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REMODELING OF THE SMALL CALIBER CORONARY VESSELS IN EXPERIMENTAL ATHEROSCLEROSIS

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Histological and morphometric studies of the small caliber coronary arteries' structural rearrangement at different stages of atherosclerosis development in the experiment were performed. Atherosclerotic damage was modeled by administration of low-density human native lipoproteins to animals. Histological staining of the coronary arteries wall was carried out using hematoxylin-eosin, sudan III and the methods of Van Gieson and Malory. It was found that the degree of structural and morphometric changes in the small caliber coronary arteries depends on the term of the study. In the early stages after antigenic influence (6-11th week of the experiment), the initial signs of damage in the coronary arteries wall were observed. At the later stages (12–20th week of the experiment), the structural rearrangement of the small caliber coronary arteries was detected, which was characterized by a progressive statistically significant thickening of their wall by 38.7 %, due to narrowing of the lumen. The Vogenvort index increased by 2.3 times ($p<0.05$) and indicated the functional insufficiency of the arteries.

Key words: coronary arteries of small caliber, morphometry, atherosclerosis.

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РЕМОДЕЛЮВАННЯ ВІНЦЕВИХ СУДИН ДРІБНОГО КАЛІБРУ ПРИ ЕКСПЕРИМЕНТАЛЬНОМУ АТЕРОСКЛЕРОЗІ

Проведені гістологічні та морфометричні дослідження особливостей структурної перебудови вінцевих судин дрібного калібру на різних етапах розвитку атеросклерозу в експерименті. Атеросклеротичне пошкодження моделювали шляхом введення тваринам нативних ліпопротеїнів низької щільності людини. Гістологічне забарвлення стінки вінцевих судин проводили гемотоксилін-еозином, за методами Ван-Гізон, Малорі і суданом ІІІ. Встановлено, що ступінь структурних та морфометричних змін вінцевих артерій залежить від терміну дослідження. В ранні терміни після антигенного навантаження (6-11-й тиждень експерименту) спостерігали початкові ознаки пошкодження судинної стінки. В пізні терміни (12-20-й тиждень експерименту) виявлена структурна реорганізація вінцевих артерій дрібного калібру, яка характеризувалась вираженим потовщенням їх стінки статистично вагомо на 38,7 %, за рахунок звуження просвіту. Індекс Вогенворта збільшувався в 2,3 рази ($p<0,05$), що свідчило про зниження пропускної здатності артерій.

Ключові слова: вінцеві артерії дрібного калібру, морфометрія, атеросклероз.

The work is a fragment of the research project "Histogenesis of the cardiovascular system components of humans and laboratory animals in normal and in experiment", state registration No. 0118U004730.

Atherosclerotic lesions of the heart and blood vessels dominate in the morbidity patterns and have the greatest clinical, medical and social significance [10]. According to modern ideas, the pathogenesis of atherosclerosis is a complex multifactorial process, the mechanisms of which involve: endothelial dysfunction, lipoprotein infiltration, inflammatory processes that cause structural changes in blood vessels, including coronary [1, 7]. Particularly negatively, the vessel wall's condition is affected by an increase in the number of structurally altered endothelial cells. The study results show that the vascular endothelium is a multifunctional system that has a regulatory effect on both blood vessels and the blood system. It provides endothelial thromboresistance, participates in hemo-tissue metabolism, ensuring its selectivity, synthesizes a number of biologically active substances, participates in lipid metabolism, as well as in the regulation of vascular wall tone [6, 9].

It is known that the development of atherosclerotic process in vessels of large and medium caliber is characterized by degenerative changes of endothelial cells, accumulation of lipids in the arterial wall, proliferation of connective tissue components, proliferation of smooth myocytes and fibroblasts and atherosclerosis plaque formation [5].

At the same time, there are contradictory data that small caliber vessels undergo more pronounced morphological changes, as they are subject to the maximum load during heart contraction and are more frequently involved in the atherosclerotic process [6].

Researchers assign an important role in deepening knowledge about the features of the functional state and capacity of the arteries to calculation of the indices, which are expressed by the ratio of different blood vessels' morphometric parameters. Among them, the most informative index is the Vogenvort index, which should be calculated to determine the degree of structural rearrangement of damaged vessels and their functional capacity [8, 9]. It is the lack of unanimous opinion as to the rearrangement of small caliber coronary vessels due to atherosclerotic lesions that requires further study.

The purpose of the work was to study the features of the structural rearrangement in coronary vessels of small caliber in experimental atherosclerosis.

Materials and methods. The studies were performed on 324 nonlinear rats, which were divided into 3 groups: group Ia was the control (n=85) – animals, injected intracutaneously with 0.1 ml of 0.9 % sodium chloride solution; Ib – comparison group (n=85) – animals injected with incomplete Freund's adjuvant at the dose of 0.1 ml intracutaneously and II – experimental group (n=154), which were immunized with native human low-density lipoprotein (LDL) diluted with incomplete Freund's adjuvant. To reproduce atherosclerotic damage to the rat coronary artery wall a proven model was used [2], according to which human LDL ("ProSpec", USA) was administered to rats intracutaneously at a single dose of 200 μg, diluted in 0.1 ml of incomplete Freund's adjuvant ("Becton Dickinson " USA), regardless of the weight. Material sampling was performed weekly, starting from the 4th week after the drug administration. Experimental animals were sacrificed by administering sodium thiopental. Animals keeping and experimental studies were carried out in compliance with the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 18 March 1986) and the Law of Ukraine on the Protection of Animals from Cruelty (No. 3447- IV of 21.02.2006). From the coronary arteries and the adjacent myocardium, microslides were made according to the generally accepted technique, which were stained with hematoxylin and eosin, according to the methods of Van Gizon, Mallory and Sudan III.

The morphometric parameters of small caliber coronary arteries, the outer diameter of which was 26–50 μm, were studied. To analyze the degree of atherosclerotic damage to the coronary artery wall of rats, the following morphometric parameters were studied: endothelial cell height (EH), inner (ID) and outer diameters (OD) of vessels, wall thickness (WT) and Vogenvort index (VI) (the ratio of the vessel wall area to the area of its lumen) [6, 7, 11, 12]. The above measurements of arteries were performed on cross-sections of vessels using "ToupView 3.2. ver. 3.5" software for analysis and processing of digital images (distributed without license restrictions). Photographing was performed using the Canon EOS 1200D digital camera. The obtained indicators were statistically processed using the software package STATISTICA 6.1. The results are presented as (M±m), where M is the mean value of the index, m is the standard error. The results at $p<0.05$ were considered reliable [4].

Results of the study and their discussion. On the 4-th week, the histological examination revealed that the intima of the arteries in the experimental group animals is represented as a monolayer of polygonally flattened endotheliocytes located on the basal membrane. The inner elastic membrane had a regular folded structure. In the media of the arteries, smooth myocytes were located in several rows, which had an oblique longitudinal direction relative to the elastic fibers. Adventitia was represented by loose fibrous connective tissue. The histostructure of the coronary arteries in experimental animals was similar to the respective vessels of the control and comparison groups rats.

When analyzing morphometric data at this term of the experiment, it was found that any statistically significant difference between morphometric parameters in all groups of observation was not recorded (table 1).

Table 1

Morphometric parameters of the small caliber coronary arteries wall in rats, (M±m)

Index	Groups of observation											
	Ia/weeks (n=85)				Ib/weeks (n=85)				II/weeks (n=154)			
	4	8	12	20	4	8	12	20	4	8	12	20
EH, μm	5.8 ±0.03	5.9 ±0.15	5.9 ±0.04	5.87 ±0.01	5.86 ±0.06	5.97 ±0.04	5.87 ±0.04	5.86 ±0.05	5.86 ±0.05	5.95 ±0.07	6.06 ±0.04	5.85 ±0.03
OD, μm	37.46 ±0.55	37.38 ±0.25	37.49 ±0.12	37.86 ±0.15	37.38 ±0.46	37.49 ±0.14	37.72 ±0.41	37.74 ±0.12	37.45 ±0.27	37.55 ±0.09	37.83 ±0.53	37.99 ±0.14
ID, μm	22.15 ±0.19	21.87 ±0.07	22.18 ±0.14	22.23 ±0.07	22.16 ±0.07	22.24 ±0.09	22.48 ±0.09	22.16 ±0.07	22.17 ±0.44	20.50 ±0.22	20.06 ±0.11*	16.28 ±0.15*
WT, μm	7.66 ±0.26	7.76 ±0.12	7.67 ±0.13	7.82 ±0.10	7.61 ±0.25	7.62 ±0.10	7.62 ±0.20	7.79 ±0.04	7.64 ±0.32	8.53 ±0.09	8.88 ±0.26*	10.85 ±0.10*
VI, %	186.4± 8.36	192.1 ±3.92	185.7± 5.44	190.18 ±3.78	184.68 ±7.97	184.07 ±3.84	181.6± 6.07	189.9 ±1.49	185.77 ±14.67	235.74 ±6.1*	255.48 ±10.0*	443.00 ±18.8*

Note: n – number of animals, * – $p<0.05$ compared to group I.

We first found signs of histological remodeling in the wall of the small caliber coronary arteries on the 6th week of the experiment. Most vessels had a narrowed lumen, densely filled with formed elements of blood (fig. 1).

The wall of the arteries maintained a uniform thickness. Endotheliocytes were characterized by the correct shape and longitudinal orientation of the nuclei. The inner elastic membrane had a regular tortuous contour. The membrane tunica media was represented by a connective tissue matrix, a small number of smooth myocytes and fibroblasts. Smooth myocytes had rod-shaped nuclei and were characterized by an ordered arrangement. Separate areas of perivascular edema and histiocytic infiltration were observed. No such changes were detected in the control and comparison groups.

Histological rearrangements in the small caliber coronary arteries' wall were reflected in the change of morphometric parameters. Thus, in group II animals, the wall inner diameter index of the studied vessels was equal to 20.72±0.07 μm and tended to decrease by 7.1 % and 6.5 %, respectively, compared to the 4th week of the experiment and to the control and comparison groups. ($p<0.05$).

The parameters of wall thickness (8.23±0.10 μm) and Vogenvort index (222.17±3.90 %) of small caliber arteries increased by 7.7 % and 19.6 %, respectively, compared to the previous term of the experiment and by 7.4 % and 19.4 %, compared to animals of group I. In groups Ia and Ib we did not find similar changes.

Histological examination in the small-caliber coronary arteries in rats of group II on the 8th week of the experiment showed an increase in the number of leukocyte elements in the lumen of blood vessels that were closely adjacent to the endothelium, which had signs of local damage. The population of leukocytes in contact with the luminal surface of endothelial cells was diverse: monocytes, neutrophilic granulocytes and lymphocytes were observed. Characteristic was the ordering impairment of the fibrous structures in the tunica media membrane and the localization of single lipid droplets in the arteries' intima. No morphological changes in the structure of the vessel wall were detected in the control and comparison groups.

The results of the morphometric study showed that during this period in rats injected with native human LDL, the outer diameter of the small caliber coronary arteries did not differ significantly from the previous term and was similar to that in the control and comparison groups. The wall thickness of the studied vessels increased by 9.9 % compared to group I animals. The Vogenvort index increased by 22.7 % compared to the control and comparative groups of animals ($p<0.05$).

On the 12th week after immunization, destruction and exfoliation of individual endothelial cells, exfoliation of the inner elastic membrane, local edema of the vascular wall, which manifested itself in the form of cytoplasm clarification of smooth myocytes and swelling of endothelial nuclei with their protrusion into the artery lumen in the stockade form, i.e. perpendicularly to the artery axis (fig. 2).

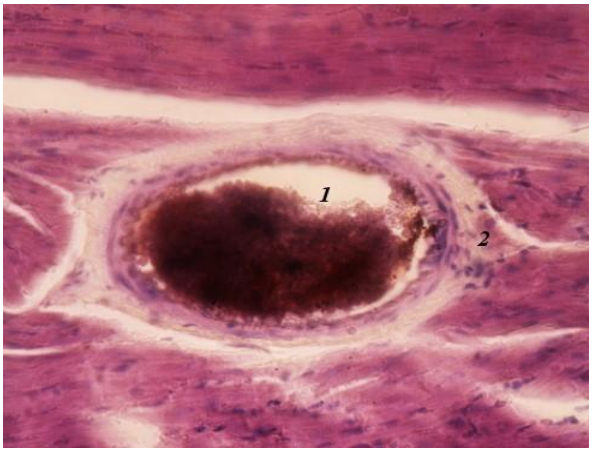

Fig. 1. Microscopic changes of the small caliber coronary artery on the 6th week of the experiment. Blood-filled lumen of the artery (1), the area of increase in the cellular composition of the adventitia (2). Hematoxylin and eosin staining. × 400.

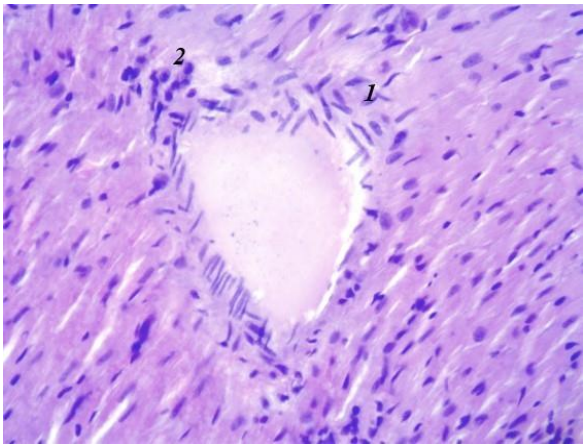

Fig. 2. Microscopic changes of the small caliber coronary artery on the 12th week of the experiment. Impairment of the cell architecture (1), their chaotic location, increase of cell populations in adventitia (2). Hematoxylin and eosin staining. × 1000.

In the control and comparison animals, no structural rearrangement of the coronary vessel wall was detected.

In the analysis of linear parameters and calculation of the vascular wall Vogenvort index on the 12th week of observation, it was found that during this period the parameter of the blood vessels inner diameter decreased against the background of their wall thickening, which was reflected in an increase in Vogenvort index by 37.6 %. $p<0.05$) compared to group 1. The index of the vessels outer diameter was stable and remained at the previous level.

Analyzing the morphometric parameters on the 16th week of the experiment, we found that during this period there were significant changes in the structure of the small caliber coronary arteries: the inner diameter of the vessels decreased (19.50±0.10 μm) against the stable outer diameter index (37.74±0.10 μm), the wall thickness increased by 18.3 % compared to group I ($p<0.05$). Also, in the group of immunized animals, the Vogenvort index increased with a significant difference, which was equal to 274.84±5.05 % and was by 1.5 times higher than the similar parameter in the control and comparison groups.

Histological examination of the small caliber coronary arteries' wall in the experimental group animals on the 20th week of the experiment revealed changes in the three membranes of blood vessels. Endothelial cells were randomly located on the basal membrane. Local ruptures of the inner elastic

membrane were associated with an increase in the number of smooth myocytes in the intima. They were arranged in a chaotic order and had the nuclei orientation perpendicular to the axis of the vessel. There was a thickening of the vessels' wall and narrowing of their lumen. The connective tissue contained a large number of disordered collagen fibers (fig. 3).

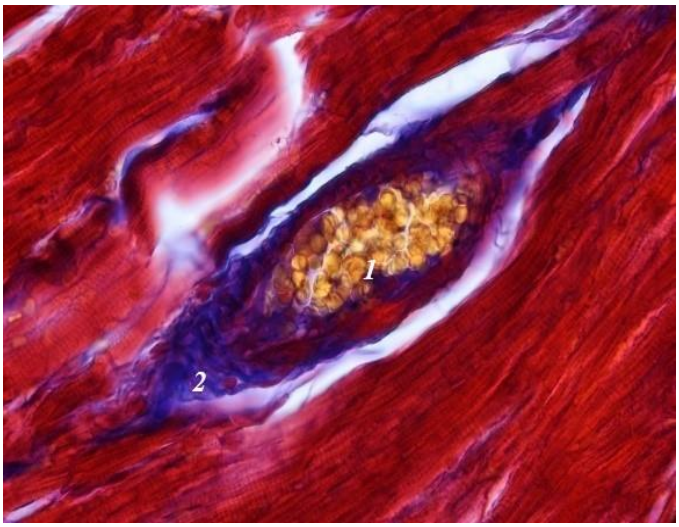

Fig. 3. Microscopic changes of the small caliber coronary artery on the 20th week of the experiment. Disruption of the endothelial contour with erythrocyte adhesion (1), disorganization of fibrous structures (2). Staining. by Mallory. × 1000.

Swelling and lysis of elastic fibers of the media were noted. The outer membrane of the coronary arteries was thickened due to the increased amount of adipose tissue. In rats of the control and comparison groups, the above changes were not detected.

Upon careful analysis of the obtained morphometric data on the 20th week of the experiment (table 1), it was found that the main parameters of the small caliber coronary vessels' wall in group II animals were statistically reliably changed in comparison with the control and comparison animals. Thus, the parameter of the coronary arteries wall's thickness acquired the greatest importance and was 38.7 % higher than in group I animals ($p<0.05$). Significant morphological changes in the coronary arteries were associated with a change in the Vogenvort index, which reached its maximum value and was by 2.3 times higher than in group I animals ($p<0.05$) and indicated even lower capacity of the vessels under study.

The results of comprehensive histological and morphometric analysis testify that small-caliber vessels showed an earlier response in the modeling of atherosclerosis than large and medium caliber vessels. According to the obtained data, which extend the results of other researchers [3, 5], the characteristic changes were: change of spatial orientation in endothelial cells and smooth myocytes in the form of a stockade, which led to a decrease in vascular lumen, which acquired an irregular shape. Narrowing of the studied arteries lumen was also caused by the thickening of their wall, which, in our opinion, is associated with the remodeling of the intima due to the growth of connective tissue components and leukocyte infiltration sites. It should be noted that there were significantly more foam cells in the wall of large and medium caliber arteries, while in small caliber arteries only single cells were visualized. The obtained results indicate that small caliber vessels undergo a pronounced structural rearrangement, which is manifested in changes of the Vogenvort index. The above index characterizes the capacity of arteries and with its growth, which is the result of a decrease in the lumen of blood vessels due to the thickening of their wall, leads to a deterioration in the heart muscle's perfusion [6, 8]. In our opinion, the prospects for further research are to study the changes in the small vessels of other organs in experimental atherosclerosis.

Conclusion

It was found that a distinctive feature of the small caliber arteries' wall was the predominance of degenerative changes in it, in contrast to the wall of medium caliber arteries, where lipid infiltration was more pronounced.

The structural reorganization was revealed in rat small caliber coronary arteries, which was characterized by a pronounced thickening of their wall by 38.7 %, due to the narrowing of the lumen, which was reflected in a significant increase of the Vogenvort index by 2.3 times ($p<0.05$) and indicated a decrease in the capacity of arteries.

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PECULIAR FEATURES OF REGENERATION AT THE END OF BONE FILING AFTER AMPUTATION OF A LIMB

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In order to evaluate the reparative processes at the end of the bone after amputation, 4 series of experiments were carried out on 64 rabbits with filing plastic cortical plate, its combination with miodesis, fascia cover and stitching of antagonist muscles. Timing of observation: 1, 3, 6 months. Examination method: histological with vessel filling in ink-gelatin mixture. It was established that in the first two series the formation of the bone closing plate and intraosseous circulation were restored by 1 month. In the series with myoplasty and fascioplasty, the shape of the residual limb was changed, the bone closing plate was absent, and the normalisation of intraosseous circulation did not occur even in remote lines. The preservation of the cylindrical shape of the residual limb, the normalisation of intraosseous circulation and the completion of the reparative process are indicators of favourable residual limb formation.

Key words: amputation, fascioplasty, muscle plastic, bone grafting, reparative regeneration.

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ОСОБЛИВОСТІ РЕГЕНЕРАЦІЇ НА КІНЦІ ОПИЛУ КІСТКИ ПІСЛЯ АМПУТАЦІЇ КІНЦІВКИ

З метою оцінки репаративних процесів на кінці кістки після її ампутації проведено 4 серії дослідів на 64 кроликах з пластикою опилу кортикальною пластинкою, поєднання її з міодезом, укриття фасцією і зшивання м'язів-антагоністів. Терміни спостереження: 1, 3, 6 місяців. Метод дослідження: гістологічний з наливкою судин туш-желатиновою сумішшю. Встановлено, що в перших двох серіях формування кісткової замикаючої пластинки і внутрішньокісткова циркуляція відновлювалися до 1 місяця. У серіях з міопластикою і фасціопластикою форма кукси змінювалася, кісткова замикальних пластинка була відсутня, нормалізація внутрішньокісткової циркуляції не наступала навіть у віддалені строки. Збереження циліндричної форми кукси кістки, нормалізація внутрішньокісткової циркуляції і завершеність репаративного процесу є показниками сприятливого формування кукси.

Ключові слова: ампутація, фасціопластика, м'язова пластика, кісткова пластика, репаративна регенерація.

The study is a fragment of the research project "Discovering the patterns of postamputation pain syndrome formation", state registration No. 0120U101372.

Amputation of a limb with the intersection of soft tissues, blood vessels, nerves, bones significantly violates the static-dynamic stereotype, which contains a potential threat of various complications. In addition, such an intersection substantially complicates the formation of a rational relationship between these anatomical formations in the future organ of support and movement. In recent years, researchers have focused on such particular issues of amputations as predicting wound healing [6], treatment of pain [3], various modifications of known methods of amputation [4, 5, 7], description of malformations and diseases of the stump [1, 2, 8, 11]. Unfortunately, such fundamental questions were not reflected in these works, as the healing of the stump of the bone, the features of reparative processes and factors that influence their course. According to the data of, unsatisfactory outcomes of bone stump healing were noted in 97.1 % of the examined, and the formation of a functional bone stump within 1–1.5 months after amputation was