia ($p<0.001$) was found in patients with EHC of non-tumoral genesis without jaundice and evidence of cholestasis development; indicators of liver stiffness depend on the level of bilirubinemia ($r = 0.84$), and in patients with hepatocyte damage also on the duration of cholestasis ($r = 0.68$). Bilirubin levels cannot be an indicator of long-term EHC.

Keywords: *extrahepatic cholestasis of non-tumor genesis, elastometry, markers of endogenous intoxication, damage to hepatocytes.*

Introduction

The term "cholestasis" of Greek origin literally means "fixed bile" (χολή - "bile" + στάσις - "stasis"). Cholestasis is one of the main processes that occur in the liver in the development of diseases of different etiology. Cholestasis syndrome is a clinical-laboratory syndrome characterized by an increase in bilirubin and bile excreted substances in the blood as a result of impaired synthesis or outflow. Actually, the term "cholestasis" in its modern sense was introduced by Popper and Schaffer only in 1970 to designate not only the mechanical causes of impaired bile flow, but also in connection with impaired secretion of its individual components, primarily impaired hepatocyte function (intrahepatic cholestasis) [1]. From the point of view of the development of the disease in accordance with the structure and structure of the bile ducts, depending on the level of the block, hepatocellular, tubular and duct cholestasis are distinguished. When viewed in a larger format, isolated hepatic and extrahepatic cholestasis (EHC) are classified. EHC have been known since ancient times, whereas intrahepatic cholestasis has caught the attention of physicians only in the mid-twentieth century. Until the 1960s, bile passages at all levels were associated solely with mechanical obstruction of the duct system, which arose as a complication of gallstone disease.

In the next 15 years, an increase in the worldwide incidence of pathology of the biliary system is projected (by 30-50%), which is caused by hereditary factors, changes in the nature of nutrition and a sedentary lifestyle. It should be

noted that over the past 10 years, there has been a steady trend of increasing incidence of cholelithiasis, which is accompanied by the development of extrahepatic cholestasis [2, 3, 4, 5, 10].

Functional EHC is most often caused by Oddi sphincter dysfunction. Obstructive EHC, or mechanical jaundice, is a persistent violation of the excretion of bile from the bile ducts and gallbladder into the lumen of the duodenum, which can be caused by obstruction of the bile ducts by gallstones, helminths or mechanical compression of the bile ducts from the outside by tumor (pancreatic head, large duodenal papilla, common bile duct, enlarged gallbladder), inflammatory swelling of the mucous membrane of the biliary passages (non-purulent, sclerosing or purulent cholangitis), bile duct stricture and other factors. Violation of the outflow of bile leads to its stagnation, increasing the pressure in the bile capillaries, their overstretching, increasing the permeability of the walls and their rupture, which promotes the flow of bile directly into the blood. The appearance of bile in the blood leads to the development of cholemic syndrome, and with complete obstruction of the bile ducts and the cessation of bile flow to the intestine (clinically, this is manifested in the acholic stool).

At detection of a syndrome of a cholestasis at the patient, for doctor it is necessary to make qualitative differential diagnostics with precise determination of degree and place of formation of the block and it is unacceptable to establish the diagnosis without excluding the possible causes of cholestasis. Diagnostic errors that occur in 12-38% of cases of cholestasis [3] lead to loss of precious time, occurrence of liver failure or other serious complications (gastrointestinal bleeding, purulent cholangitis, liver abscesses, sepsis) that in 14-27% of observations lead to death [1, 3, 8, 9, 10].

To date, prevention and treatment of EHC at various stages of development remains one of the most pressing problems of biliary surgery. There is evidence [11, 12, 13] that EHC affect the elasticity of the liver, but to date, the possibilities of using elastography to assess the functional and morphological status of the liver in EHC of non-tumorigenesis have not been sufficiently studied. Recently, several ultrasound and radiological methods have been developed to quantify liver elasticity. One of the most accurate and common among them is the method of shear wave elastography (SWE).

The basis of the method of shear wave elastography is the property of the ultrasonic beam to generate transverse mechanical shear waves in the direction of its propagation. The speed of their passage through the fabric depends on its stiffness or visco-elastic properties. The digital expression of liver stiffness is determined in kilopascals (kPa) from a specific control volume of the liver parenchyma [11-13]. However, the effect of EHC on liver elasticity in comparison with non-invasive serum markers of endotoxemia, inflammation, and fibrosis has not been studied.

Purpose of the study: to determine structural changes of the liver according to shear wave elastometry and ultrasound in comparison with biochemical indicators of endotoxemia, inflammation, markers of fibrosis in patients with non-hepatic cholestasis of non-tumoral genesis.

Materials and methods

The data of the examination of 121 patients with EHC of non-tumoral genesis who were undergoing treatment at the department of digestive surgery of the State Institution "Institute of Gastroenterology of the National Academy of Medical Sciences of Ukraine" for the period from 2013 to 2019 were analyzed.

Extrahepatic cholestasis in 22 patients (18.18%) patients was caused by chronic calculous cholecystitis with the phenomena of Oddi sphincter, in 28 patients (23.14%) - choledocholithiasis after cholecystectomy, in 18 patients (14.87%) - stenotic papillitis after cholecystectomy, in 16 patients (13.22%) by residual choledocholithiasis, in 12 (9.91%) by choledocholithiasis with chronic calculous cholecystitis and stenosis papillitis, in 6 (4.95%) by choledocholithiasis with stenosis of the terminal choledochitis, in 3 (2.47%) fixed concrement of terminal choledochus and chronic calculous cholecystitis, in 2 (1.65%) - choledocholithiasis with chronic calculous cholecystitis, complicated by cholecystoduodenal fistula, in 5 patients (4.13%) Mirizzi syndrome and in 9 patients (7.43%) exacerbation of pancreatitis on the background of choledocholithiasis with chronic calculous cholecystitis.

The duration of EHC with jaundice was determined according to the E. V. Smirnov classification (1974). Acute cholestasis (up to 10 days) was observed in 35.5% of patients (n=43), prolonged cholestasis (from 10 to 30 days, which after a severe attack took a stable character and lasted up to 4 weeks) was observed in 44.62% of patients (n=54), and chronic (more than 30 days) - in 19.83% (in 24 patients).

To solve the tasks, depending on the type of EHC of non-tumoral genesis of patients was divided into 4 groups: EHC type 1 - without jaundice and without damage to hepatocytes (n=50); type 2 EHC - no jaundice with hepatocyte damage (n=38); type 3 EHC - with jaundice without damage to hepatocytes (n=17); type 4 EHC with jaundice and hepatocyte damage (n=16).

The study excluded patients who had concomitant diseases such as viral and autoimmune hepatitis, Carroll's disease, Wilson-Konovalov's disease, Gilbert's syndrome, and oncological genesis of jaundice. The mean age of the examined patients was 58.23±1.69 years. The youngest patient was 27 years old, the oldest was 83. The largest number of patients were patients aged 40 to 69 years, ie most patients were of working age. Most patients were women (87 (71.9%)).

All patients with simultaneous shear-wave elastometry (SWE) on a Soneus P7 apparatus (Kharkiv, Ukraine-Switzerland) with a sensor with a frequency of 2-5 MHz at a depth of not more than 70 mm from the capsule were performed ultrasound. 7 successful measurements (Σ up

to 10%, deviation up to 1) were evaluated, followed by determination of the median, which characterized the stiffness of the liver tissue in kilopascals (kPa). Stratification of reliable results was performed depending on the ratio of IQR/median - less than 30%.

The concentration of total bilirubin in the serum was determined according to the instructions to the kits of EliTech (France). The activity of alanine-aminotransferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (ALP), γ-glutamyltransferase (GGT) serum was determined by ultraviolet kinetics (ALT/GPT, AST - AST/GOT), recommended by the International Federation of Clinical Chemistry (IFCC) according to the instructions of the EliTech kits (France). The presence of endogenous intoxication (EI) was determined by the content of medium molecular weight peptides (MMP) according to V.V. Nikolaychuk. The MMP fraction consists of aromatic amino acids, which are part of proteins, collagen fibers, aromatic amino acids, among which tyrosine and tryptophan occupy a significant place, and therefore the increase in the content of MMP in serum is a marker of activation of catabolic processes in the body. Fibrosis processes were evaluated by the content of hydroxyproline free (HPf) and glycosaminoglycans (GAG). In serum, the content of GAG was determined according to Rimington, HPf - by Osadchuk, the activation of the inflammatory process in patients was evidenced by a change in the level of alpha-1-acid glycoprotein, the content of which was determined by Weimer [16]. Assessment of biochemical parameters was given according to their content in blood of relatively healthy 20 persons (control group).

In order to optimize the mathematical processing, all the input data was entered into a database built using Microsoft Excel spreadsheets. Statistical processing of the obtained data was performed using SPSS 16.0. For statistical analysis of the data used: M - mean value, m - error in determining the mean, comparison of mean values of the variables was performed using parametric method (Student's t-test) for the normal distribution of these features, expressed in the interval scale. The correspondence of the type of distribution of signs of the law of normal distribution was checked using the Shapito-Wilk method. In other cases, a nonparametric method (the Mana-Whitney U test) was used. The difference in mean values was considered significant at $p<0.05$. To determine the relationship between the data, a correlation analysis was performed with the calculation of the Spearman correlation coefficient (r) [19].

Results

According to shear wave elastography in patients with EHC of non-tumoral genesis, the stiffness of the liver varied depending on the increase in the intensity of jaundice and was in patients of group I - 6,021±0,223 kPa, II - 6,383±0,171 kPa, III - 7,812±0,321 kPa, in IV - 8,022±0,364 kPa ($p<0.001$), respectively.

It was found that liver stiffness indices for SWE in patients with EHC differed significantly between the study groups and depended on the level of bilirubinemia (r=0.84) (Fig. 1). The indicators of liver stiffness according to the SWE and the diameter of the choledoch according to the ultrasound are shown in Table 1.

It was found that the liver stiffness indices correlated well with the established choledoch diameter parameters during ultrasound (r = 0.69): I group - 7.582±0.121 mm; II - 8.112±0.191 mm; III - 9.312±0.242 mm; IV - 13.06±0.72 mm (see Table 1 and Figure 2).

Serum bilirubin levels were found to be significantly different in patients with EHC of non-tumorigenic genesis depending on the available jaundice and hepatocyte damage rates, both compared to controls and between the study groups (Fig. 3). Thus, in group II the bilirubin level was 1.5 times higher (34.71±3.23 μmol/l), in III - 6.7 times (159.0±12.7 μmol/l), in IV - 13 times (313.8±28.1 μmol/l). I) relative to patients of group I ($p<0.05$).

The activity of serum enzymes confirmed cytolytic processes in the liver. Hyperalanine aminotransferasemia and hyperaspartate aminotransferasemia were observed in patients in all groups compared to controls, but changes

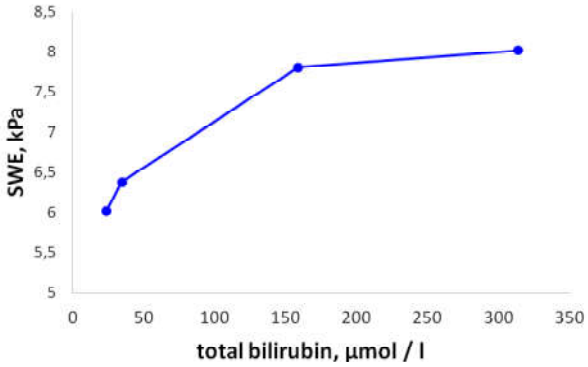

Fig. 1. Dependence of SWE level (kPa) on total serum bilirubin content in patients with non-tumorigenic EHC.

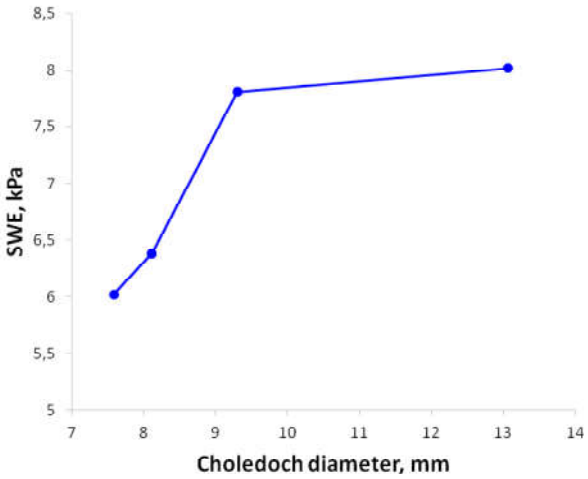

Fig. 2. Dependence of SWE level (kPa) on mean choledoch diameter (mm) in patients with EHC of non-tumorigenic genesis.

Table 1. Indicators in patients with different types of extrahepatic cholestasis.

Indicator	I group (n=50)	II group (n=38)	III group (n=17)	IV group (n=16)
SWE, kPa	6.021±0.223	6.383±0.171	7.812±0.321***###	8.022±0.364***###
The diameter of the common bile duct, mm	7.582±0.121	8.112±0.191*	9.312±0.242 *** ###	13.06±0.72***###°°°

Notes: * - p<0.01, *** - p<0.001 - reliability of differences between indicators of patients of groups II, III, IV and I group; ## - p<0.01, ### - p<0.001 - reliability of differences between indicators of patients of III and IV groups and II group; °°° - p<0.001 - the reliability of the differences between the indicators of patients III and IV group.

in the II and IV groups were more pronounced and probably significant (p<0.001) (Fig. 4).

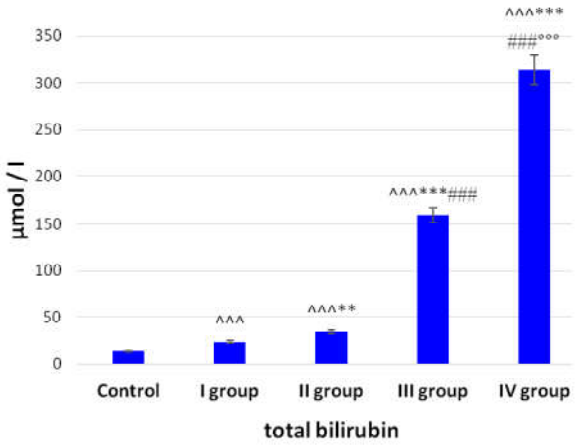

Fig. 3. Total bilirubin level in serum of patients with non-tumor genesis of EHC. ^^^ - p<0.001 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; ** - p<0.01, *** - p<0.001 - significance of differences between patients of groups II, III, IV and I; ### - p<0.001 - significance of difference between indicators of patients of III and IV groups and group II; °°° - p<0.001 - significance of the difference between indicators of patients of III and IV groups.

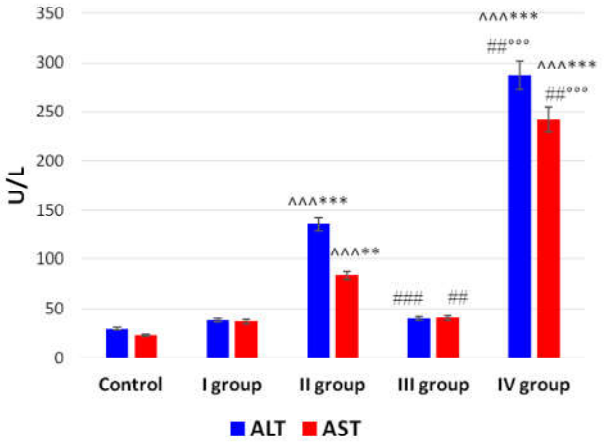

Fig. 4. The activity of liver enzymes in the serum of patients with EHC non-tumoral genesis. ^^^ - p<0.001 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; ** - p<0.01, *** - p<0.001 - significance of the difference between the indicators of patients of II, III, IV groups and group I; - p<0.01, - p<0.001 - significance of the difference between indicators of patients of III and IV groups and group II; °°° - p<0.001 - significance of difference between indicators of patients of III and IV groups.

The determined values of alanine transaminase in serum by groups: 38.22±3.45 U/L in group I; 136.0±27.8 U/L in group II; 39.82±5.11 in group III and 287.0±44.6 U/L in group IV (p<0.001). Serum blood alanine transaminase activity was 36.93±4.14 U/L in group I; 84.13±15.50 U/L in group II; 40.41±6.80 in group III, and 242.0±49.7 U/L in group IV (p<0.001). Such indicators testify to disturbance of permeability of membranes of hepatocytes and their destruction.

Serum cholestasis markers, in contrast to cytolysis indices, had more pronounced pathological changes in patients with non-tumorigenic EHC, both in comparison with the control group and in patients with group IV: their activity increased - alkaline phosphatase to 284.0±34.6 U/L in group I; 262.0±32.8 U/L in group II; 310.0±59.1 U/L in group III and 630.0±81.2 U/L in group IV, respectively, compared with patients in group I (p<0.001) and γ-glutamyltransferase up to 247.0±34.6 U/L in group I; 345.0±37.6 U/L - in group II; 260.0±41.4 U/L in group III; to 610.0±69.9 U/L in group IV, respectively, compared with patients in group I (p<0.001) (Fig. 5).

Patients with EHC of non-tumorigenic genesis had activity of inflammatory processes according to the content of alpha-1-acid glycoprotein in blood serum, which was increased 1.9 times to 0.451±0.041 g/l, (p<0.001) in patients of group I; 2.6 times to 0.632±0.053 g/l - group II;

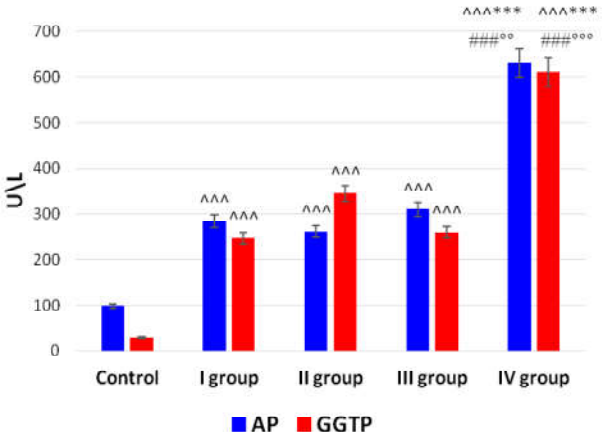

Fig. 5. The activity of liver enzymes in the serum of patients with EHC non-tumoral genesis. ^^^ - p<0.001 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; *** - p<0.001 - significance of differences between patients of groups II, III, IV and I; - p<0.001 -significance of difference between indicators of patients of III and IV groups and group II; °° - p<0.01, °°° - p<0.001 - significance of the difference between indicators of patients of III and IV groups.

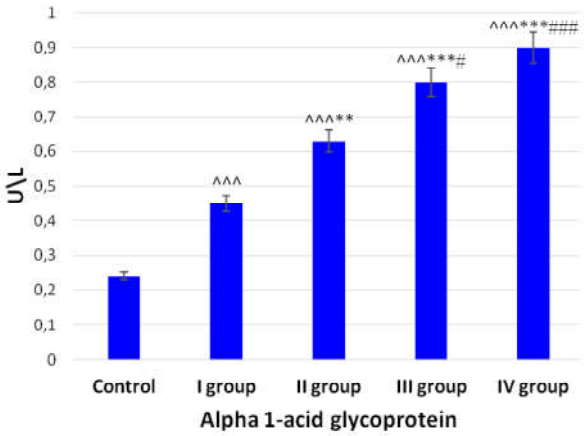

Fig. 6. The content of alpha-1-acid glycoprotein in the serum of patients with EHC non-tumorigenesis. ^^^ - p<0.001 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; ** - p<0.01, *** - p<0.001 - significance of the difference between the indicators of patients of II, III, IV groups and group I; # - p<0.05, ### - p<0.001 - significance of the difference between the indicators of patients of III and IV groups and group II.

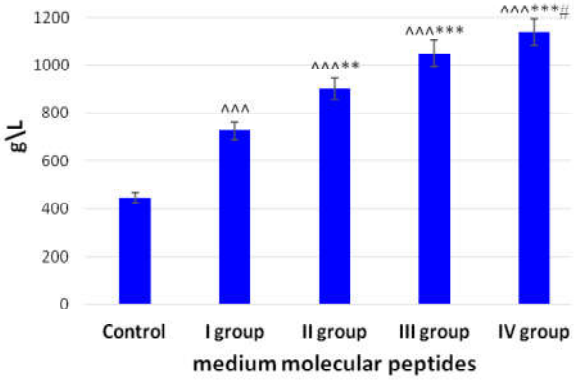

Fig. 7. The level of the average molecular peptides of serum of patients with EHC of non-tumoral genesis. ^^^ - p<0.001 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; ** - p<0.01, *** - p<0.001 - significance of the difference between the indicators of patients of II, III, IV groups and group I; # - p<0.05 - significance of difference between indicators of patients of III and IV groups and group II.

3.3 times to 0.801±0.053 g/l - group III; 3.8 times to 0.902±0.053 g/l - group IV (p<0.001), respectively, more pronounced pathological changes were characteristic of patients of III and IV groups compared with patients of group I and control indicators, these changes were accompanied by an increase of ESR in patients of group III to 26.70±4.80 mm/g and group IV up to 31.20±3.60 mm/g (p<0.05), as well as an increase in leukocyte levels, which reflected the general inflammatory response of the body (Fig. 6).

The analysis of the data obtained showed a gradual increase in the content of medium-molecular peptides in the serum of patients, depending on the intensity of EHC. The presence of endotoxemia was present in patients of all groups: significant changes were observed in patients of group II, which showed an increase in the concentration of medium-molecular peptides in the serum in 1.3 times to 902.0±56.4 g/l (p<0.01) relative to patients of group I (Fig. 7).

It should be emphasized that in patients of group I, in which the patency disorders were associated with long-term phenomena of EHC without jaundice and lesions of hepatocytes - endogenous intoxication syndrome was formed already at the initial stage of EHC development, and with the increase in bilirubinemia, the content of medium molecular peptides, accordingly, in groups III and IV almost: 1.5 to 1050±76 g/l (p<0.001) and 1.7 times to 1139±78 g/l (p<0.01), respectively, relative to patients of group I; 2.4 and 2.6 times relative to the control group (p<0.001) (see Fig. 7).

There was a gradual decrease in the serum hydroxyproline free content of patients in all groups compared to the control group (Fig. 8). It is impossible to exclude the fact that with the progression of non-neoplastic EHC, there is an imbalance between the synthesis and disintegration of collagen in these patients, which indicates a decrease in the process of collagen degradation, which was more pronounced in patients of group IV, where its content decreased in 2-times to 5,232±0,631 μmol/l (p<0.01) relative to group I (10.72±1.90 μmol/l) and in 1.8 times relative to group II (9.633±1.201 μmol/l, p<0.01) (see Fig. 8).

Increase in GAG concentration in serum was observed in patients of all groups. In group II, this indicator increased 1.4 times to 6.430±0.671 mmol/l (p<0.05); in group III - 1.6 times to 7.133±0.711 mmol/l (p<0.05); in group IV - 1.8 times to 8.140±0.482 mmol/l (p<0.001), respectively, in comparison with patients of group I, indicating the increased breakdown of carbohydrate-protein components of connective tissue, increasing their content in the serum

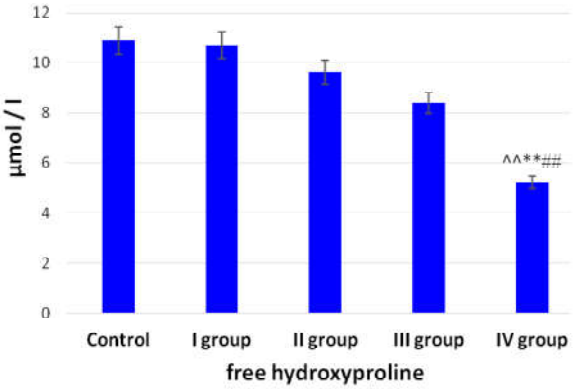

Fig. 8. Level of hydroxyproline free in serum of patients with EHC of non-tumoral genesis. ^^ - p<0.01 - significance of the difference between the indicators of patients I, II, III, IV groups and indicators of the control group; ** - p<0.01 - significance of the difference between the indicators of patients of II, III, IV groups and group I; ## - p<0.01 - significance of difference between indicators of patients of III and IV groups and group II.

indicates the activity of the inflammatory process, the duration of which leads to the destruction of liver tissues.

Discussion

The results of the study indicate that in patients with EHC significantly disrupt the biochemical composition of blood serum, which is determined before all types of EHC, which are significantly different in terms of choledochal diameter, liver stiffness, severity of hepatic and cellular insufficiency, EI markers and nonspecific fibrosis markers.

According to SWE, EHC, depending on the available jaundice and hepatocyte damage, definitely affects the elasticity of the liver, regardless of the presence of liver fibrosis/cirrhosis, with an increase in choledoch diameter, liver stiffness and their ratio (r = 0.69) in the case of existing EHC processes are associated with worsening of bile flow, inflammatory phenomena in the ductal system and, as a consequence, increased hydrostatic pressure in the ducts and edema of the liver parenchyma, which is confirmed by other studies [11-13, 20-22, 24-27].

The liver is one of the first organs whose functions are altered as a result of impaired bile flow in EHC of non-tumorigenesis. The presence of signs of cholestasis was associated with more significant changes in the activity of serum enzymes of serum, with a more pronounced cytolytic and cholestatic syndrome with increasing intensity of cholestasis. A similar pattern was observed with regard to the content of α1-acid glycopeptide in blood serum, the value of which was highest in patients of group IV with non-neoplastic EHC and showed activation of inflammatory processes in the body, revealed changes were accompanied by an increase in leukocyte and ESR levels inflammatory response of the body.

An important pathogenetic syndrome of homeostasis disorders in virtually all diseases is endogenous intoxication - an integral concept that includes a number of components: the accumulation of intermediates of impaired metabolism and metabolites, endogenous and bacterial toxins, aggressive biologically active substances, inflammatory factors in combination with hypoxia and disorders of microcirculation [14, 15, 23].

Occurrence in the blood of non-oxidized products of lipid metabolism (acetone, acetoacetic and β-Hydroxybutyric acid), increase in the level of ketones, medium-molecular peptides cause the development of the clinical picture of endogenous intoxication syndrome. The obvious important role of the liver, as a body of metabolic detoxification, in the process of formation of EI in the body, as well as the processes of cytolysis and cholestasis.

Therefore, extrahepatic cholestasis of non-tumoral genesis is accompanied by certain metabolic disorders and ultrastructural changes that lead to impaired cell-tissue metabolism, the appearance of a large number of aggressive active substances, the reduction of the inactivation and elimination of toxins, that accumulate in the patient and leads to the development of general endotoxemia.

Given the data obtained, it is promising to further study liver stiffness under conditions of surgical correction as a non-invasive way of monitoring the syndrome of "rapid discharge" of bile in patients with non-hepatic cholestasis of non-tumoral genesis.

Conclusions

1. Indicators of liver stiffness according to SWE data in patients of all groups with extrahepatic cholestasis of non-tumoral genesis depend on the level of bilirubinemia (r = 0.84), and in patients with hepatocyte damage (II and IV group) also on the duration of cholestasis (r = 0.69) but cannot serve as an objective indicator of liver functional status.

2. Patients with extracorporeal cholestasis of non-tumoral genesis without the appearance of jaundice and hepatocyte damage revealed an increase in endotoxemia indicators (p<0.001), which indicates developing of EHC.

3. Extrahepatic cholestasis of non-tumorigenesis is accompanied by a gradual increase in alkaline phosphatase and γ-glutamyltransferase activity, while bilirubin levels cannot serve as an indicator of long-term EHC.

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СТРУКТУРНО-ФУНКЦІОНАЛЬНИЙ СТАН ПЕЧІНКИ У ХВОРИХ З ПОЗАПЕЧІНКОВИМ ХОЛЕСТАЗОМ НЕПУХЛИННОГО ГЕНЕЗУ

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На сьогоднішній день діагностика та лікування позапечінкового холестазу (ПХС) на різних етапах розвитку залишається однією з найактуальніших проблем сучасної біліарної хірургії. Мета дослідження - визначити структурні зміни печінки у пацієнтів з позапечінковим холестазом непухлинного ґенезу за даними зсувнохвильової еластометрії та ультразвукового дослідження у співставленні з біохімічними показниками ендотоксемії, запалення та маркерами фіброзу. Передопераційно обстежили 121 хворого з ПХС непухлинного генезу: провели стандартні загальноклінічні дослідження, ультразвукове дослідження печінки та її зсувнохвильову еластометрію. В залежності від наявності жовтяниці та симптомів пошкодження гепатоцитів усіх досліджених розподілили на 4 групи. Визначали вміст у сироватці крові середньомолекулярних пептидів, вільного гідроксипроліну та глікозаміногліканів. Статистичну обробку отриманих даних проводили з використанням програми SPSS 16.0. У пацієнтів з ПХС непухлинного генезу за даними зсувнохвильової еластографії жорсткість печінки змінювалася в залежності від збільшення інтенсивності жовтяниці. Встановлено, що рівень білірубіну у сироватці крові у таких пацієнтів достовірно відрізнявся в залежності від наявної жовтяниці та симптомів пошкодження гепатоцитів як порівняно з даними контрольної групи, так і між дослідженими групами. При ПХС структурні зміни печінки залежать від порушень біохімічного складу сироватки крові, які суттєво змінюються при тривалому позапечінковому холестазі та проявляються вираженою печінковою недостатністю. Таким чином, у пацієнтів з наявним позапечінковим холестазом непухлинного генезу без жовтяниці та пошкодження гепатоцитів виявлено збільшення показників ендотоксемії ($p<0,001$), що свідчить про розвиток холестазу; показники жорсткості печінки залежать від рівня білірубінемії ($r = 0,84$), а у пацієнтів з пошкодженням гепатоцитів ще і від тривалості холестазу ($r = 0,68$). Рівень білірубіну не може бути індикатором тривалості позапечінкового холестазу.

Ключові слова: *позапечінковий холестаз непухлинного генезу, еластометрія, маркери ендогенної інтоксикації, пошкодження гепатоцитів.*

СТРУКТУРНО-ФУНКЦИОНАЛЬНОЕ СОСТОЯНИЕ ПЕЧЕНИ У БОЛЬНЫХ С ВНЕПЕЧЕНОЧНЫМ ХОЛЕСТАЗОМ НЕОПУХОЛЕВОГО ГЕНЕЗА

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На сегодняшний день диагностика и лечение внепеченочного холестаза (ВПХ) на разных этапах развития остается одной из самых актуальных проблем современной билиарной хирургии. Цель исследования - установить структурные изменения печени у пациентов с внепеченочными холестазом неопухолевого генеза по данным сдвиговолновой эластометрии и ультразвукового исследования в сопоставлении с биохимическими показателями эндотоксемии, воспаления и маркерами фиброза. Предоперационно обследовали 121 больного с ВПХ неопухолевого генеза провели стандартные общеклинические исследования, ультразвуковое исследование печени и ее сдвиговолновую эластометрию. В зависимости от наличия желтухи и симптомов повреждения гепатоцитов всех исследованных разделили на 4 группы. Определяли содержание в сыворотке крови среднемолекулярных пептидов, свободного гидроксипролина и гликозаминогликанов. Статистическую обработку полученных данных проводили с использованием программы SPSS 16.0. У пациентов с ВПХ неопухолевого генеза по данным сдвиговолновой эластографии жесткость печени менялась в зависимости от увеличения интенсивности желтухи. Установлено, что уровень билирубина в сыворотке крови у таких пациентов достоверно отличался в зависимости от имеющейся желтухи и симптомов повреждения гепатоцитов как по сравнению с данными контрольной группы, так и между исследованными группами. При ВПХ структурные изменения печени зависят от нарушений биохимического состава сыворотки крови, которые существенно изменяются при длительном внепеченочном холестазе и проявляются выраженной печеночной недостаточностью. Таким образом, у пациентов с имеющимся ВПХ неопухолевого генеза без желтухи и повреждения гепатоцитов выявлено увеличение показателей эндотоксемии ($p<0,001$), что свидетельствует о развитии холестаза; показатели жесткости печени зависят от уровня билирубинемии ($r = 0,84$), а у пациентов с повреждением гепатоцитов еще и от длительности холестаза ($r = 0,68$). Уровень билирубина не может быть индикатором длительности внепеченочного холестаза.

Ключевые слова: *внепеченочный холестаз неопухолевого генеза, эластометрия, маркеры эндогенной интоксикации, повреждение гепатоцитов.*

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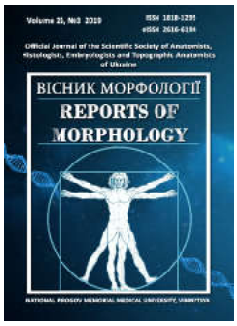

Features of the relationship between cardiointervalographic indices and constitutional characteristics in highly skilled mesomorphic somatotype wrestlers

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Mechanisms of regulation of cardiac rhythm have many individual features, which are conditioned by age, sex, training of the organism, strength and nature of external influence, constitutional features of the organism. The purpose of the work is to determine the relationship between cardiointervalographic indices and parameters of the external structure of the body in highly skilled wrestlers of the mesomorphic somatotype. The study involved 24 wrestlers between the ages of 17 and 21 with a high level of sportsmanship and more than 3 years of experience. All of the wrestlers were of medium weight and engaged in free and Greco-Roman wrestling. We conducted a study of heart rate variability on the cardiac computer diagnostic complex "OPTW" following the recommendations of the European and North American Cardiac Association (1996). The indices of vegetative homeostasis according to Bayevsky, variational heart rate, statistical and spectral cardiointervalographic indicators were determined. Anthropometry was performed according to the method of V.V. Bunak (1941), somatotypological study - by the calculated modification of the Heath-Carter method (1990), determination of the component composition of body weight by the method of Matejko (1992). In the package "STATISTICA 5.5" correlation analysis was performed using the nonparametric Spearman statistical method. It was found that in the wrestlers of the mesomorphic somatotype, the variations of the pulsometry had the highest number and strength of reliable correlations with constitutional parameters, most of which were inverse of the mean force. All statistical indicators of heart rate variability with indicators of the external structure of the body had only inverse significant correlations. The least significant correlations were found for spectral indices and parameters of vegetative homeostasis. According to the results of the correlation analysis in the wrestlers of the mesomorphic somatotype, we can assume that with the increase of total, longitudinal, circumferential, transverse body sizes and muscle and bone mass, the variability of the heart rhythm of the sympathetic department of the autonomic nervous system will be more pronounced.

Keywords: *correlations, cardiointervalographic indices, anthropometric dimensions, somatotype, wrestlers.*

Introduction

Achieving high athletic performance is closely linked to the effective training of athletes in specialized training centers [8, 24]. One of the most important principles of building a training process is to match the loads to the current functional state [2, 24]. In order to improve and predict the athletic performance of athletes today, a simple method of examination, such as determining heart rate variability, is used in sports medicine and cardiology to effectively evaluate the balance of all nervous system components and make prognosis in reaching sports peaks [14, 22, 23].

Thus, the use of excessive daily physical training by young athletes causes not only a feeling of general fatigue, but also requires a significant overall mobilization of all structural and functional resources of the body and systems, which leads to maladaptation, physical overstrain and overtraining [1]. All these factors overstress the mechanisms of adaptation over time lead to a decrease in the protective mechanisms of the body of athletes and provoke diseases

of the cardiovascular system [1, 20].

According to some authors [13, 16], the objective criteria for assessing the current functional status and physical fitness of athletes are physiological indicators that reflect the state of the mechanisms of vegetative regulation of cardiac activity. A non-invasive method of analyzing heart rate variability, such as cardiointervalography, has become widespread. It is used to evaluate the vegetative regulation of physiological functions, from the point of view of objectivity there is no doubt [17, 25]. This method allows to record disturbances of neurohumoral equilibrium, participation of sympathetic and parasympathetic, nervous and humoral units in regulation of heart rate, degree of centralization of its management [17]. Mechanisms of regulation of cardiac rhythm have many individual features, which are due to age, sex, body training, strength and nature of external influence [6, 9, 11]. In recent years, studies have been conducted to identify the relationship between heart rate variability and features of the external structure of the body [7, 19]. However, studies on the relationship between heart rate variability and anthropometric parameters in wrestlers of a particular constitutional type have not been conducted at all.

The aim of our work was to determine the relationship between cardiointervalographic indices and parameters of the external structure of the body in highly trained wrestlers of the mesomorphic somatotype.

Materials and methods

We hane conducted on the basis of the research center of National Pirogov Memorial Medical University, Vinnytsya comprehensive survey of highly skilled (from the first adult category to masters of sports) athletes aged 17 to 21 years, who have been engaged in more than three years of wrestling (free and Greco-Roman). All athletes belonged to the middle weight categories, were in the preparatory period of the training annual macrocycle. The determination of the constitutional features of the body of wrestlers was based on an anthropometric study conducted by the Bunak method [4] and somatotypological - by Heath-Carter [5]. In addition, the component composition of body weight was determined by the method of Matejko [10]. After the somatotypological analysis, 24 wrestlers with a mesomorphic somatotype were selected.

Determination of heart rate variability in wrestlers was performed on the "OPTW" computer diagnostic complex, following the recommendations of the European and North American Cardiac Association [12]. Four groups of indicators were defined: statistical (SDNN is the standard deviation of the length of normal R-R intervals, RMSSD is the square root of the sum of squares of the difference of successive pairs of normal R-R intervals, PNN50 is the percentage of the number of pairs of consecutive normal R-R intervals that is more than 50 ms the total number of consecutive pairs of intervals); variational heart rate (mode, mode amplitude, mean, minimum and maximum R-R intervals, variational swing); spectral (total recording power in all bands, power in very low frequencies, power in low frequencies, power in high frequencies, power ratio in the low and high frequencies); indices of vegetative homeostasis, which was determined by the Baevsky method (vegetative rhythm index and voltage indices of regulatory systems and vegetative equilibrium).

Correlation analysis was performed in the "STATISTICA 5.5" package (license number AXXR910A374605FA) using the nonparametric Spearman method.

Results

After performed correlation analysis, it was found that most indices of variational heart rate with anthropometric parameters of the body had a considerable amount of average strength of reliable connections. It was found that the mode had significant inverse correlations of medium and high strength with the following parameters: body length (r=-0.45, p=0.027), body weight (r=-0.51, p=0.012), body surface area (r=-0.45, p=0.026), height of the upper thoracic point (r=-0.59, p=0.002), height of the pubic point (r=-0.40, p=0.050), height of the shoulder point (r=-0.43, p=0.035), finger point height (r=-0.61, p=0.001), width of distal shoulder epiphyses (r=-0.40, p=0.050), hips (r=-0.67, p=0.001), lower legs (r=-0.67, p=0.001), shoulder girths in the unstressed state (r = -0.40, p = 0.050), upper arms at the top (r = -0.45, p = 0.028), at the bottom (r=-0.40, p=0.050), hips (r=-0.61, p=0.002), lower legs (r=-0.46, p=0.024), waist (r=-0.42, p=0.039), chest on inhalation (r=-0.49, p=0.015), exhalation (r=-0.50, p=0.013), at rest (r=-0.56, p=0.005), anterior-posterior mid-thoracic diameter (r=-0.77, p=0.001); transverse thorax diameter (r=-0.48, p=0.018), transverse thorax diameter (r=-0.52, p=0.009), interspinous distance (r=-0.54, p=0.006), intercristal distance (r=-0.58, p=0.003), intertrochanteric distance (r=-0.70, p=0.001), mesomorphic component of the somatotype (r=-0.47, p=0.021), muscular (r=-0.68, p=0.001) and bone (r=-0.74, p=0.001) body weight. Fairly straight, the average strength of the relationship was between the indices of mode in the thickness of the skin and fat folds on the abdomen (r=0.41, p=0.049), on the thigh (r=0.58, p=0.003) and the lower leg (r=0.41, p=0.050).

The amplitude of the mode had only one significant mean feedback in relation to the width of the shoulders (r=-0.45, p=0.028), with all other anthropometric indicators found to be unreliable correlations. It was found that the *average R-R interval* in mesomorph wrestlers had numerous inverse mean and individual strong relationships with the external structure of the body, in particular: with length (r=-0.43, p=0.034), mass (r=-0.47, p=0.022) and the surface area of the body (r=-0.43, p=0.038), height of the upper thorax (r=-0.56, p=0.005), pubic (r=-0.40, p=0.050), shoulder (r=-0.40, p=0.050), finger (r=-0.48, p=0.019) anthropometric points, width of the distal femoral epiphysis (r=-0.47, p=0.020) and lower leg (r=-0.61, p=0.002), shoulder girth in a non-stressed condition (r=-0.41, p=0.049), upper arm girth (r=-0.41, p=0.049), girth

of the thigh (r=-0.61, p=0.001), shin girth at the top (r=-0.40, p=0.050), chest girth on inhalation (r=-0.47, p=0.022), exhalation (r=-0.42, p=0.043) and calm state (r=-0.46, p=0.025), transverse lower thoracic diameter (r=-0.45, p=0.026), anterior-posterior mid-thoracic diameter (r=-0.57 p=0.004), interspinous (r=-0.41, p=0.050), intercristal (r=-0.45, p=0.027), intertrochanteric (r=-0.54, p=0.007) pelvis distances, muscle (r=-0.58, p=0.003) and bone (r=-0.55, p=0.006) body weight.

The maximum R-R interval in wrestlers correlated with body length (r=-0.41, p=0.050), body weight (r=-0.42, p=0.040), body surface area (r=-0.41, p=0.050), and upper-thoracic height (r=-0.46, p=0.023) and finger (r=-0.39, p=0.050) points, width of the distal tibial epiphysis (r=-0.43, p=0.035), hip girth (r=-0.48, p=0.017), interspinous distance (r=-0.40, p=0.050), intercristal (r=-0.41, p=0.049) and intertrochanteric distance (r=-0.53, p=0.007), muscle (r=-0.57, p=0.004) and bone mass (r=-0.40, p=0.050) of the body. *And the minimum R-R interval* had both feedbacks and direct correlations, in most cases they were average. Feedbacks were found with the height of the upper thoracic (r=-0.44, p=0.031) and finger (r=-0.60, p=0.002) points, the width of the distal femoral epiphysis (r=-0.52, p=0.010), the lower leg (r=-0.58, p=0.003), hip girth (r=-0.43, p=0.037), anterior-posterior mid-thoracic diameter (r=-0.56, p=0.004), interspinous (r=-0.44, p=0.032), intercristal (r=-0.41, p=0.050) and intertrochanteric (r=-0.50, p=0.013) distances of the pelvis, muscle (r=-0.47, p=0.022) and bone (r=-0.57, p=0.004) weight; and direct connections with the thickness of the skin-fat folds on the forearm (r=0.49, p=0.016), at the lower blade angle (r=0.42, p=0.040), abdomen (r=0.57, p=0.004), hips (r=0.61, p=0.002), the shin (r=0.55, p=0.006), and the endomorphic component of the somatotype (r=0.40, p=0.050).

Variation span with indicators of external structure of the body had significant only inverse correlations, in particular with the circumference of the hips (r=-0.45, p=0.028), the thickness of the skin-fat folds on the back (r=-0.41, p=0.047) and the front (r=-0.44, p=0.028) of the shoulder surface, the thickness of the skin-fat folds on the chest (r=-0.46, p=0.023), with body fat (r=-0.43, p=0.038).

The SDNN heart rate variability statistic was significantly correlated with the thickness of the skin and fat folds on the posterior surface of the shoulder (r=-0.43, p=0.037) and chest (r=-0.41, p=0.049) and muscle mass (r=-0.41, p=0.049). *RMSSD* had numerous significant inverse correlations: with body length (r=-0.58, p=0.003), body weight (r=-0.43, p=0.037), body surface area (r=-0.55, p=0.006), height of upper thoracic (r=-0.50, p=0.012) and finger (r=-0.40, p=0.050) anthropometric points, width of distal shoulder epiphysis (r=-0.41, p=0.049), shoulder girth in tension (r=-0.47, p=0.022), non-stressed state (r=-0.51, p=0.012), upper arm circumference (r=-0.58, p=0.003) and lower (r=-0.63, p=0.001), hip circumference (r=-0.54, p=0.007), lower legs in the upper (r=-0.47, p=0.020) and lower (r=-0.46, p=0.023) parts, neck (r=-0.42, p=0.043), waist (r=-0.49, p=0.016), foot (r=-0.44, p=0.031), chest inhalation (r=-0.53, p=0.008), exhalation (r=-0.43, p=0.036) and in a calm state (r=-0.44, p=0.034), interspinous (r=-0.41, p=0.046), intercristal (r=-0.45, p=0.028), intertrochanteric (r=-0.58, p=0.003) distances, muscle (r=-0.69, p=0.001) and bone (r=-0.46, p=0.023) body weight. *PNN50* had significant, inverse, mean correlation strength with body length (r=-0.41, p=0.049), suprathoracic height (r=-0.43, p=0.035) and finger (r=-0.41, p=0.047) points, the circumferential dimensions of the forearm in the upper (r=-0.41, p=0.048) and lower (r=-0.39, p=0.050) parts, hips (r=-0.46, p=0.025), lower legs in the upper (r=-0.39, p=0.050) and lower (r=-0.43, p=0.034) parts, waist (r=-0.40, p=0.050), chest on inhalation (r=-0.43, p=0.037), anterior-posterior mid-thoracic diameter (r=-0.45, p=0.027), interspinous (r=-0.41, p=0.047), intertrochanteric (r=-0.51, p=0.010) pelvis distances and bone mass determined by the Matejko method (r=-0.55, p=0.005).

Most spectral indicators of heart rate variability in wrestlers of the mesomorphic somatotype did not have numerically significant correlations with anthropometric and somatotypological parameters.

Total recording power in all ranges had only two significant direct correlations: the width of the distal shoulder epiphyses (r=0.47, p=0.021) and the forearm (r=0.46, p=0.024). *The spectral index, which reflects the power at very low frequencies*, had reliable direct relations of the average force with the body weight (r=0.47, p=0.019), the area of the body (r=0.40, p=0.050), the heights of the upper thorax (r=0.40), p=0.050), pubic (r=0.56, p=0.005), brachial (r=0.50, p=0.013), and finger (r=0.50, p=0.014) points, width of distal shoulder epiphyses (r=0.54, p=0.006) and forearm (r=0.67, p=0.001), lower arm circumference (r=0.48, p=0.019), lower leg (r=0.41, p=0.049), brush (r=0.41, p=0.044), bone mass (r=0.51, p=0.011).

It was found that the *power in the low-frequency* range of mesomorph wrestlers had a reliable feedback only with the height of the trochanter point (r=-0.41, p=0.049) and the intercristal distance of the pelvis (r=-0.41, p=0.047). *Power in the high-frequency range* had only one significant indirect relationship with the width of the distal tibial epiphysis (r=0.41, p=0.046). *The ratio of power in the low and high frequencies* had significant inverse mean force due to the height of the upper thoracic (r=-0.49, p=0.015) and finger (r=-0.42, p=0.039) points and anterior-posterior mid-thoracic diameter (r=-0.43, p=0.036).

Indicators of vegetative homeostasis, determined by the Bayevsky method, had only direct reliable correlations with constitutional characteristics. Thus, the *voltage index of the regulatory systems* was correlated with the height of the upper thoracic (r=0.52, p=0.009), shoulder (r=0.45, p=0.027), finger (r=0.41, p=0.050) points and muscle mass (r=0.40, p=0.050). The vegetative equilibrium index had a small number of direct reliable mean correlation strengths: with the height of the upper thoracic point (r=0.43, p=0.038), the intertrochanteric distance (r=0.41, p=0.044), muscular

(r=0.42, p=0.042) and bone mass (r=0.44, p=0.037). *Vegetative rhythm index* had the highest number of relationship reliability; it correlated with length (r=0.40, p=0.050), mass (r=0.49, p=0.014), and body surface area (r=0.46, p=0.025), height of the upper thoracic (r=0.56, p=0.004), pubic (r=0.41, p=0.050), humerus (r=0.49, p=0.015), finger (r=0.41, p=0.050) points, distal width shoulder epiphysis (r=0.42, p=0.039), girth of thigh (r=0.45, p=0.027), thighs (r=0.43, p=0.038), chest inhalation (r=0.47, p=0.020), exhalation (r=0.48, p=0.019) and at rest (r=0.46, p=0.022), anterior-posterior mid-thoracic size (r=0.43, p=0.036), the intercristal (r=0.44, p=0.032), the intertrochanteric distance (r=0.49, p=0.015), the thickness of the skin-fat folds on the back surface of the shoulder (r=0.41, p=0.047), and the chest (r=0.45, p=0.029), muscle (r=0.52, p=0.009) and bone (r=0.52, p=0.009) body weight.

Discussion

Analyzing the peculiarities of correlations between cardiointervalographic parameters and indicators of external body structure in mesomorph wrestlers, it is necessary to note a much greater number and strength of revealed reliable relationships, unlike the group of athletes who also belonged to this somatotype, as noted in our previous studies [18]. In addition, it was found that the wrestlers of the mesomorphic somatotype had more numerical correlations between the femur rheographic parameters and constitutional characteristics than the athletes [15].

To summarize our results, it should be noted that most indicators of variational heart rate have numerous reliable correlations with anthropometric dimensions and components of somatotype and body weight. In particular, *mode* among youth wrestlers is correlated with the majority of constitutional parameters (31 out of 49), which is 63.3%, of which inverse strong correlations were 16.3%, inverse mean strength - 40.8%, only with a thickness of 3 skin-fat folds (6.1%) correlations were direct, medium power. Based on this, we can assume that by increasing the total, longitudinal, girth size of the body, width of the distal epiphyses of the thigh and tibia, diameters of the chest and pelvis, muscle and bone components of somatotype and body weight, and at the same time reduce the fat deposition in the lower ankles of the mesomorphic somatotype wrestlers, the mode will decrease, and thus the level of functioning of the sinus node will be more pronounced [3, 25]. In athletes of the mesomorphic somatotype, the mode only had feedback with a thickness of 1 skin-fat fold (on the side) [18].

The amplitude of the wrestlers' mode had only 1 (2.0%) reliable average feedback. The average R-R interval, which reflects the balance of parasympathetic and sympathetic influences [24], had reliable feedbacks with 42.8% of the external body structure, including 21 medium strengths and 2 strong ones. It should be noted that this indicator of variational heart rate has significant correlations with almost the same constitutional parameters as mode, but the strength of the connections is somewhat smaller.

The maximum R-R interval for mesomorph wrestlers correlated statistically with 12 (24.5%) anthropometric indicators, all correlations were inversely proportional to the mean force. We can predict that with decreasing total body size, pelvic diameters, muscle mass, this indicator will increase, and thus the variability of the heart rate of the parasympathetic department of the autonomic nervous system will be more pronounced [3, 25].

The minimal value of the R-R interval was varied with significant correlations with 17 parameters of the external structure of the body, among them the average mean force was 10 (20.4%), the strong inverse - 1 (2.0%), the direct average force - 5 (10.2%), the strong direct - 1 (2.0%). The peculiarities of established relationships between this indicator and constitutional characteristics confirm the established pattern that wrestlers of mesomorphic somatotype with increase of bone and muscle mass, individual body diameters and decrease of skin and fat folds will be observed decrease of the distance R-R and the greater will be the impact of the sympathetic department of the autonomic nervous system [13, 17]. The variational range had only significant mean power inverse correlations with 5 (10.2%) indicators of the external structure of the body.

It should be noted that all statistical indicators of heart rate variability with constitutional parameters in young wrestlers of mesomorphic somatotype had only inverse significant correlations, mainly of average power. The SDNN was only associated with the value of only 3 (6.1%) anthropometric indicators indicating subcutaneous fat deposition and muscle size. RMSSD had the most numerous and highest correlation strength among all statistical cardiointervalographic parameters, it was associated with 26 (53.1%) somatic dimensions, of which 2 had strong relationships. The established nature of the relationships suggests that with decreasing anthropo-somatotypological parameters in mesomorph wrestlers, the standard deviation of the difference of consecutive R-R intervals will increase and will be dominated by parasympathetic activity, which will reflect sinus arrhythmia associated with respiratory movements [21].

The PNN50 had a significant mean association with 14 (28.6%) constitutional parameters, with the strongest correlation with muscle mass. It should be noted that all heart rate variability statistics had reliable feedback on muscle size.

Spectral indices of mesomorphic type wrestlers, which in athletes [18], were found to have the least significant correlation with external body size compared to other cardiointervalographic parameters.

The total recording power in all ranges had only 2 significant direct average correlation strength with the external body structure index (4.0%). Power at very low frequencies, which reflects the activity of neurohumoral

regulation of angiotensin, chemoreceptive and thermoregulatory systems [3, 13], was directly correlated with 12 (24.5%) anthropometric parameters. The established nature of the relationship indicates that with the increase of total and longitudinal body sizes, the massiveness of the segments of the upper extremity and bone mass will increase the magnitude of this spectral index, and therefore will increase sympathetic influence through humoral regulation and increased activity [3]. The power in the low frequency range of mesomorph wrestlers correlated with 2 body sizes, but it is noteworthy that these connections were inverse. Power in the high-frequency range, which reflects the activity of the parasympathetic link [13], also had a small number of significant correlations (4.0%), all correlations were direct.

We found that the indicators of vegetative homeostasis by the Baevsky method in wrestlers of adolescent mesomorphic somatotype with constitutional parameters had only direct reliable correlations. The voltage index of the regulatory systems was interrelated with 4 (8.2%) constitutional parameters, the nature of the detected connections indicates that the activity of sympathetic regulation mechanisms will increase with the increase of longitudinal body size and muscle mass [3, 13]. The vegetative equilibrium index is also linked to 4 constitutional parameters, including the height of the upper thoracic point, intertrochanteric, muscular, and bone. Vegetative rhythm index had the highest number of significant correlations (40.8%) in comparison with other spectral parameters. It has been found that with increasing total, longitudinal body sizes, chest and pelvis size, bone and muscle mass, this indicator will increase and thus the vegetative balance will be less shifted to the parasympathetic side [13].

The obtained results make it possible to apply the stepwise regression analysis method to develop normative individual cardiointervalographic indicators in the wrestlers of the mesomorphic somatotype depending on the features of the body structure.

Conclusions

1. It was found that in youth wrestlers belonging to the mesomorphic somatotype, the indicators of variational heart rate had the highest number and strength (r=0.42-0.74) of statistically significant correlations with external body size and components of somatotype and body weight. The most reliable relationships were found for mode (63.3% of all possible), average R-R interval (42.8%), and minimum R-R interval (34.7%). Most anthropometric parameters have inverse correlations of average strength, except for the thickness of the skin-fat folds where the correlations are straight.

2. It was found that among statistical indicators of heart rate variability, RMSSD with constitutional parameters had the highest number (53.1%) and strength (r=-0.41 - -0.69) of significant correlations. All the trusted correlations found were feedback. All statistics data had significant correlations with muscle size.

3. The least significant correlations with the constitutional characteristics of mesomorph wrestlers had spectral indices, except for the power at very low frequencies (24.5%) and vegetative homeostasis indices by Baevsky, except for the vegetative rhythm index (40.8%).

4. The revealed nature of the relationship in the wrestlers of the mesomorphic somatotype between all cardiointervalographic parameters and indicators of the external structure of the body indicates that with the increase of total, longitudinal, circumferential, transverse body sizes and muscle and bone mass, the activity of sympathetic regulation mechanisms will increase.

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ОСОБЛИВОСТІ ВЗАЄМОЗВ'ЯЗКІВ МІЖ КАРДІОІНТЕРВАЛОГРАФІЧНИМИ ПОКАЗНИКАМИ ТА КОНСТИТУЦІОНАЛЬНИМИ ХАРАКТЕРИСТИКАМИ У ВИСОКОКВАЛІФІКОВАНИХ БОРЦІВ МЕЗОМОРФНОГО СОМАТОТИПУ

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Механізми регуляції серцевого ритму мають безліч індивідуальних особливостей, котрі обумовлені віком, статтю, тренованістю організму, силою і характером зовнішнього впливу, конституціональними особливостями організму. Мета роботи - визначити взаємозв'язки між кардіоінтервалографічними показниками та параметрами зовнішньої будови тіла у висококваліфікованих борців мезоморфного соматотипу. У дослідженні взяли участь 24 борців у віці від 17 до 21 року з високим рівнем спортивної майстерності і стажем більше 3 років. Всі борці були середніх вагових категорій і займалися вільною та греко-римською боротьбою. Нами було проведене дослідження варіабельності серцевого ритму на кардіологічному комп'ютерному діагностичному комплексі "OPTW" за рекомендаціями Європейської та Північноамериканської кардіологічної асоціації (1996). Визначали показники вегетативного гомеостазу за Баєвським, варіаційної пульсометрії, статистичні та спектральні кардіоінтервалографічні показники. Антропометрію проводили за методом В.В. Бунака (1941), соматотипологічне дослідження - за розрахунковою модифікацією метода Heath-Carter (1990), визначення компонентного складу маси тіла за методом Матейко (1992). У пакеті "STATISTICA 5.5" був проведений кореляційний аналіз із використанням непараметричного статистичного методу Спірмена. Виявлено, що у борців мезоморфного соматотипу показники варіаційної пульсометрії мали з конституціональними параметрами найбільшу кількість та силу достовірних зв'язків, більшість з яких були зворотні середньої сили. Всі статистичні показники варіабельності серцевого ритму з показниками зовнішньої будови тіла мали лише зворотні достовірні кореляції. Найменшу кількість достовірних кореляцій виявлено для спектральних показників і параметрів вегетативного гомеостазу. За результатами кореляційного аналізу у борців мезоморфного соматотипу ми можемо припустити, що із збільшенням тотальних, поздовжніх, обхватних, поперечних розмірів тіла та м'язової та кісткової маси буде більш вираженим вплив на варіабельність серцевого ритму симпатичного відділу автономної нервової системи.

Ключові слова: *кореляції, кардіоінтервалографічні показники, антропометричні розміри, соматотип, борці.*

ОСОБЕННОСТИ ВЗАИМОСВЯЗЕЙ МЕЖДУ КАРДИОИНТЕРВАЛОГРАФИЧЕСКИМИ ПОКАЗАТЕЛЯМИ И КОНСТИТУЦИОНАЛЬНЫМИ ХАРАКТЕРИСТИКАМИ У ВЫСОКОКВАЛИФИЦИРОВАННЫХ БОРЦОВ МЕЗОМОРФНОГО СОМАТОТИПА

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Механизмы регуляции сердечного ритма имеют огромное количество индивидуальных особенностей, которые обусловлены возрастом, полом, тренированностью организма, силой и характером внешнего влияния, конституциональными особенностями организма. Цель работы - определить взаимосвязи между кардиоинтервалографическими показателями и параметрами телосложения у высококвалифицированных борцов мезоморфного соматотипа. В исследовании участвовали 24 борца в возрасте от 17 до 21 года высокого уровня спортивного мастерства и со стажем более 3 лет. Все борцы были средних весовых категорий и занимались вольной и греко-римской борьбой. Нами было проведенное исследование вариабельности сердечного ритма на кардиологическом компьютерном диагностическом комплексе "OPTW" по рекомендациям Европейской и

Североамериканской кардиологической ассоциации (1996). Определяли показатели вегетативного гомеостаза по Баевскому, вариационной пульсометрии, статистические и спектральные кардиоинтервалографические показатели. Антропометрию проводили по методу В.В. Бунака (1941), соматотипологическое исследования - по расчетной модификации метода Heath-Carter (1990), определение компонентного состава массы тела по методу Матейко (1992). В пакете "STATISTICA 5.5" был проведен корреляционный анализ с использованием непараметрического статистического метода Спирмена. Выявлено, что у борцов мезоморфного соматотипа показатели вариационной пульсометрии имели с конституциональными параметрами наибольшее количество и силу достоверных связей, большинство из которых были обратные средней силы. Все статистические показатели вариабельности сердечного ритма с показателями телосложения имели лишь обратные достоверные корреляции. Наименьшее количество достоверных корреляций выявлено для спектральных показателей и параметров вегетативного гомеостаза. По результатам корреляционного анализа у борцов мезоморфного соматотипа мы можем допустить, что с увеличением тотальных, продольных, охватных, поперечных размеров тела, мышечной и костной массы будет более выраженное влияние на вариабельность сердечного ритма симпатичного отдела автономной нервной системы.

Ключевые слова: *корреляции, кардиоинтервалографические показатели, антропометрические размеры, соматотип, борцы.*

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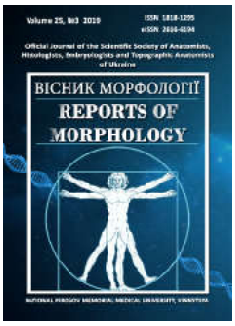

Meibomian gland dysfunction and dry eye disease symptoms in patients with type 2 diabetes mellitus

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An important problem to date is the dysfunction of meibomian glands in patients with type 2 diabetes. The aim of our work was to analyze the prevalence of meibomian gland dysfunction and signs of dry eye disease in patients with type 2 diabetes. We examined 40 patients (80 eyes) with compensated type 2 diabetes mellitus and symptoms of dry eye disease, who made up the main group at the age of 54.00±8.00 years, and 30 patients (60 eyes) of practically healthy people, aged 51.00±8.00 years. All patients underwent visiometry, biomicroscopy, ophthalmoscopy, tonometry, compression test to evaluate the secretion of meibomian glands, Schirmer's test for compression before and after 30 min. Afterwards, Norn test, meibography, Demodex mite test, laboratory tests (lipidogram, blood glucose, glycosylated hemoglobin). In addition, standard OSDI, McMonnies Dry Eye Questionnaire questionnaires were used and an average clipping rate of1 min was determined. Statistical processing of the obtained results was carried out using the license package "Statistica 8.0" using parametric estimation methods. Our study has shown that in patients with diabetes for more than 5 years, dry eye disease occurs more often and is dominated by moderate and severe severity. Each patient in the study group, together with the symptoms of dry eye, had signs of dysfunction of the meibomian glands of varying degrees of severity according to the compression test and objective symptoms of the disease. A significant difference was found between the clipping frequency in the control group and the main one ($p \leq 0.01$). Compared with the control group, patients with diabetes have a longer interval between the clipping movements. Meibography data show that patients in the primary group have changes in the meibomian glands in 90% of cases, while in the control group - 44%. Thus, it was found that patients with type 2 diabetes had dysfunction of meibomian glands of moderate severity in 90% of cases, severe - in 10%, which is associated with the duration of diabetes.

Keywords: *diabetes mellitus, meibomian gland dysfunction, dry eye disease, meibography.*

Introduction

Diabetes mellitus is a metabolic disorder that occurs with chronic hyperglycemia, insulin deficiency, insulin resistance and is accompanied by complications from the visual organ: development of retinopathy, cataracts, dry eye disease. According to the 2015 IDF Diabetes Atlas, about 91% of patients in high-income countries have type 2 diabetes. In 193 million cases, diabetes remains undiagnosed and causes a high risk of complications [16]. The manifestations of dry eye disease occur in 60-72% in patients with diabetes mellitus, while in the general population - 53% [10]. The mechanism of dry eye disease in such patients has not yet been studied [8, 9], among the reasons are considered autoimmune dysfunction, reduction of corneal and conjunctival sensitivity as a result of neuropathy with the involvement of the lacrimal glands, increased osmolarity of the lacrimal function. A new understanding of this problem is reflected in the definition of dry eye syndrome: "Dry eye is a multifactorial disease of the ocular surface, characterized by the loss of homeostasis of the lacrimal film and accompanied by ocular symptoms in which instability of the lacrimal film and hyperosmolarity, inflammation and damage to the ocular surface and neurosensory abnormalities play an etiological role" [2, 12].

Many studies aimed at studying the pathogenesis of dry eye syndrome indicate that one of the important risk

factors for dry eye disease is dysfunction of the meibomian glands [6, 21-23].

Dysfunction of the meibomian glands is one of the main causes of the development of posterior blepharitis [19] and dry eye disease [14]. Its frequency in the general population is 39-50% [13]. At blockage of openings of glands or stasis of lipids inside the gland, the secretion decreases and the composition of the lipid layer of the precorneal film changes, which normally stabilizes it and reduces evaporation [1, 16].

Chronic conditions such as cancer, diabetes and kidney failure are known to directly affect immunity. Skin infestations caused by Demodex sp. show progress in the case of humoral and cellular immune disorders. The formation of Demodex infection depends on internal factors such as sebaceous gland dysfunction and T-cell inhibition, as well as certain external factors. C. Gökçe et al. [4] found demodicosis in 24.6% of 69 patients with type 2 diabetes and concluded that impaired blood glucose regulation increased the sensitivity to D. Folliculorum infection. Some studies found a statistically significant ($p<0.001$) difference in the determination of demodicosis in pregnant women with gestational diabetes (24.2%) and without (3.3%) [7].

The purpose of the study is to analyze the prevalence of dysfunction of the meibomian glands and the signs of dry eye disease in patients with type 2 diabetes.

Materials and methods

The main study group included 40 patients (80 eyes) with type 2 diabetes mellitus and symptoms of dry eye disease aged 54.00±8.00 years, among them men - 18 (45%), women - 22 (55%). Duration of diabetes mellitus type 2 averaged 18 years. The main group was divided into two subgroups: №1 - with type 2 diabetes experience < 5 years (34 eyes) and №2 - duration of diabetes > 5 years (46 eyes). The control group consisted of 30 individuals (60 eyes) of healthy subjects, aged 51.00±8.00 years.

The study followed the basic bioethical standards of the Helsinki Declaration of Human Rights and Biomedicine (1977), the requirements of the Helsinki Declaration of Human Rights (1975) and the Vancouver Convention (1979, 1994), relevant to the provisions of WHO, the International Council of Medical Scientific Societies, The International Code of Medical Ethics (1983) and the laws of Ukraine. All patients were informed and voluntary informed consent was obtained.

All patients underwent visiometry, biomicroscopy, ophthalmoscopy, tonometry, compression test to evaluate the secretion of meibomian glands, Schirmer's test for compression before and after 30 min., Norn test, meibography, Demodex tick detection test, laboratory tests (lipidogram, blood sugar, glycosylated hemoglobin). In addition, standard OSDI, McMonnies Dry Eye Questionnaire questionnaires were used and an average clipping rate of 1 min was determined.

Meibography is a method of patient examination that allows to study in vivo the structure and morphological status of the meibomian glands. There are various techniques in the world, both contact and contactless screening. In our practice, we use a self-developed device based on photographing the turned eyelid in the infrared radiation spectrum (patent No. 127795 "Portable device for the study of the condition of the meibomian glands", patent No. 126656 "Method of obtaining images of the meibomian glands", 2018) [23]. This technique is non-invasive, non-contact, can be used with this device portably in all conditions and does not require prior preparation of the patient. The obtained data were estimated using the meibograde scale [20]. The method is based on three major changes in the meibomian glands: curvature, shortening and complete loss of glands [20]. The evaluation was performed on a 3-point scale: 0 - changes in the meibomian glands are absent, 1 - there are changes in the meibomian glands at 33% of the eyelid area, 2 - impressions of the meibomian glands take up 33-66% of the eyelid area, 3 - more than 66% of the eyelid area have pathological changes in the meibomian glands.

Statistical processing of the obtained results was carried out using the license package "Statistica 8.0" using parametric estimation methods.

Results

All patients of subgroup № 1 had a decrease in the number of functioning glands by an average of 28.0% (according to the compression test) and revealed dysfunction of the meibomian glands of I-II severity. Dry eye disease of the mild degree in this subgroup was detected in 29.4% and the average degree in 47.0% of patients.

Patients of subgroup № 2 had a decrease in the number of functioning glands by an average of 48.2%, grade III dysfunction of meibomian glands was observed in 28.2% (22 eyes); middle-grade dry eye disease was reported in 69.5%, and three patients were diagnosed with a severe degree.

In the control group, the results of the compression test were normal.

The Demodex test was positive in 61.3% of the patients in the main group.

The main complaints that patients presented were: discomfort, tearing, heaviness of the eyelids, sensation of sand, redness, pain, itching. Moreover, most patients with type 2 diabetes had complaints of heaviness of the eyelids (94.6%), discomfort in the eyes (82.6%), redness that occurred periodically, more in the evening (70.6%), sensation of sand and lacrimation (68.0% and 62.6% respectively).

The results of the Schirmer test before compression and after in subgroup № 1 were 5.910±0.100 mm/5min. and 7.400±0.100 mm/5min.; in subgroup № 2 - 4.910±0.100 mm/5min. and 5.810±0.100 mm/5min.; in the control group - 15.30±0.10 mm/5min. and 17.70±0.10 mm/5min., respectively ($p\leq0.05$).

The Norn test was in subgroup №1 - 9.410±0.500 s and in subgroup №2 - 8.150±0.500 s; in the control group -

10.50±0.50 s ($p \leq 0.05$).

The average clipping frequency was 14.00±0.80 per min. in the main group and 29.00±1.00 per min. in the control.

A significant difference was found between the clipping frequency in the control group and the main one ($p \leq 0.01$). Compared with the control group, patients with type 2 diabetes have a longer interval between clipping movements.

Functional test scores correlate with duration of type 2 diabetes lasting > 5 years (r1=-0.68, r2=-0.56).

According to meibography data, patients in the main group showed changes in the meibomian glands in 90.0% of cases, while in the control group - in 44.0%. According to indicators of the "meibograde" scale in patients of the main group amounted to 5.000±0.900 points, which indicates the predominance of dysfunction of meibomian glands of moderate severity, and in those patients, who had diabetes for more than 5 years severe dysfunction of meibomian glands was detected in 14.0%. In patients of the control group according to the indicators of the scale "meibograde" was 2.800±0.800, which means that in patients of this group in 44.0% is dominated by dysfunction of meibomian glands of mild degree [22, 23].

According to lipidograms, 87.1% of patients with type 2 diabetes and dysfunction of the meibomian glands had a low level of high-density lipoproteins.

Discussion

In the studies of Figueroa-Ortiza L.C. et al. [3] found that dry eye disease in patients with diabetes is more often manifested on the basis of objective features than subjective ones. Their research has shown that most patients with dry eye disease have a decrease in goblet cell count. The higher prevalence of dry eye disease detected on the basis of examination and coloring data may also explain the presence of diabetic corneal neuropathy in patients with diabetes mellitus. An increased risk of infection in patients with diabetes [5] contributes to the combination of dry eye disease symptoms with chronic blepharitis in about 50% of patients. Such a course of diseases causes difficulties in establishing the primary link of pathogenesis and their etiology, causing difficulties for finding an effective treatment regimen [15].

Our study showed that in patients with type 2 diabetes for more than 5 years, dry eye disease occurs more often and is dominated by moderate and severe severity. The same pattern is observed in the study of the Nakonechnyi D.O. and Bezkorovaina I.M. [10]. M.R. Manaviat et al. [8] indicate that dry eye syndrome was present in 100% of patients with diabetes for 15 years or more. The results of Figueroa-Ortiz L.C. et al. [3] among patients with diabetes without subjective symptoms of dry eye syndrome, according to the McMonnies questionnaire, found that 67.0% of the Schirmer test was normal, whereas in our study, almost every patient had signs of dry eye syndrome associated with prolonged suffering from diabetes mellitus and possibly lack of proper control over the course of the disease.

According to the DEWS classification, there are water-deficient and water-vaporizing forms of dry eye disease, "dry eye" may be due to insufficient water component of the tear or increased evaporation of it [2]. Tear evaporation is a consequence of dysfunction of the meibomian glands [11, 18]. It is believed that the trigger of primary inflammation is dysfunction of the meibomian glands, which leads to pathological changes of the lacrimal film [15, 17].

Each patient in the study group, together with dry eye syndrome, had signs of dysfunction of the meibomian glands of varying degrees of severity according to the compression test and objective symptoms of the disease. A reliable relationship between the degree of dry eye syndrome and dysfunction of meibomian glands has been established in the studies by Tomlinson A. et al. [19] and Qiao J. and Yan X. [14], who believe that the main cause is the rapid evaporation of the aqueous layer of the lacrimal film due to lack of lipid. A study by Pathan R. [13] found that dysfunction of meibomian glands in patients with diabetes mellitus is a precursor to other eye diseases, including dry eye syndrome, and is found in 56.0%, compared with the general population.

According to our previous studies [22], more than half of the patients had a Demodex test positive. In the study by G?k?e C. et al. [5] found that 24.6% (of 69) of patients with diabetes had a positive Demodex test regardless of age and gender. The authors suggest that inadequate blood glucose control increases the susceptibility to tick infection.

Conclusions

1. Metabolic disorders and dysfunction of the immune system in patients with type 2 diabetes contributes to the emergence of dysfunction of the meibomian glands and activation of conditionally pathogenic flora.

2. The degree of dry eye disease has been found to correlate with the duration of type 2 diabetes in patients suffering more than 5 years. Symptoms of a dry eye of moderate severity were observed 1.5 times more often in patients in whom type 2 diabetes lasted more than 5 years.

3. Changes in meibomian glands in patients with type 2 diabetes were found to be 2 times more frequent than in people of the same age without diabetes, and in patients with diabetes for more than 5 years there was mainly a dysfunction of meibomian glands and amounted to 90.0%.

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ДИСФУНКЦІЯ МЕЙБОМІЄВИХ ЗАЛОЗ ТА ОЗНАКИ СУХОГО ОКА У ХВОРИХ З ЦУКРОВИМ ДІАБЕТОМ 2 ТИПУ

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Важливою проблемою на сьогоднішній день є дисфункція мейбомієвих залоз у хворих з цукровим діабетом 2 типу. Метою нашої роботи було проаналізувати поширеність дисфункції мейбомієвих залоз та ознак хвороби сухого ока у хворих з цукровим діабетом 2 типу. Було обстежено 40 пацієнтів (80 очей) з компенсованим цукровим діабетом 2 типу та симптомами хвороби сухого ока, які склали основну групу віком 54,00±8,00 років та 30 пацієнтів (60 очей) практично здорових людей, віком 51,00±8,00 років. Всім пацієнтам проводили візіометрію, біомікроскопію, офтальмоскопію, тонометрію, компресійний тест для оцінки секреції мейбомієвих залоз, пробу Ширмера до компресії та через 30 хвилин після неї, пробу Норна, мейбографію, тест на виявлення кліща Demodex, лабораторні дослідження (ліпідограма, глюкоза крові, глікозильований гемоглобін). Крім того, використовували стандартні опитувальники OSDI, McMonnies Dry Eye Questionnaire та визначали середню частоту кліпання за 1 хвилину. Статистична обробка отриманих результатів проведена за допомогою ліцензійного пакету "Statistica 8.0" з використанням параметричних методів оцінки. Проведене нами дослідження показало, що у пацієнтів з тривалістю цукрового діабету більше 5 років хвороба сухого ока виникає частіше та переважає середній та важкий ступінь тяжкості. За даними компресійного тесту та об'єктивними симптомами захворювання кожен пацієнт досліджуваної групи разом із симптомами сухого ока мав ознаки дисфункції мейбомієвих залоз різного ступеня вираженості. Виявлена

достовірна різниця між частотою кліпання у контрольній групі та у основній ($p \leq 0,01$). У порівнянні з контрольною групою, хворі на цукровий діабет мають довший інтервал між кліпальними рухами. За даними мейбографії встановлено, що у пацієнтів основної групи зміни мейбомієвих залоз зустрічаються у 90% випадків, тоді як в контрольній групі - у 44%. Таким чином, встановлено, що у хворих з цукровим діабетом 2 типу виявлено дисфункцію мейбомієвих залоз середнього ступеня важкості у 90% випадків, важкого ступеня - у 10%, що пов'язано з тривалістю діабету.
Ключові слова: *цукровий діабет, дисфункція мейбомієвих залоз, хвороба сухого ока, мейбографія.*

ДИСФУНКЦИЯ МЕЙБОМИЕВЫХ ЖЕЛЕЗ И ПРИЗНАКИ СУХОГО ГЛАЗА У БОЛЬНЫХ С САХАРНЫМ ДИАБЕТОМ 2 ТИПА

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Важной проблемой сегодняшнего дня является дисфункция мейбомиевых желез у больных сахарным диабетом 2 типа. Целью нашей работы было проанализировать распространенность дисфункции мейбомиевых желез и признаков болезни сухого глаза у больных с сахарным диабетом 2 типа. Было обследовано 40 пациентов (80 глаз) с компенсированным сахарным диабетом 2 типа и симптомами болезни сухого глаза, которые составили основную группу возрастом 54,00±8,00 лет и 30 пациентов (60 глаз) практически здоровых людей в возрасте 51,00±8,00 лет. Всем пациентам проводили визиометрию, биомикроскопию, офтальмоскопию, тонометрию, компрессионный тест для оценки секреции мейбомиевых желез, пробу Ширмера перед компрессией и через 30 минут после нее, пробу Норна, мейбографию, тест на клеща Demodex, лабораторные исследования (липидограмма, глюкоза крови, гликозилированный гемоглобин). Кроме того, использовали стандартные опросники OSDI, McMonnies Dry Eye Questionnaire и определяли среднюю частоту моргания за 1 мин. Статистическая обработка полученных результатов проведена с помощью лицензионного пакета "Statistica 8.0" с использованием параметрических методов оценки. Проведенное нами исследование показало, что у пациентов с длительностью сахарного диабета более 5 лет болезнь сухого глаза возникает чаще и преобладают средние и тяжелые степени тяжести. По данным компрессионного теста и объективных симптомов заболевания каждый пациент исследуемой группы вместе с симптомами сухого глаза имел признаки дисфункции мейбомиевых желез различной степени выраженности. Выявлена достоверная разница между частотой моргания пациентов контрольной группы и основной ($p \leq 0,01$). По сравнению с контрольной группой, больные сахарным диабетом имеют более длительный интервал между мигательными движениями. По данным мейбографии установлено, что у пациентов основной группы изменения в мейбомиевых железах встречаются в 90% случаев, тогда как в контрольной группе - у 44%. Таким образом, установлено, что у больных с сахарным диабетом 2 типа выявлена дисфункция мейбомиевых желез средней степени тяжести в 90% случаев, тяжелой степени - в 10%, что связано с длительностью диабета.
Ключевые слова: *сахарный диабет, дисфункция мейбомиевых желез, болезнь сухого глаза, мейбография.*

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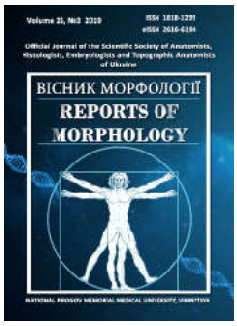

Organometric parameters of hepatoduodenal ligament n the perinatal period

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During any surgery, in addition to pathology-related factors, it is necessary to evaluate the individual features of the anatomy of a particular area or complex of organs. The relevance of such data is confirmed by the results of numerous studies that show that half of patients are characterized by certain anatomical variants, including the location of the arteries and biliary tract. The purpose of the work is to establish the peculiarities of the morphometric parameters of the components of the hepatic-duodenal ligament. The study was based on 50 preparations of fetus corpses (4 to 10 months) and 10 newborns without external signs of anatomical abnormalities or congenital malformations. Adequate anatomical methods were used during the study: macropreparation, injection of blood vessels, making topographic anatomical sections, morphometry. Statistical analysis of the obtained data was performed using the licensed program RStudio. During the perinatal period, the length of the hepatic-duodenal ligament was found to increase from 5.224±0.572 mm in fetuses for 4 months to 32.12±1.77 mm in newborns. The chart of change of the average values of its length by months of prenatal development indicates uneven increase of organometric parameters. From 4 to 5 months observed a significant increase in the length of the hepatic-duodenal ligament, while from 5 to 7 months of development observed a period of relative slowdown of its length, and from 5 to 6 months of development even a relative decrease in length. The period from 7 to 10 months determines the period of accelerated growth of ligament. The indices of the width of the hepatic-duodenal ligament in the perinatal period increased from 3.292±0.227 mm in fetus of 4 months to 21.25±0.938 mm in newborns. The width increases are not uniform. The periods of accelerated development (4-5 months and 9 months - newborns) and the period of slow development (5-8 months) were observed. It was proved that there are periods of accelerated and slow development, in which during periods of intensive development, organometric indicators always differed significantly, were smaller than the previous ones and outweighed the following ones respectively (4-5 months and 7-9 months, $p<0.05$). Regarding the periods of slow development, the organometric indicators in these periods did not differ significantly ($p>0.05$). Therefore, analyzing the dynamics of changes in the morphometric parameters of the components of the hepatic-duodenal ligament, revealed periods of their accelerated and slow growth.

Keywords: *hepatoduodenal ligament, fetus, anatomy, human.*

Introduction

Hepatic-duodenal ligament, portal hepatic vein and gallbladder are target structures during laparoscopic cholecystectomy - one of the most common surgical interventions. This manipulation is for the purpose of treating gallstone disease, which, although a benign disease, is potentially dangerous to the patient. As with any surgical manipulation, cholecystectomy is at risk for complications and errors for the surgeon. Considering the importance of the communication structures: biliary tract, portal hepatic vein, hepatic arteries, etc., any damage to them is dangerous to the life of the patient [21-23]. That is why, along with surgical skills, knowledge of variants and features of the topography of structures of the hepatic-duodenal ligament is equally important for the favorable course of surgery. The ductal system of the liver, gallbladder and pancreas develop from the endodermal diverticula of the duodenum, which are

immersed in the transverse septum, which promotes the formation of a ligamentous apparatus - the small omentum and hepatic-duodenal ligament [24, 25]. Further growth and rotation of the duodenum lead to displacement of the rudiments of the pancreas and extrahepatic bile ducts more dorsally than the duodenum [1-6].

The gallbladder and bladder duct develop from the vesical diverticulum. Certain features of its development lead to the formation of certain variants of the structure. By this term we can mean the doubling of the gallbladder, the septum of the bladder, the bladder deeply immersed in the parenchyma of the liver, the gallbladder with mesentery and the left-sided position of the gallbladder. It should be noted that most of these anatomical features (except for the deep position of the bladder) do not present complications for the surgeon during cholecystectomy [7-12].

The purpose of the study was to determine the peculiarities of the morphometric parameters of the components of the hepatic-duodenal ligament of fetuses and newborns.

Materials and methods

The study was based on 50 preparations of corpses of fetus (4 to 10 months) and 10 newborns without external signs of anatomical abnormalities or congenital malformations. Adequate anatomical methods were used during the study: macropreparation, injection of blood vessels, making topographic anatomical sections, morphometry.

The work was performed in compliance with the main provisions of the Declaration of the World Medical Association on the ethical principles of conducting scientific and medical research with human participation (1964-2000) and the order of the Ministry of Health of Ukraine No. 690 of 23.09.2009 and is a fragment of a comprehensive planned initiative scientific research work of department anatomy of human named after M.G. Turkevych, anatomy, topographic anatomy and operative surgery of the Higher Medical Institute of Ukraine "Bukovinian State Medical University": "Features of morphogenesis and topography of organs and systems in prenatal and postnatal ontogeny periods" (State registration no. 0115U002769).

Statistical analysis of the obtained data was performed using the licensed program RStudio. The null hypothesis was tested that the samples were taken from one distribution or from distributions with the same median:

H_0: {each group has the same distribution}

H_1: {each group does not have the same distribution}.

The nature of the distributions was estimated for each of the variations obtained, the average for each trait being studied, the standard deviation, the percentile span of the indicators. Used Student's t test, nonparametric Kruskal-Wallis test (answers the question whether there are differences between group distributions, but does not specify which groups are different), Conover-Iman test for comparison of stochastic dominance and results between different pairwise comparisons after test for stochastic dominance among groups. Statistically significant values were considered to be $p<0.05$.

Results

Analyzing the organometric parameters of the hepatic-duodenal ligament by constructing a box diagram (Fig. 1), it can be seen that the difference between the medians of the samples (horizontal line in the box) is statistically significant. The Kruskal-Wallis test performed the following results: since $p<0.05$, the difference between the medians of the groups is statistically significant. Using the Conover-Iman test, when comparing the morphometric parameters of the length of the hepatic to duodenal ligament of fruits of different age groups and newborns, revealed certain features of their dynamics. The length of the hepatic-duodenal ligament of the fetus of 4 months significantly shorter than in fetus of 5 months ($p<0.05$). However, this parameter is not significantly different from such fruits for 6 months ($p>0.05$), the latter, in turn, is significantly smaller than the length of the hepatic-duodenal ligament of the fetus for 5 months. Further, from the 6th to the 9th month of development, the morphometric indices differ significantly with each other, and the index of the length of the hepatic-duodenal ligament of each subsequent month significantly exceeds that of the previous month. Median difference for couple: "10 months" - "newborns" are not statistically significant ($p>0.05$), that is, the length of the hepatic-duodenal ligament of the fetus is 10 months and newborns were not significantly different, although they were by far the highest among all study groups.

Descriptive statistics of the length of the hepatic-duodenal ligament during the perinatal ontogeny period are presented in Table 1.

The graph of the mean values of hepatic-duodenal ligament by age group (Fig. 2) of the perinatal period indicates the intensity of changes in the parameters of hepatic-duodenal ligament during the perinatal ontogeny period.

Column diagram for the group of medium hepatic-duodenal ligament widths looks like (Fig. 3).

Analyzing the morphometric parameters of the width of the hepatic-duodenal ligament by constructing a box

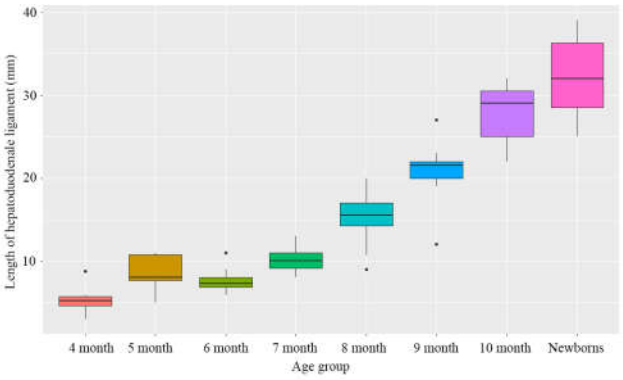

Fig. 1. Box diagram of length of hepatic-duodenal ligament by age groups.

Table 1. Descriptive statistics of hepatic-duodenal ligament length during the perinatal ontogeny period.

Age group	Average	Standard error for the average	Confidence interval for the average
4 month	5.224	0.572	(4.090; 6.352)
5 month	8.551	0.681	(7.021; 10.08)
6 month	7.627	0.485	(6.543; 8.753)
7 month	10.25	0.48	(9.171; 11.33)
8 month	15.17	1.04	(12.81; 17.53)
9 month	20.80	1.20	(18.09; 23.51)
10 month	27.91	1.09	(25.48; 30.34)
Newborns	32.12	1.779	(27.94; 36.30)

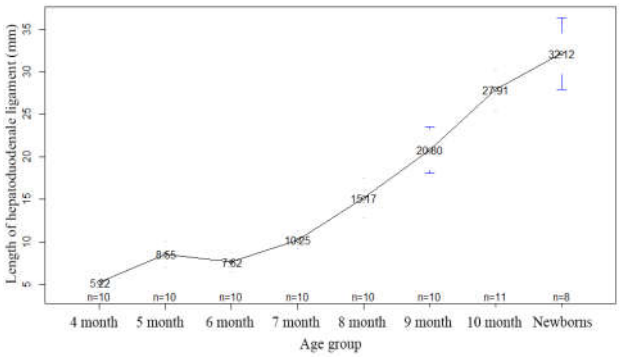

Fig. 2. Schedule average values of hepatic-duodenal ligament length in age groups.

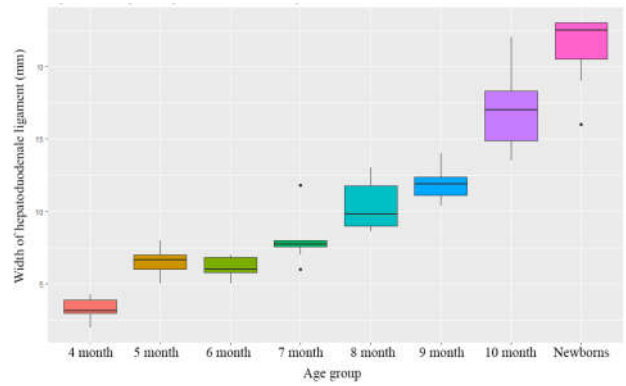

Fig. 3. Box diagram of hepatic-duodenal ligament width by age groups.

Table 2. Descriptive statistics of hepatic-duodenal ligament width during the perinatal ontogeny period.

Age group	Average	Standard error for the average	Confidence interval for the average
4 month	3.292	0.227	(2.811; 3.786)
5 month	6.495	0.282	(5.862; 7.127)
6 month	6.184	0.211	(5.754; 6.667)
7 month	7.952	0.471	(6.894; 9.015)
8 month	10.28	0.51	(9.134; 11.43)
9 month	11.92	0.37	(11.08; 12.76)
10 month	16.99	0.79	(15.24; 18.74)
Newborns	21.25	0.90	(19.26; 23.38)

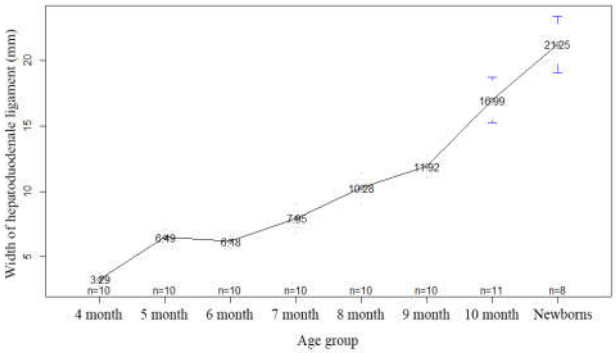

Fig. 4. Graph of mean hepatic-duodenal width by age groups.

diagram (see Fig. 3), it can be seen that the difference between the medians of the samples (horizontal line in the box) is statistically significant. The Kruskal-Wallis test performed the following results: since $p<0.05$, the difference between the medians of the groups is statistically significant. Using the Conover-Iman test, when comparing the organometric parameters of the width of the hepatic-duodenal ligament of fetus of different age groups and newborns revealed certain peculiarities of their dynamics. Width of hepatic-duodenal ligament of fetus 4 months significantly less than in fetus of all subsequent age groups (5 months - newborns) ($p<0.05$). However, this parameter is not significantly different from such fetus for 5 months and 6 months ($p>0.05$), the latter in turn significantly smaller than the width of the hepatic-duodenal ligament of the fetus for 7 months - newborns. In the following, the parameters of the width of the hepatic-duodenal ligament from fetus 7 months to newborns significantly outweigh those of the previous month and significantly less than the parameters of the following months.

Descriptive statistics of the width of the hepatic-duodenal ligament during the perinatal ontogeny period are presented in Table 2.

The graph of mean hepatic-duodenal ligament lengths by age group (Fig. 4) of the perinatal period indicates the intensity of change in hepatic-duodenal ligament width during the perinatal period.

Discussion

Comparing the graphs of the average values of the length and width of the hepatic-duodenal ligament at each of the stages of the perinatal period, it is worth noting that some processes of increasing organometric parameters are not synchronous.

The graph of average values of the length of the hepatic-duodenal ligament by age groups of the perinatal period indicates the presence of two periods of accelerated development (4-5 months and 7-9 months) and a period of slow development (5-7 months). Regarding the width of the ligament, we found that there were two periods of accelerated development (4-5 months and 9 months - newborns) and a period of slow development (5-8 months).

It is worth noting that at some stage of development (6 months), the length of the hepatic-duodenal ligament is even slightly reduced, which can probably be associated with an increase in the adjacent organs - the liver and the duodenum in this period [13-20].

The presence of periods of accelerated and delayed development is confirmed by the validity of the differences in indicators of width and length of hepatic-duodenal ligament. The period of slow increase in the ligament length is 5-7 months, coincides with periods of no significant difference between organometric parameters ($p>0.05$). At the same time, the periods of accelerated growth coincide with the periods of significant difference in the morphometric parameters of the ligament (4-5 months and 7-9 months, $p<0.05$). A similar situation was observed with periods of intensive and slow growth of the link width. For 4-5 months we observe a period of accelerated increase in the width of the hepatic-duodenal ligament at the same time, the morphometric parameters significantly increase and differ ($p<0.05$). While in the period of slow development (5-6 months), the morphometric parameters of the ligament width do not differ ($p>0.05$).

Conclusions

1. During the perinatal period, the length of the hepatic-duodenal ligament increases from 5.224±0.572 mm in fetuses for 4 months to 32.12±1.77 mm in newborns. The chart of change of the average values of its length by months of prenatal development indicates uneven increase of organometric parameters. From 4 to 5 months observed a significant increase in the length of the hepatic-duodenal ligament, while from 5 to 7 months of development observed a period of relative slowdown of its length, and from 5 to 6 months of development even a relative decrease in length. The period from 7 to 10 months determines the period of accelerated growth of ligament.

2. The indices of the width of the hepatic-duodenal ligament in the perinatal period increase from 3.292±0.227 mm in the fetus in 4 months to 21.25±0.93 mm in newborns. The width increases are not uniform. The periods of accelerated development (4-5 months and 9 months - newborns) and the period of slow development (5-8 months) were observed.

3. It is proved that there are periods of accelerated and delayed development, according to which, during periods of intensive development, organometric indicators always differed significantly, were smaller than the previous ones and outweighed the following ones respectively (4-5 months and 7-9 months, $p<0.05$). Regarding the periods of slow development, the organometric indicators in these periods did not differ significantly ($p>0.05$).

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ОРГАНОМЕТРИЧНІ ПАРАМЕТРИ ПЕЧІНКОВО-ДВАНАДЦЯТИПАЛОЇ ЗВ'ЯЗКИ У ПЕРИНАТАЛЬНОМУ ПЕРІОДІ

Юзько Р.В., Слободян О.М.

Під час проведення будь-якого оперативного втручання, окрім факторів, що викликали патологію, необхідно оцінити індивідуальні особливості анатомії тієї чи іншої ділянки, або комплексу органів. Актуальність таких даних підтверджується результатами численних досліджень, котрі показують, що половині пацієнтів характерні певні анатомічні варіанти, зокрема, і розташування артерій та жовчовивідних шляхів. Мета роботи - встановити особливості морфометричних параметрів компонентів печінково-дванадцятипалої зв'язки. Матеріалом дослідження послужили 50 препаратів трупів плодів (від 4 до 10 місяців) та 10 новонароджених без зовнішніх ознак анатомічних відхилень або вроджених вад розвитку. Під час дослідження використовували адекватні анатомічні методи: макропрепарування, ін'єкцію кровоносних судин, виготовлення топографо-анатомічних зрізів, морфометрію. Статистичний аналіз отриманих даних проводили за допомогою ліцензованої програми RStudio. Встановлено, що впродовж перинатального періоду довжина печінково-дванадцятипалої зв'язки збільшується від 5,224±0,572 мм у плодів 4 міс. до 32,12±1,77 мм у новонароджених. Графік зміни середніх значень її довжини по місяцям внутрішньоутробного розвитку вказує на нерівномірне збільшення органометричних параметрів. Так, з 4 по 5 міс. спостерігали істотне збільшення довжини печінково-дванадцятипалої зв'язки, в той час як з 5 по 7 місяць розвитку спостерігали період відносного уповільнення збільшення її довжини, а в період з 5 по 6 місяці розвитку навіть і відносне зменшення довжини. У період з 7 по 10 місяці визначається період прискореного росту зв'язки. Показники ширини печінково-дванадцятипалої зв'язки у перинатальному періоді збільшуються з 3,292±0,227 мм у плодів 4 міс. до 21,25±0,938 мм у новонароджених. Збільшення ширини відбувається нерівномірно. Спостерігали наявність періодів прискореного розвитку (4-5 міс. та 9 міс. - новонароджені) та період уповільненого розвитку (5-8 міс.). Було доведено наявність періодів прискореного та уповільненого розвитку, за котрими у періоди інтенсивного розвитку органометричні показники завжди достовірно відрізнялись, були меншими за попередні та переважали наступні, відповідно (4-5 міс. та 7-9 міс., p<0,05). Щодо періодів уповільненого розвитку, то органометричні показники в дані періоди достовірно не відрізнялись (p>0,05). Отже, аналізуючи динаміку змін морфометричних параметрів компонентів печінково-дванадцятипалої зв'язки, виявлені періоди їх прискореного та уповільненого росту.

Ключові слова: *печінково-дванадцятипала зв'язка, плід, анатомія, людина.*

ОРГАНОМЕТРИЧЕСКИЕ ПАРАМЕТРЫ ПЕЧЕНОЧНО-ДВЕНАДЦАТИПЕРСТНОЙ СВЯЗКИ В ПЕРИНАТАЛЬНОМ ПЕРИОДЕ

Юзько Р.В., Слободян А.Н.

Во время проведения оперативных вмешательств, кроме факторов, повлекших за собой патологию, необходимо учитывать и индивидуальные особенности анатомии той или иной области, или комплекса оперируемых органов. Актуальность таких данных подтверждается результатами множества исследований, которые указывают на то, что в половине случаев пациентам характерны определенные анатомические варианты размещения артерий и желчевыводящих путей. Цель работы - установить особенности морфометрических параметров компонентов печеночно-двенадцатиперстной связки. Материалом исследования послужили 50 препаратов трупов плодов (от 4 до 10 месяцев) и 10 новорожденных без внешних признаков анатомических отклонений или врожденных пороков развития. Во время исследования использовали адекватные анатомические методы: макропрепарирование, инъекцию кровеносных сосудов, изготовление топографоанатомических срезов, морфометрию. Статистический анализ полученных данных проводили с помощью

лицензированной программы RStudio. Установлено, что в течение перинатального периода длина печеночно-двенадцатиперстной связки увеличивается от 5,224±0,572 мм у плодов 4 мес. до 32,12±1,77 мм у новорожденных. График изменения средних значений ее длины по месяцам внутриутробного развития указывает на неравномерное увеличение органометрических параметров. Так, с 4 по 5 мес. наблюдали существенное увеличение длины печеночно-двенадцатиперстной связки, в то время как с 5 по 7 месяц развития наблюдали период относительного замедления увеличения ее длины, а в период с 5 по 6 месяц внутриутробного развития даже относительное уменьшение длины. В период с 7 по 10 месяц определяется период ускоренного роста связки. Показатели ширины печеночно-двенадцатиперстной связки в перинатальном периоде растут с 3,292±0,227 мм у плодов 4 мес. до 21,25±0,938 мм у новорожденных. Увеличение ширины происходит не равномерно. Наблюдали наличие периодов ускоренного развития (4-5 мес. и 9 мес. - новорожденные) и период замедленного развития (5-8 мес.). Было доказано наличие периодов ускоренного и замедленного развития, по которым в периоды интенсивного развития органометрические показатели всегда достоверно отличались, были меньше предыдущих и преобладали над последующими, соответственно (4-5 мес. и 7-9 мес., $p<0,05$). Относительно периодов замедленного развития, то органометрические показатели в данные периоды достоверно не отличались ($p>0,05$). Итак, анализируя динамику изменений морфометрических параметров компонентов печеночно-двенадцатиперстной связки, выявлены периоды их ускоренного и замедленного роста.

Ключевые слова: *печеночно-двенадцатиперстная связка, плод, анатомия, человек.*

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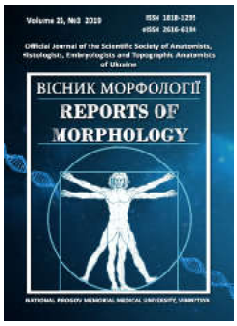

Morphological changes of a great omentum at implantation of polypropylene and composite prostheses at allohernioplasty

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To date, allohernioplasty of giant postoperative ventral hernias retains high postoperative mortality and a significant number of postoperative complications. The purpose of the study is to investigate the possibility of intraperitoneal use of polypropylene and composite implants in allohernioplasty of postoperative giant ventral hernias by studying the morphological changes of the great omentum. The results of surgical treatment of 146 patients with postoperative giant ventral hernias were investigated. To this group of patients with allohernioplasty by the method onlay was performed 22 (15.1%) patients, by the method sublay - 46 (31.5%), by the method inlay - 52 (35.6%), with intra-abdominal placement of the mesh by the method onlay (intraperitoneal onlay mesh) - 26 (17.8%). 32 (21.9%) patients who underwent surgery using the sublay method polypropylene implant was fenced off from the abdominal organs with a great omentum, 22 (15.1%) patients operated on by the intraperitoneal onlay mesh method composite implant was also fenced off from the abdominal organs by a great omentum. In 8 (5.5%) patients from the group of patients who were operated on by the sublay method and in 6 (4.1%) - operated by the intraperitoneal onlay mesh method for 14-18 days patients developed signs of chronic intestinal obstruction, where the cause was postoperative adhesive illness. The study of the effect of polypropylene prosthesis on a great omentum was performed on 8 outbred dogs and 6 outbred mature dogs, who were implanted with a composite mesh unilaterally covered with oxycellulose. The polypropylene implant has been shown to have a greater capacity for the development of adhesive processes. However, if a great omentum to protect this prosthesis from the abdominal cavity then the first zone (active inflammation) spread in 155 microns (increase in the number of blood capillaries of the microcirculatory bed, thickening of the arterioles wall, venous full blood flow, diapedesis of leukocytes through the wall of the blood cells), the second zone (sclerosis) - up to 40 microns (increase in the number of macrophages and lymphocytes. proliferation of fibroblasts). In the future, the structure of the omentum was almost indistinguishable, so with allohernioplasty by the developed method, it was possible to prevent the development of adhesive disease on the intestine. This study confirmed the limited spread of the inflammatory response, which allows the implant of a polypropylene mesh on a great omentum. In the composite mesh, the composite mesh was spliced with surrounding tissues from the side where there was no gel coating, and from the side of the salivary coating, the "readiness" to spread the inflammatory process (vasculitis with pronounced lympho-plasmocytic infiltration of the vessel walls and perivascular ductus), therefore, left it is also necessary to enclose it with a large omentum from intestium.

Keywords: *polypropylene prosthesis, composite mesh, allohernioplasty, great omentum.*

Introduction

The incidence of postoperative ventral hernias after laparotomy, according to various authors, is from 10% to 30% [1]. According to many researchers, a special place is occupied by defects of large and giant size, involving two or more sections of the abdominal wall, make up 18-45% [9, 13]. Of particular concern to surgeons is the degree of destruction of the anatomical structures of the abdominal wall, which can be considered a real " catastrophe " when

conducting reconstructive operations. First of all, this is due to the fact that the scar formation in the area of hernial defect runs parallel to the atrophic degenerative processes in the muscles and fascial-aponeurotic structures of the abdominal wall [5]. According to some authors, the use of auxiliary materials has allowed to significantly improve the results of treatment of patients with giant postoperative ventral hernias [2, 12]. However, with different methods of alloplasty, the recurrence of the disease ranges from 10% to 20% [8].

However, there are studies that state that synthetic tissue introduced into the body still remains a "foreign" body to it. The possibility of contact of the implanted prosthesis with the intestine remains unexplored, and as a result there is a risk of splicing with the intestine and impaired patency [3]. Also, some surgeons have noted that the above methods of alloplasty have a common disadvantage, because they do not consider the need to attach the prosthesis to the tendon fixation of the abdominal wall muscles, and thus exclude the pathogenetic aspect of restoration of function [5] in cases where the location of polypropylene implant in the retromuscular or preperitoneal space was impossible [14, 18]. To solve these problems, consider the choice of a method of intraperitoneal plastics - an alternative option of closing the defects of the abdominal wall with localization M and L (according to the classification Chevrel - Rath) [7]. The use of composite materials has become widespread [16].

The purpose of the study is to investigate the possibility of intraperitoneal use of polypropylene and composite implants in allohernioplasty of postoperative giant ventral hernias by studying the morphological changes of the great omentum.

Materials and methods

For the period 2002-2018 at the Department of Surgery №1, 146 patients with postoperative giant ventral hernias were operated (according to the classification of J.P. Chevrel and A.M. Rath). The age of patients ranged from 40 to 88 years. This group of patients with allohernioplasty was performed by the method onlay 22 (15.1%), sublay - 46 (31.5%), inlay - 52 (35.6%), IPOM - 26 (17.8%). Preference was given to operations that did not reduce abdominal volume. Of the 46 (31.5%) patients operated on by the sublay method, 32 (21.9%) patients with a polypropylene implant ("Ukrainian chain mail", "Lintex") were fenced off from the abdominal cavity by great omentum. Of the 26 (17.8%) patients operated on by the IPOM method, 22 (15.1%) patients used a composite implant ("Proseed"), which was also fenced off from the abdominal cavity by great omentum. In 8 (5.5%) patients from the group of patients operated on the sublay method and in 6 (4.1%) operated on the IPOM method, signs of chronic intestinal obstruction developed on 14-18 days, where postoperative adhesive disease became the cause.

To determine this reason, it was decided to conduct an experimental study. Experimental studies selected 14 outbred dogs. The experiment obtained permission of the Commission on Bioethics National Pirogov Memorial Medical University, Vinnytsya (protocol №1 of 13.01.2011), which established that the conducted studies meet the ethical and moral requirements in accordance with the order of the Ministry of Health of Ukraine №281 of 01.11.2000. During the research, the basic rules of good laboratory practice GLP (1981), Law of Ukraine №3447-IV "On the Protection of Animals against Cruelty" of 21.02.2006 were followed. The experiment was carried out in accordance with the standards of the Council of Europe Convention on Bioethics (1997), the World Health Association Declaration of Helsinki (1996), the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (1985). The operative part of the experiment was performed in the operating room with all the requirements of asepsis. After surgery, the animals were kept in the vivarium on a standard diet. There was no harm to the dogs' health.

From the selected experimental material were cut fragments of tissue 1 x 1 x 0.5 cm so that the block had implants and a great omentum with a border between them. Similarly, sized tissue fragments were obtained from the clinical operating material, which was fully analyzed. For fixation of tissues used a 10% solution of neutral formalin. After dehydration according to the standard scheme, paraffin blocks were made, sections of which 5-7 μm thick were examined by histological methods (staining with hematoxylin and eosin, as well as by Van Gieson and Verhoff methods). Separate histochemical techniques have been used to verify certain chemical compounds in tissue structures, as described in the relevant guidelines. For the verification of protein-synthesizing cells (in particular immunocompetent) used the Brasche reaction. The presence of glycogen and glycoproteins was determined by the method of PAS reaction, lipofuscin - by the method of detection of acid-resistant lipofuscin in semi-thin sections according to Ziehl-Nielsen.

Preparations were viewed under the OLYMPUSBX 41 microscope at magnifications of 40, 100, 200 and 400. Photographs of the microproducts were performed using a video system and a licensed Olympus Quick Photo Micro 2.3 software using an Olympus E-410 digital camera.

The morphological study of the effect of polypropylene mesh on a great omentum was performed on 8 outbred dogs, which were divided into 2 groups. The control group consisted of 2 dogs who had a laparotomy and were taken biopsy from a great omentum. In the study group (6 animals), a laparotomy was performed with simultaneous modeling of the defect of the parietal peritoneum, where a polypropylene mesh was sewn, the strand being sewn to a great omentum. Further, a relaparotomy was performed to collect the biopsy specimen from the great omentum at the point of its adherence to the mesh. Two dogs were re-operated after 1 week, 2 dogs after 2 weeks, and 2 dogs

after 3 weeks.

We also conducted an experimental study of the effect of alloprosthetics composite mesh on the great omentum and intestine. To find out the effectiveness of different ways of strengthening the abdominal wall and their possible complications during surgical treatment of hernias, an experiment was conducted on 6 outbred sexually mature dogs, which were implanted with polypropylene mesh unilaterally covered with oxycellulose. The material for pathohistological examination was obtained during the following operations 3 weeks later and 3 months after the first surgery.

Results

Morphological study of the effect of polypropylene mesh on a great omentum. One week after surgery, in 6 outbred dogs were allocated 2 zones on histological preparations of the omentum: 1 - the omental area, which was directly adjacent to the mesh, up to 40 μm thick with signs of inflammatory processes; 2 - the zone of the omentum adjacent to the intestine was virtually unchanged.

In the first zone, the following phenomena were observed: increase in the number of vessels of the microcirculatory bed, arterial and venous plethora, thickening of the walls of arterioles and venules, marginal placement of leukocytes in venules, diapedesis of leukocytes through the walls of venules and capillaries. The lumen of the lymphatic capillaries was greatly expanded. The connective tissue contains neutrophils, macrophages, lymphocytes, tissue basophils, giant macrophages. The proliferation of fibroblasts and the growth of collagen fibers were expressed, especially the concentration of these cells around the vessels of the microcirculatory bed.

In the second zone, there was a slight increase in collagen fibers (Fig. 1). The structure of the omentum in this area did not differ from the structure of the omentum of intact animals. A slight thickening of collagen fibers was found.

After 2 weeks after surgery, the first zone expanded significantly - up to 120 μm. The growth of fibroblasts and fibrocytes, coarse fibrous scar tissue was observed, and the number of vessels of the microcirculatory bed with pronounced venous plethora was significantly increased (Fig. 2). Accumulations of macrophages, lymphocytes, and lymphoblasts, which were similar in structure to lymphatic follicles, were also established (Fig. 3).

In the second zone, venous plethora, thickening and fibrosis of the walls of the arterioles were observed and their lumen was reduced compared to the norm. The lymphatic capillaries were enlarged. Against the background of increasing numbers of macrophages and lymphocytes, fibroblast proliferation occurred.

At the boundary of these areas was noted area of tender sclerosis. It observed infiltration of the interstitium by macrophages and lymphocytes, as well as single tissue basophils, proliferation of fibroblasts and growth of collagen fibers (Fig. 4).

3 weeks after surgery, there is a significant expansion of the first zone to 140-155 microns and an increase in the number of blood vessels in the microcirculatory bed; enlargement of the lumen of the lymphatic vessels, gross

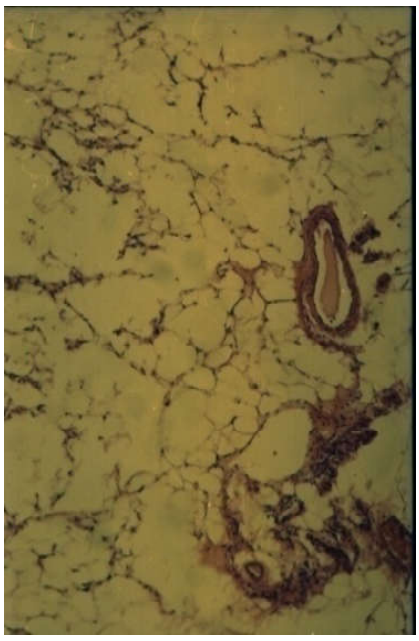

Fig. 1. The concentration of collagen fibers around the vessels of the microcirculatory bed (yellow arrow). Hematoxylin-eosin, x100.

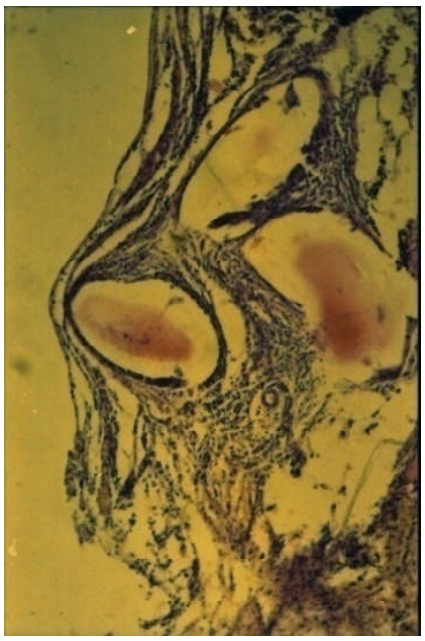

Fig. 2. Venous plethora on the background of the growth of coarse fibrous connective tissue (yellow arrows). Hematoxylin-eosin, x100.

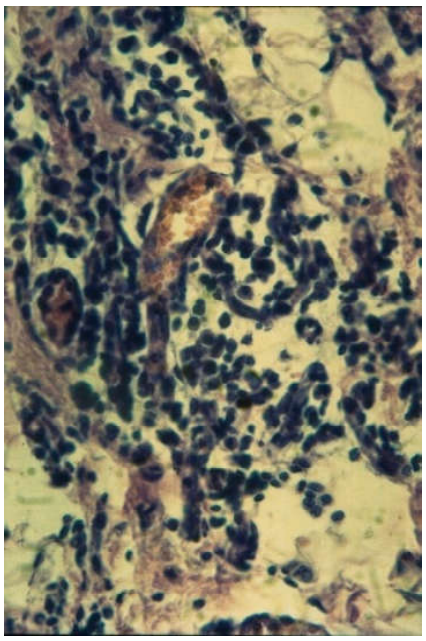

Fig. 3. The accumulation of neutrophils, macrophages, lymphocytes and tissue basophilia is more pronounced around the vessels (yellow arrow). Hematoxylin-eosin, x200.

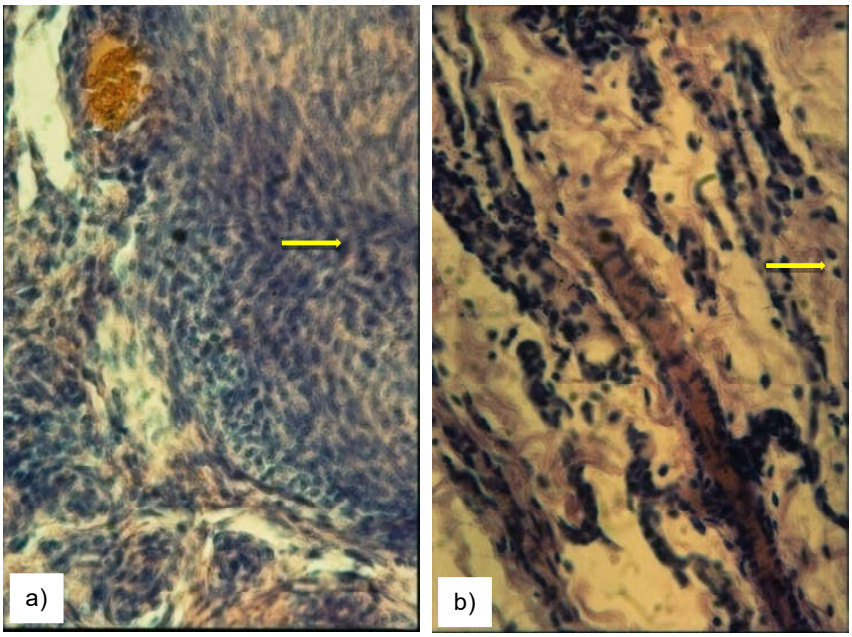

Fig. 4. Collagen fiber growth with the formation of tender sclerosis (yellow arrows). a - Van Gieson method, x200; b - Van Gieson method, x200.

sclerosis of the interstitium, pronounced diapedesis of erythrocytes, marginal standing and diapedesis of leukocytes, numerous accumulations of lymphocytes, macrophages, as well as basophils in the interstitium.

Significant reduction of the thickness of the unchanged adipose tissue area, increase and expansion of the intermediate region was established. Moreover, it increases the number of blood vessels of the microcirculatory bed, which are enlarged and full-blooded. Very enlarged lymphatic vessels. There is diapedesis of leukocytes through the wall of arterioles and venules, capillaries, as well as tender mesh sclerosis.

In the interstitium, a large number of macrophages, leukocytes and tissue basophilia are located near the vessels of the microcirculatory bed. Changes observed in the dynamics, manifested in the form of an inflammatory process, the signs of which were an increase in the number of vessels, an increase in infiltration of interstitium by macrophages, lymphocytes, tissue basophils, diapedesis of erythrocytes, which tended to increase and achieve the highest magnitude on the third week, collagen fibers, which led to tender mesh sclerosis and then to the development of fibrous tissue and fibrosis.

Experimental study of the influence of alloprosthetics by composite mesh on surrounding tissues. At implantation of a composite mesh on a great omentum of a dog in 3 months after operation its full merging with it was observed. Around the mesh weave, they revealed fibrous tissue filling the cells, forming a single dense structure ("patch") (Fig. 5).

In some places, granulomas of foreign bodies were observed around the elements of the grid, granulation tissue islands were observed. As it matured, it turned into fibrous. Large extraneous granulomas were found around the suture that fixed the graft on the great omentum and not around its elements. Fuchsinophilic collagen fibers and pyroninophilic fibroblasts (collagen synthesis) were found in the newly formed connective tissue, and the elastic fibers were not traced.

The fatty tissue of a great omentum was also drawn into the honeycomb mesh of the polypropylene mesh, which created the idea of its "ingrowth". From the free surface of

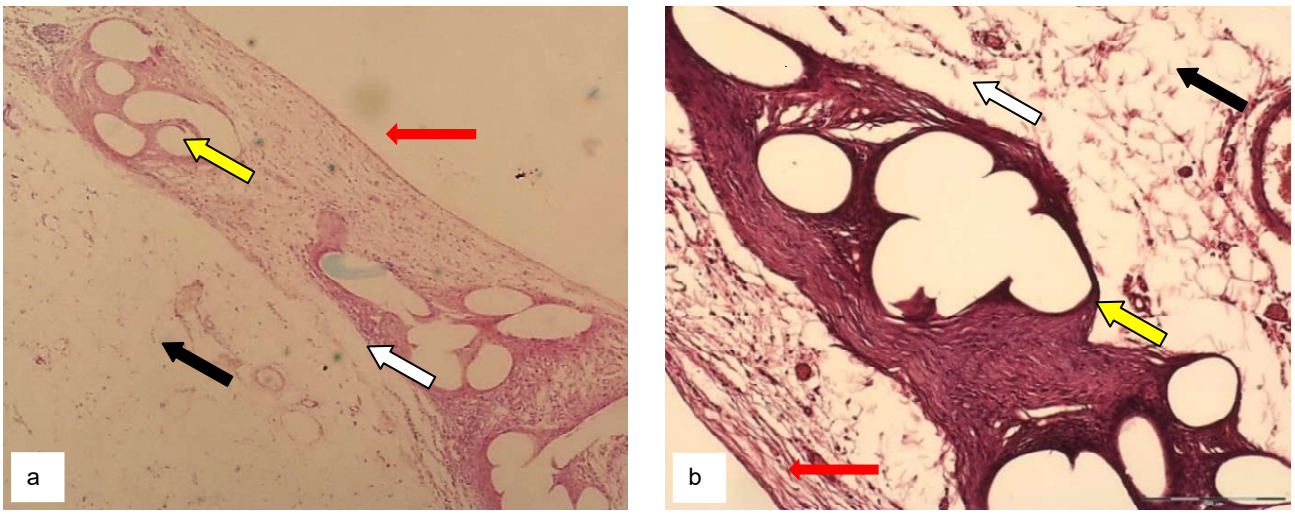

Fig. 5. Fusion of cellulose-coated polypropylene mesh with a great omentum of dog after 3 months after implantation: mesh cavities (white arrows), fibrous tissue "binds" the weave, filling the honeycombs (yellow arrows), great omentum (black arrows), peritoneum on the surface of the formed "patch" (red arrows). a - Brasche method, x40; b - hematoxylin-eosin, x100.

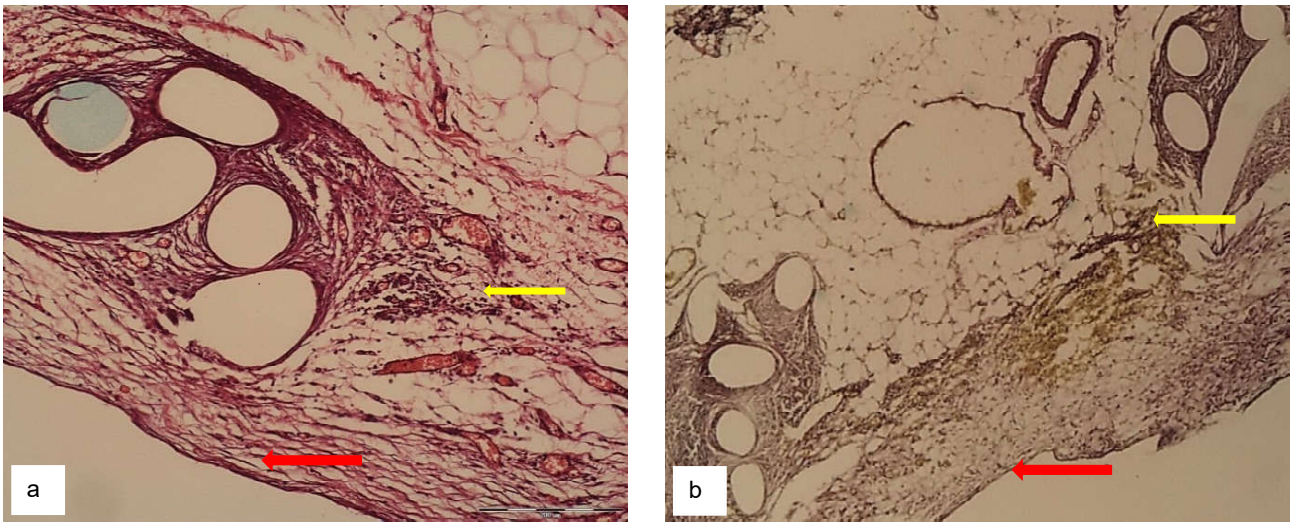

Fig. 6. "Ingrown" adipose tissue of the great omentum (black arrows) into the cells of the implanted mesh and spread by peritoneum that formed the "patch" (red arrows) 3 months after surgery. a - hematoxylin-eosin, x100, b - Verhoff method, x100.

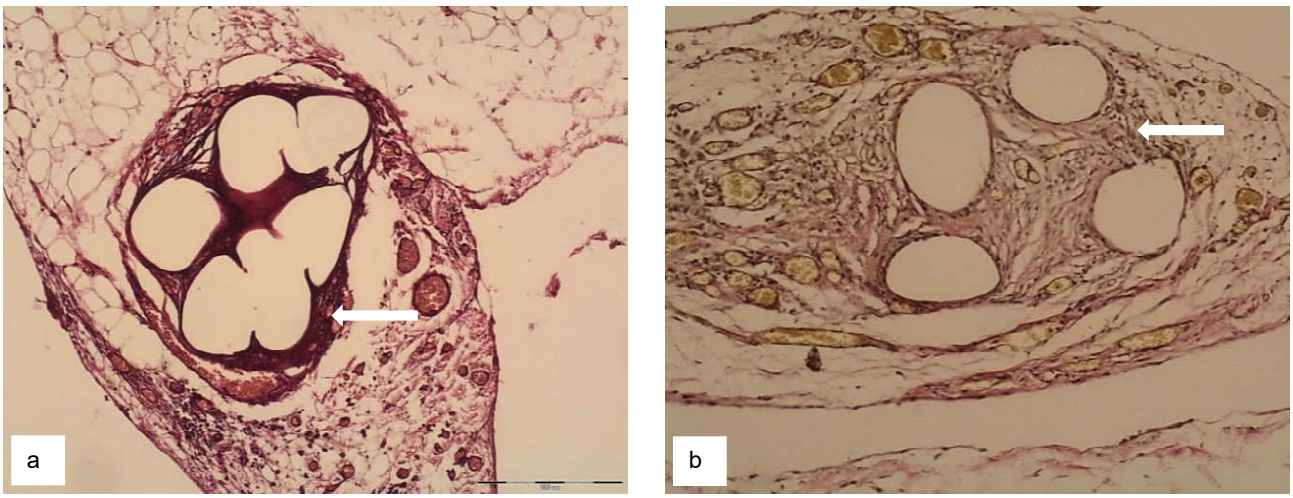

Fig. 7. Multiple full blood vessels (arrows) surrounded by a cavity with elements of implanted cellulose coated polypropylene mesh 3 months after surgery. a - hematoxylin-eosin, x100, b - Verhoff method, x100.

the mesh facing the abdominal cavity, the fat and connective tissues that filled the cells were coated with gel material, which prevented adhesions from forming with the adjacent small intestine loops (Fig. 6).

In the adipose tissue of the great omentum in the implant around the areas of fibrosis in the cells and around the actual mesh elements were observed vasculitis with pronounced lymphoplasmacytic infiltration of the walls of blood vessels and perivascular zones, the marginal standing of leukocytes (neutrophils), which is caused by gel coating of polypropylene mesh.

It should be noted that the tissues around the mesh structure were well vascularized and poorly infiltrated by individual lymphocytes, plasma cells and macrophages (Fig. 7).

Discussion

According to the morphological analysis of some authors, the use of collagen-treated polypropylene mesh after the plastic defect is accompanied by an acceleration of reparative processes and an improvement in the alterations of the connective, muscular and vascular components of the anterior abdominal wall 4 weeks after intervention [10].

The revealed signs of chronic inflammation with the presence of giant multinucleated foreign body cells in the implanted mesh area served as a basis for the researchers to develop postoperative complications and relapses. In alloplasty by the method "onlay" exudative inflammation prevails, with "sublay" - productive, which confirms the higher efficiency of the retromuscular placement of the mesh implant.

Several authors have experimentally established that intraperitoneal placement of a composite mesh implant with anti-adhesive coating does not cause splicing with internal organs, provides optimal integration into the tissues of the abdominal wall with a slight deformation, promotes the formation of neoperitoneum, without stitching of straight muscles [11].

In the study of allografts (polypropylene mesh with and without anti-adhesive coating, polytetrafluoroethylene mesh with nitinol framework), some authors argue for a comparative effect on the level of adhesive process in the study groups of rats. Others indicate that the level of adhesive process in the abdominal cavity when intraabdominal placement of polytetrafluoroethylene mesh with nitinol framework is 34.8% and 46.3% lower than the corresponding figure after intraabdominal placement of polypropylene mesh with anti-adhesive and without it ($p<0.05$).

The commissure in rats that were intraabdominally placed with a polypropylene mesh with an anti-adhesive coating are represented by a loose connective tissue, and when used with polypropylene mesh, are composed mainly of collagen fibers, their structure is unregulated. The introduction of tension free plastic with the use of mesh implants has allowed to solve a number of issues of modern surgery. The variety of mesh implants makes it possible to use new treatments reliably and, in most cases, close abdominal wall defects without reducing the volume of the abdominal cavity. Recently, reports of intraperitoneal onlay mesh (IPOM) have begun to appear, but data on the most effective type of implant are missing [11, 16]. Therefore, we need a new individual approach to the selection of mesh material, its fixation features and location. The use of special nets with their intraperitoneal location can help improve the results of treatment of abdominal wall defects. Implants with anti-adhesive coating when using the IPOM technique provide optimal integration into the tissues of the abdominal wall with a slight deformity, promote the formation of the neoperitoneum and minimal adhesive process in the abdominal cavity [6].

Finding mesh material that meets the requirements of an ideal hernioprosthesis remains an urgent task of treating ventral hernias. In experiments on Wistar rats, the nature of the local proliferative inflammatory response induced by a foreign body was investigated at different terms after implantation of 4 polypropylene, 1 teflon and 1 metal mesh hernioprosthesis from different manufacturers. In parallel with the histological examination of the encapsulated mesh implant, two morphometric parameters of aseptic proliferative inflammation were evaluated, namely: the mass of the connective tissue capsule around the foreign body and its thickness. It has been established that the minimal fibrous capsule is formed after implantation of a polypropylene mesh endoprosthesis within 3 months [10].

According to a number of authors it is believed that pronounced inflammatory reaction is caused by polypropylene implants. The maximum fibrous capsule is formed after implantation of mesh-implant to rats from porous two-layer polytetrafluoroethylene. Poor results are associated with a high incidence of purulent-inflammatory complications in the postoperative wound (up to 49.2% of cases) and in the postoperative spaces of the abdominal wall [6]. Suppuration of postoperative wounds causes a high incidence of disease recurrences, which, according to various authors, ranges from 14% to 50%, and after repeated operations there is an increase in their number from 65% to 80% [15].

It is believed that special prospects may rely on the creation of prostheses that combine the positive properties of both polyvinylidene fluoride and polypropylene materials. The inflammatory response induced by implantation into the abdominal wall of these prostheses is associated with a minimal adhesive process in the area of polyvinylidene fluoride-based material introduction and more pronounced in the area of polypropylene prostheses. Undoubtedly, the development of implants with such properties is important for the needs of herniologists [4, 13].

Despite the above research results, most authors note that with allohernioplasty of giant postoperative ventral hernias, today we have a high postoperative mortality rate, which is 1.3-12.0% and a significant number of postoperative complications - 20-63% [7, 17, 19].

In our study, within 3 weeks, changes in the microscopic structure of the omentum were examined after the polypropylene mesh was sewn. Study of histological preparations after 1 week allowed to distinguish two zones: in the first, close to the mesh, an inflammatory process was expressed, which was manifested in the increase, in comparison with the control study, the number of blood capillaries of the microcirculatory bed, thickening of the walls of arterioles, venous plethora, diapause through the wall of the blood capillaries. The omentum adipose tissue was replaced by a coarse fibrous connective tissue that revealed the proliferation of fibroblasts and the spread of collagen fibers, ie, sclerosis was present; in the second, highlighted more than 40 microns, the structure of the omentum was almost indistinguishable from the structure of the omentum in norm.

The study of the drugs 2 weeks after surgery, in addition to the above areas, made it possible to distinguish another - the intermediate zone of tender sclerosis, which separated the two previous zones. After 3 weeks after surgery, the first area with corresponding histological changes significantly expands. The interstitium marked a significant reduction of the second zone. Also, there was a significant increase in the number of microcirculatory vessels, marginal standing of leukocytes, their diapedesis through the vein wall, adhesion and diapedesis of erythrocytes through the capillary wall, a large number of macrophage and lymphocyte clusters, which were subordinated to the lymphoid follicles. Thus, this study confirmed the limited spread of the inflammatory response that made it possible to implant a polypropylene mesh on a great omentum.

In the remote postoperative period (3 months), with the spread of sclerotic changes, there was an enlargement of the composite mesh with surrounding tissues from the side where there was no gel coating. On the other hand, where it was present, sclerotic processes were absent, however, there was a "readiness" for the spread of the inflammatory

process, which could be manifested after the shelf life of this coating.

Therefore, from the results of the experimental material it is established that irrespective of the type of implant, its adhesion with the surrounding tissues is observed. The largest adhesive processes were observed with the polypropylene mesh, there were no sclerotic processes when using composite meshes and the process depended on the shelf life of the gel coating.

This study requires further accumulation of material to confirm the above findings.

Conclusions

The polypropylene implant has a greater capacity for the development of adhesive processes, but if the great omentum is shielded from the abdominal organs by the developed method, it is possible to prevent the development of adhesive bowel disease. Composite nets with a one-sided gel coating remain for a long time "ready" for the propagation of the inflammatory process, so it is also necessary to shield it with a great omentum from the intestine. Therefore, no type of prosthetic material guarantees the avoidance of adhesive process in the abdominal cavity in the postoperative period after allohernioplasty of giant postoperative hernias, which indicates the need to use a great omentum on the border between the allograft and the abdominal cavity to prevent the above complications.

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МОРФОЛОГІЧНІ ЗМІНИ ВЕЛИКОГО ЧЕПЦЯ ПРИ ІМПЛАНТАЦІЇ ПОЛІПРОПІЛЕНОВИХ ТА КОМПОЗИТНИХ ПРОТЕЗІВ ПРИ АЛОГЕРНІОПЛАСТИЦІ

Воровський О.О., Шапринський В.О., Садик І.М.

До теперішнього часу при алогерніопластиці гігантських післяопераційних вентральних гриж зберігається висока післяопераційна летальність і значна кількість післяопераційних ускладнень. Мета дослідження - дослідити можливість

інтраперитонеального застосування поліпропіленових та композитних імплантатів при алогерніопластиці післяопераційних гігантських вентральних гриж за рахунок вивчення морфологічних змін великого чепця. Досліджено результати хірургічного лікування 146 хворих з післяопераційними гігантськими вентральними грижами. У даній групі хворих алогерніопластика за методом onlay була виконана 22 (15,1%) хворим, за методом sublay - 46 (31,5%), за методом inlay - 52 (35,6%), з внутрішньочеревним розташуванням сітки за методом onlay (intraperitoneal onlay mesh) - 26 (17,8%). У 32 (21,9%) хворих, що були прооперовані за методом sublay, поліпропіленовий імплантат був відгороджений від органів черевної порожнини великим чепцем, у 22 (15,1%) хворих, що прооперовані за методом intraperitoneal onlay mesh, композитний імплантат також був відгороджений від органів черевної порожнини великим чепцем. У 8 (5,5%) пацієнтів із групи хворих, прооперованих за методом sublay та у 6 (4,1%), прооперованих за методом intraperitoneal onlay mesh, на 14-18 добу розвинулися ознаки хронічної кишкової непрохідності, де причиною стала післяопераційна спайкова хвороба. Дослідження впливу поліпропіленового протезу на великий чепець проводили на 8 безпородних собаках та композитного імплантату - на 6 безпородних статевозрілих собаках, котрим було імплантовано композитну сітку, однобічно вкриту оксицелюлозою. Встановлено, що поліпропіленовий імплантат має більшу здатність до розвитку адгезивних процесів. Проте, якщо великим чепцем відгородити даний протез від органів черевної порожнини, то перша зона (активного запалення) поширювалася до 155 мкм (встановлено збільшення чисельності кровоносних капілярів мікроциркуляторного русла, потовщення стінки артеріол, венозного повнокрів'я, діапедез лейкоцитів крізь стінку кровоносних капілярів), друга зона (склерозування) - до 40 мкм (встановлено збільшення чисельності макрофагів і лімфоцитів, проліферація фібробластів). Подалі будова чепця майже не відрізнялась, тому при алогерніопластиці за розробленою методикою вдалося запобігти розвитку спайкової хвороби кишечника. Це дослідження підтвердило обмежене розповсюдження запальної реакції, що дає можливість імплантувати поліпропіленову сітку на великий чепець. У композитних сіток відбувалося їх зрощення з навколишніми тканинами з боку, де відсутнє гелеве покриття, а з боку гелевого покриття зберігалася "готовність" до розповсюдження запального процесу (васкуліт з вираженою лімфоплазмоцитарною інфільтрацією стінок судин і периваскулярних зон, крайове стояння лейкоцитів), а тому його також необхідно відгородити великим чепцем від кишки.

Ключові слова: *поліпропіленовий протез, композитна сітка, алогерніопластика, великий чепець.*

МОРФОЛОГИЧЕСКИЕ ИЗМЕНЕНИЯ БОЛЬШОГО САЛЬНИКА ПРИ ИМПЛАНТАЦИИ ПОЛИПРОПИЛЕНОВИХ И КОМПОЗИТНЫХ ПРОТЕЗОВ ПРИ АЛЛОГЕРНИОПЛАСТИКЕ

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До настоящего времени при аллогерниопластике гигантских послеоперационных вентральных грыж сохраняется высокая послеоперационная летальность и значительное количество послеоперационных осложнений. Цель исследования - исследовать возможность интраперитонеального применения полипропиленовых и композитных имплантатов при аллогерниопластике послеоперационных гигантских вентральных грыж за счет изучения морфологических изменений большого сальника. Исследованы результаты хирургического лечения 146 больных с послеоперационными гигантскими вентральными грыжами. В данной группе больных аллогерниопластика по методу onlay была выполнена 22 (15,1%) больным, по методу sublay - 46 (31,5%), по методу inlay - 52 (35,6%), с внутрибрюшным расположением сетки по методу onlay (intraperitoneal onlay mesh) - 26 (17,8%). У 32 (21,9%) больных, которые были прооперированы по методу sublay, полипропиленовый имплантат был отгорожен от органов брюшной полости большим сальником; у 22 (15,1%) больных, прооперированных по методу intraperitoneal onlay mesh, композитный имплантат также был отгорожен от органов брюшной полости большим сальником. У 8 (5,5%) пациентов из группы больных, которые были прооперированы по методу sublay и у 6 (4,1%), прооперированных по методу intraperitoneal onlay mesh, на 14-18 сутки развились признаки хронической кишечной непроходимости, где причиной стала послеоперационная спаечная болезнь. Исследование влияния полипропиленового протеза на большой сальник проводили на 8 беспородных собаках и композитного имплантанта - на 6 беспородных половозрелых собаках, которым была имплантирована композитная сетка, односторонне покрытая оксицеллюлозой. Установлено, что полипропиленовый имплантат имеет большую способность к развитию адгезивных процессов. Однако, если большим сальником оградить данный протез от органов брюшной полости, то первая зона (активного воспаления) распространилась до 155 мкм (установлено увеличение численности кровеносных капилляров микроциркуляторного русла, утолщение стенки артериол, венозное полнокровие, диапедез лейкоцитов через стенку кровеносных капилляров), вторая зона (склерозирования) - 40 мкм (установлено увеличение численности макрофагов и лимфоцитов, пролиферация фибробластов). В дальнейшем строение сальника почти не отличалось, поэтому при аллогерниопластике по разработанной методике удалось предотвратить развитие спаечной болезни кишечника. Это исследование подтвердило ограниченное распространение воспалительной реакции, что позволяет имплантировать полипропиленовую сетку на большой сальник. В композитных сетках происходило их сращивание с окружающими тканями со стороны, где отсутствует гелевое покрытие, а со стороны гелевого покрытия сохранялась "готовность" к распространению воспалительного процесса (васкулит с выраженной лимфоплазмоцитарной инфильтрацией стенок сосудов и периваскулярных зон, краевое стояние лейкоцитов), а поэтому также его необходимо оградить большим сальником от кишки.

Ключевые слова: *полипропиленовый протез, композитная сетка, алогерниопластика, большой сальник.*

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For publication, scientific articles are accepted only in English only with translation on Ukrainian or Russian, which contain the following necessary elements: UDC code; title of the article (in English, Ukrainian and Russian); surname, name and patronymic of the authors (in English, Ukrainian and Russian); the official name of the organization (institution) (in English, Ukrainian and Russian); city, country (in English, Ukrainian and Russian); structured annotations (in English, Ukrainian and Russian); keywords (in English, Ukrainian and Russian); introduction; purpose; materials and methods of research; research results; discussion; conclusions; bibliographic references.

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In the discussion, it is necessary to summarize and analyze the results, as possible, compare them with the data of other researchers. It is necessary to highlight the novelty and possible theoretical or practical significance of the results of the research. You should not repeat the information already listed in the "Introduction" section. At the end of the discussion, a separate paragraph should reflect the prospects for using the results obtained by the author.

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