Myocardial interstitial matrix as novel target for succinic acid treatment strategies during experimental hypobaric hypoxia

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Abstract

The myocardial extracellular matrix is not a passive entity, but rather a complex and dynamic microenvironment which represents an important structural and signaling system within the myocardium. Understanding the fundamental role of hypoxia and peroxidation in the genesis of many cardiovascular diseases has stimulated the development of strategies that can enhance the energy-producing functions of cells. Revealing the alterations in cardiac metabolism and function associated with sustained exposure to high altitude advances our understanding of hypoxia-related disease. The study was conducted on 26 adult males of Wistar rats weighing 220-310 g, divided into 3 groups. The first control group consisted of 6 intact animals, the second group included 10 rats which were exposed to hypobaric hypoxia without medication for 30 days. Third group was composed of 10 rats, which were medicated by succinic acid solution which was injected intraperitoneally once a day at the rate of 0.5 ml/100 g of animal body weight 15 minutes before hypoxic exposure for 30 days. Fibrosis in the myocardium inevitably leads to increased myocardial stiffness, resulting in systolic and diastolic dysfunction, neurohormonal activation and, ultimately, heart failure Changes in cardiac highenergy phosphate metabolism may underlie the myocardial dysfunction caused by hypobaric hypoxia. Reduced oxygen delivery by microvascular damage, increased perivascular fibrosis associated with reduced cellular oxygen availability may contribute to contractile failure. Succinic acid combined with inosine acts as a high-energy phosphate reserve, to maintain adenosine triphosphate at levels sufficient to support contractile function.

Background. Cardiovascular diseases continue to be the leading cause of death worldwide. Many of them are associated with sclerotic, fibrotic, autoimmune and inflammatory processes where the extracellular myocardial matrix plays the crucial role determining the adaptive and regenerative capabilities of myocardium [1]. In this regard, the unresearched connective tissue component of the myocardium continues to draw the attention of scientists. The myocardium demonstrates two morphological components: muscular part, represented by cardiomyocytes, and non-muscular or extracellular matrix. The second one is represented by the layers of fibrillar collagens and matricellular proteins. Fibroblasts (90 ... 95%) and mast cells (4 ... 9%) are most common among the interstitial cells. Collagen and elastic fibers of the intercellular substance form a kind of skeleton, constantly changing its shape during the cardiac cycle. The main, or amorphous, substance of matrix shows polyanionic nature providing trophic and metabolic functions and contains glycosaminoglycans and proteoglycans in a large amount [2]. In addition to fibrous structures, the composition of the intercellular substance includes the main, or amorphous, substance that surrounds the cardiomyocytes, cellular and fibrous structures, and nerve and vascular elements. The basic substance includes plasma proteins, water, inorganic ions, products of cell metabolism, soluble precursors of collagen and elastin, proteoglycans, glycoproteins and the complexes formed by them. All these substances are in constant motion and renewal [3].

The network of collagen and elastic fibers, which forms the basis of the intercellular substance, binds the components of the myocardium together, retains the desired shape of the heart muscle and determines the mechanical properties of the myocardium during the cardiac cycle. From fibrillar proteins, collagen I and type III collagen predominate. About 80% of the total collagen is represented by type I collagen, which is part of the thick fibers of the myocardium, ensuring its strength. About 11% falls on type III collagen, which is associated with myocardial distensibility. The ratio of collagens I and III types in a normal myocardium is quite stable [4].

The relevance of the interstitial matrix (IM) lays in the onset of cardiovascular pathologies. This is determined mainly by the fact that it allows to assess the severity of structural changes to identify the myocardial reserve and functional cardiac dysfunction in the pathogenesis and clinical manifestations of cardiomyopathies. The results of recent studies have shown that the myocardial IM has vere specific spatial organization, its own system of regulation and reproduction, and is capable of quick response to changes in the myocardial functioning. It has

been established that structural changes of IM are deeply involved in embryogenesis, angiogenesis, tissue involution, wound healing, as well as in pathological remodeling associated with the degradation of its proteins [5]. A number of authors have convincingly demonstrated that IM plays an independent and significant role in the progression of cardiac dysfunction, rupture and mortality [6].

Morphologically, such changes were represented by increased amount of collagen and fibrous tissue in the myocardium. Later it was observed that acute and chronic myocardial ischemia, the action of toxic agents and some congenital pathological conditions were accompanied by the excessive accumulation of fibrillar collagen in the interstitial space and miscommunication of myocytes, fibroblasts, and endothelial cells [5, 7]. It has been shown that the development of fibrosis may be accompanied by the deterioration of the contractile properties of the heart affecting both systolic and diastolic output [4].

Exposure to hypobaric hypoxia is associated with a number of adaptive responses, such as increased ventilation and erythropoiesis, which help maintain oxygen delivery [8]. However, the concomitant increases in cardiac output may not be matched by increased oxygen availability to the heart tissue itself. During hypoxia and ischemia there is inhibition of energy production in cells and the development of acidosis is observe, this triggers free radical processes and inhibits antioxidant protection. Reactive oxygen species (ROS) damage membranes (including mitochondrial), exacerbate the disturbances of energy metabolism, i.e. a vicious circle is created. In this regard, the use of funds that contribute to the normalization of energy metabolism, allows to break the vicious circle in its primary link, while the action of antioxidants occurs at the level of production of reactive oxygen species (ROS), exerting a mediated effect on the cell energy [4, 7].

Understanding the fundamental role of hypoxia and peroxidation in the genesis of many cardiovascular diseases has stimulated the development of strategies that can enhance the energy-producing functions of cells [9]. In the treatment of the pathology of the cardiovascular system, a whole range of blood pressure lowering drugs with antianginal and antihypoxic properties that affect hemostasis is currently used. In fact, these are just symptomatic drugs that do not eliminate the triggering point of the disease. Succinic acid is the endogenous natural substrate of the cell. Its antioxidant effect is realized in the acceleration of the circulation of the dicarboxylic part of the tricarboxylic acid cycle, which results in a decrease in the concentration of lactate and contributes to the normalization of the metabolic component of the acid-base balance. As a result of the acceleration (increase) of the cycle in the tricarboxylic acid cycle, the amount of energy required for ATP synthesis increases. As a result, increased oxygen consumption by tissues [9, 10]. Particularly marked effects of succinic acid are manifested in combination with Inosin (Riboxin), an agonist of purine-ergic receptors that activate glycolysis, which helps to maintain energy in the cell. Inosin-induced activation of glycolysis helps to maintain the energy balance in the cell. An important aspect of riboxin action is its participation in the formation of nitric oxide (NO) which appears to be very powerful vasodilator that provides better cellular access to oxygen and the energetic substrates by improving microcirculation [11].

Synthesis of proteins of the main substance alternates with their destruction, which ensures the constant renewal of the matrix during growth, remodeling and restoration. Thus, ideas about the spatial organization of the intercellular substance of the myocardium remain controversial and are based mainly on stereological studies that do not allow to give a complete picture of the architectonics of the connective tissue component of the myocardium. Quantitative morphological studies of cellular elements of the myocardium require the search for new methods for the identification of various types of cells and their interrelations.

Materials and Methods

Hypobaric hypoxia modeling

The study was conducted on 26 adult males of Wistar rats weighing 220-310 g, divided into 3 groups. The **first control** group consisted of 6 intact animals, the **second group** included 10 rats which were exposed to hypobaric hypoxia without medication for 30 days. **Third group** was composed of 10 rats, which were medicated by succinic acid solution Cytoflavin which was injected intraperitoneally once a day at the rate of 0.5 ml / 100 g of animal body weight 15 minutes before hypoxic exposition for 30 days [12]. A solution of 0.9% NaCl was administered intraperitoneally to the rats of the second group in a similar dosage.

Cytoflavin was provided by Scientific technological pharmaceutical company "POLYSAN". The active ingredients of Cytoflavin solution contain the following substances:
1. Succinic acid - 100 mg in 1 ml of solution;
2. Inosine (Riboxin) - 20 mg in 1 ml of solution;
3. Nicotinamide (Vitamin PP) –10 mg in 1 ml of solution;
4. Riboflavin mononucleotide (Vitamin B2) - 2 mg in 1 ml.

Riboflavin (vitamin B2) is a coenzyme of FAD that activates succinate dehydrogenase and other redox reactions of the Krebs cycle.

Nicotinamide (vitamin PP) - nicotinic acid amide. Nicotinamide in cells, through a cascade of biochemical reactions, is transformed into the form of nicotinamide adenine nucleotide (NAD) and its phosphate (NADP), activating the nicotinamide-dependent Krebs cycle enzymes necessary for cellular respiration and stimulation of ATP synthesis.

Throughout the experiment, animals of the second and third groups were daily immersed for 1 hour in a transparent hypobaric chamber equipped with a manometer, safety valve, alkaline absorber to eliminate excess carbon dioxide, where the pressure was below atmospheric, which corresponded to a rise of 6000 m above sea level: (354,2 mm Hg), which is equivalent to a moderately intense hypoxic exposure. The animals were housed in standard conditions with free access to food and water. Prolonged hypobaric hypoxia was modeled after determining the individual sensitivity, so we have used low-resistant rats. Animals were kept in a vivarium, they were cared for in accordance with the rules and regulations for handling laboratory animals.

Ethics approval

All efforts were made to minimize animal suffering and to reduce the number of animals used. Rats were sacrificed by decapitation under anesthesia (ether with chloroform) in accordance with the "International Recommendations (Code of Ethics) for conducting biomedical

research using animals" (1985) and laboratory practice rules in the Russian Federation (order of the Ministry of Health of the Russian Federation from 19.06.2003 №267).

Animal experiment was approved by the Bioethics committee of Crimea Federal University Center (Protocol № 8 from 15.03.2016) according to the permission of the Academic Council of the Crimean Medical Institute (No. 103 of 30.11.77). The research was approved by the Institutional Committee on Bioethics and is consistent with the International Guidelines for the Care and Use of Laboratory Animals published by the US NIH (No. 85-23, 1985) and Guide for the Care and Use of Laboratory Animals (2009).

Study of myocardial interstitial matrix

In the end of experiment the hearts of the above-mentioned males after thoraco-and pericardiotomy were removed and immediately placed in a cardioplegic solution (0.9% KCl at a temperature of 0 ° C) to achieve the relaxed myofibers due to the cardiac arrest in diastole.

Fragments of right and left ventricle after sampling was immediately placed in 4% formaldehyde solution buffered to a pH of 7.2–7.4 with monosodium phosphate and processed through the usual technique for paraffin inclusion [13]. For the immunohistochemical study, sections were cut using the same equipment, but with a thickness of 3 μm. Sections were collected on poly-lysine coated slides, dried in a thermostat at 370 C for 24 hours in order to obtain a perfect adhesion of the biological material to the surface of the histological slide, and then stained using different antibodies: Anti-alpha smooth muscle Actin antibody (ab5694), Anti-MMP9 antibody [5G3] (ab119906). After antigen retrieval, sections were cooled down to room temperature and were incubated for 30 minutes in a 1% hydrogen peroxide solution. The sections were next washed in phosphate-buffered saline (PBS), followed by a blocking step of 30 minutes in 2% skim milk. Phosphate-buffered saline (abbreviated PBS) is a buffer solution commonly used in biological research. It is a water-based salt solution containing disodium hydrogen phosphate, sodium chloride and, in some formulations, potassium chloride and potassium dihydrogen phosphate. The osmolarity and ion concentrations of the solutions match those of the human body (isotonic). Next, the slides were incubated with the primary antibodies overnight at 40^0 C, and the next day, the signal was amplified for 30 minutes using a peroxidase polymeric secondary detection system (EnVision, Dako).

Electron microscopy

Electron microscopy fixation of myocardium was performed by immersion in the mixture of 1% paraformaldehyde and 1.5% glutaraldehyde in 0.1 M phosphate buffer at PH 7.4. After fixation, the specimens were postfixed in a 1% osmium tetroxide solution, and then washed 3 times in the buffer solution, continuing with dehydration from 50% ethanol, then with uranyl acetate (2% in 60% ethanol) with ascending graded ethanols up to 100%, and then washed in propylene oxide [14]. They were embedded in an Epon-Araldite composite, after that semithin sections and ultrathin sections were cut on an ultra-microtome UltraCut. Semithin sections were stained with tolouidine blue and ultrathin sections, with lead citrate, and examined on SELMI electron microscope

Statistical analysis

Continuous data are presented as a mean±SEM. Comparisons between groups were determined by a one-way ANOVA with a post-hoc Student t-test (SPSS Inc., Chicago, IL). Probabilities of $p < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Interstitial matrix and capillary measurements

The volume ratio of cardiomyocytes (parenchyma) and the connective tissue component (stroma) in the definitive myocardium varies, according to different sources, from 3:1 (75% and 25%) to 4:1 (80% and 20%). In quantitative ratio the cellular elements of the connective tissue (65 -75%) predominate in the myocardium, ensuring that the cardiomyocytes perform their main contractile function [16].

Differences in response of the experimental groups to hypoxia were also revealed by the quantitative analysis of the extracellular (stromal) components of the myocardium. The nuclei of these cells became dense. Without treatment in the second group the quality analysis of microphotographs showed that in contrast to the previous group almost the entire stroma was consisting of the thickened collagen fibers and proliferating cells of the connective tissue. Their total number increased relatively to the control by 31% initiating the processes of collagenases which may lead in future to cardiosclerosis if pronged hypoxic exposure. Hypoxic effects in the third group receiving treatment were not significantly different from control according to the observed changes in the stroma. Most of it demonstrated the dilated capillaries and edematous intercellular space. The number of connective tissue cells was not significantly different from control (Table 1).

Table 1

Morphometric analysis of myocardial interstitial matrix in rats expose to hypobaric hypoxia (Mean±SEM)

Parameter	Control group n=6	Hypobaric Hypoxia group n=10	Hypobaric Hypoxia+Cytoflavin group n=10
Number of nucleated cells in the interstitial matrix	31,2±0,36	41,9±0,37*	32,7±0,57
Average area of stromal cells nuclei (μm^2)	12,37±1,72	11,14±1,79	9,37±1,45*
Average area of interstitial matrix (μm^2)	328,35±16,53	262,9±14,48*	341,133±18,76

*$p < 0.05$ compared with the control group

Other structural changes of the myocardium were represented by swelling of contractile cardiomyocytes, homogenization of their sarcoplasm. In polarization microscopy, cells had fuzzy contours, they did not trace transverse striation, there was a marked disintegration of myofibrils, hyperrelaxation of sarcomeres and some areas of myocytolysis. The capillary network was uneven: areas of constriction alternated with the expansion of the lumens of the vessels with plasma soaking their walls, the development of paravascular edema and erythrocyte diapedesis into the surrounding tissue. Separate groups of cardiomyocytes were thinned, their sarcoplasma was uneven in color. There were also signs of intermuscular edema (Fig. 1a).

The administration of succinic acid combined with inosine and vitamins diminished the dystrophic and necrobiotic changes of cardiomyocytes in the third group rats. Histological examination of myocardial samples showed diminished groups of some muscle fibers, homogenization of sarcoplasma and reduction of glycogen granules. Vacuolization of cardiomyocyte sarcoplasm occurred in isolated cases. Most of the cardiomyocytes with clear cell boundaries, uniformly stained sarcoplasm, large nuclei (Fig. 1b). When polarizing microscopy traced myofibrils, intercalated discs. In cardiomyocytes with signs of destruction, I-II degree contractures were detected, less frequently, there was a marked collapse of the myofibrils.
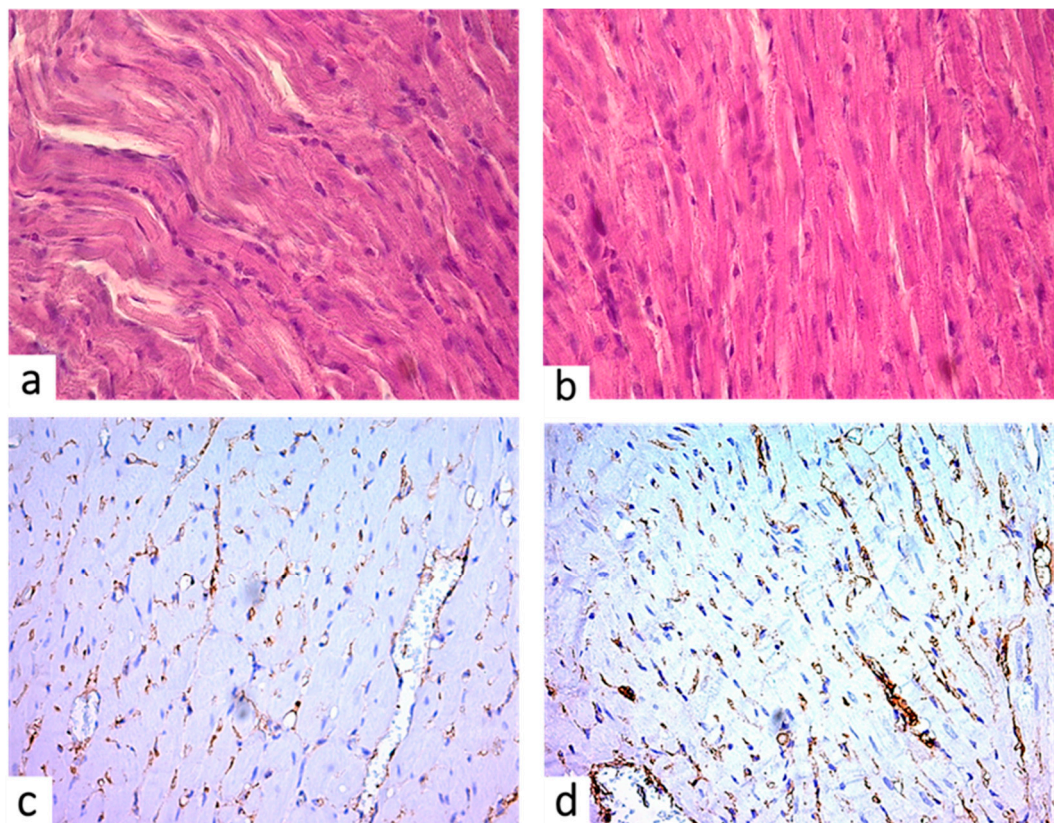

Fig. 1. Rat myocardium general overview. a - swelling of contractile cardiomyocytes, signs of cell dystrophy, interstitial edema. H&E, x400. **b** - **c** - CD31 expression showing reduction of capillary patters, x 400. **d** - CD31 expression demonstrates abundant capillaries, x 400

In rats of the second group without correction, the vasculature was represented by dilated and spasmed capillaries. Against the background of narrowed capillaries, zones devoid

of them were encountered. (Fig. 1c). The average diameter of the capillary was significantly different from the control by 34.4% (Table 1). The numerical density of capillaries during this period of the experiment was 27.51 ± 0.34. The cross-sectional area of the capillary was 57.0% lower than the initial level, and the total area of their cross-sections was 61.3%. The relative surface area of the vascular bed was 21.5 ± 0.18%, which was less than the control by 32.0%.

Against the background of administration of Cytoflavin, it was found that in rats the third group of the walls of some vessels were thickened, endothelial cells protruded into the lumen in the form of a paling. In some fields, a "discontinuity" of the vascular pattern was observed (Fig. 2b). The capillary diameter exceeded the indicator of the second group (p <0.05), which indicates the relative safety of the hemomicrocirculatory bed, but was significantly lower than the control indicator (p <0.05). Accordingly, compared with the series without correction, the cross-sectional area of the capillaries was increased - 28.44 ± 0.14 μm^2 against 24.53 ± 0.20 μm^2, (p <0.05). The total cross-sectional area of the capillaries is 0.77 ± 0.10 x10^3 μm2. The relative surface area of the vascular area was lower than the control by 21.8% (p <0.05) (Table 2).

Table 2

Microcapillary measurements in rats exposed to hypobaric hypoxia (Mean±SEM)

Parameter	Control group n=6	Hypobaric Hypoxia group n=10	Hypobaric Hypoxia+Cytoflavin group n=10
Diameter of the capillary (μm)	8,52±0,12	5,59+0,07*	6,02±0,07*
The cross-sectional area of the capillary (μm^2)	56,98±0,23	24,53+0,20*	28,44±0,14*
The numerical density of the capillaries	30,32+0,16	27,51±0,34*	27,15+0,16*
Total. cross-sectional area of cross sections of capillaries (x10^3 μm^2)	1,73±0,18	0,67±0,10*	0,77±0,10*
Relative superficial area of vessels (%)	31,6±0,12	21,5+0,18*	24,7±0,23*

*p<0.05 compared with the control group

Fibrogenesis in the interstitial space

After exposure to hypobaric hypoxia in second group rat myocardium lysis of single cardiomyocytes was noted. At the site of the dead cardiomyocytes along the capillaries and around the larger vessels proliferation of connective tissue was noted (Fig. 2a). This was due to the fact that in response to ischemia fibroblast activation occurs resulting in an increase in collagen synthesis. A disproportionate increase in the synthesis and inhibition of the collagen degradation in the extracellular matrix can cause subsequent fibrogenesis — a disproportionate accumulation of fibrillar collagen (Fig. 2b). Reparative (compensatory) fibrogenesis develops at

the site of the dead cardiomyocytes. In turn, the reactive fibrogenesis we observed which is not directly related to cardiomyocyte necrosis, was presented as interstitial (accumulation of collagen in the intermyofibrillar space) and perivascular (accumulation of collagen within the advent of coronary arteries and arterioles) fibrogenesis [11].

Undoubtedly developing fibrogenesis has a negative impact on the state of the myocardium, contributing to the remodeling process. The accumulation of collagen causes an increase in the rigidity of the ventricular wall, which leads to a violation of contractility and relaxation [7]. The progressive accumulation of connective tissue leads to a decrease in capillary density and increases the distance required for diffusion of oxygen, which can determine the development of subsequent ischemia of cardiomyocytes. Fibrosis can also disrupt the electrical interaction between cardiomyocytes, contributing to the development of arrhythmias [1].

Third experimental group cardiac interstitium demonstrated mild edema around the arterioles and venules. Electron microscopy revealed many fibroblasts were involved in myocardial tissue compartmentalisation, separating certain groups of cardiomyocytes from adjacent capillaries without overexpression of collagen fibers (Fig. 2b). The formation of fibroblasts of thin plate-like processes delimiting cardiomyocytes from adjacent capillaries, as well as adjacent bundles of cardiomyocytes, has been found. However near necrotic cardiomyocytes and around capillaries foci of newly formed connective tissue were also detected (Fig. 2d). Hypertrophied cardiomyocytes with large nuclei were located near them. The need to maintain the stroke volume against the loss of a part of the contractile myocardium determines the launch of an internal, compensatory program aimed at adapting functioning cardiomyocytes to increased workload by hypertrophy. Cardiomyocyte hypertrophy provides adaptation of the myocardium to new working conditions by increasing the number of contractile units and reducing the tension of the thickened heart wall in accordance with the Laplace law [3]. Mechanical tension of interstitial matrix can regulate the direction of cellular hypertrophy while providing adequate tissue architect. A reference point, or "matrix", for directed extracellular fibrogenesis are microfibrils (glycosaminoglycans and structural glycoproteins) and cell surface relief. It should be noted that the enhancement of collagen-synthesizing activity of fibroblasts requires an advanced accumulation of glycosaminoglycans, glycoproteins, which is observed in fibrotic processes in the myocardium [4, 6].
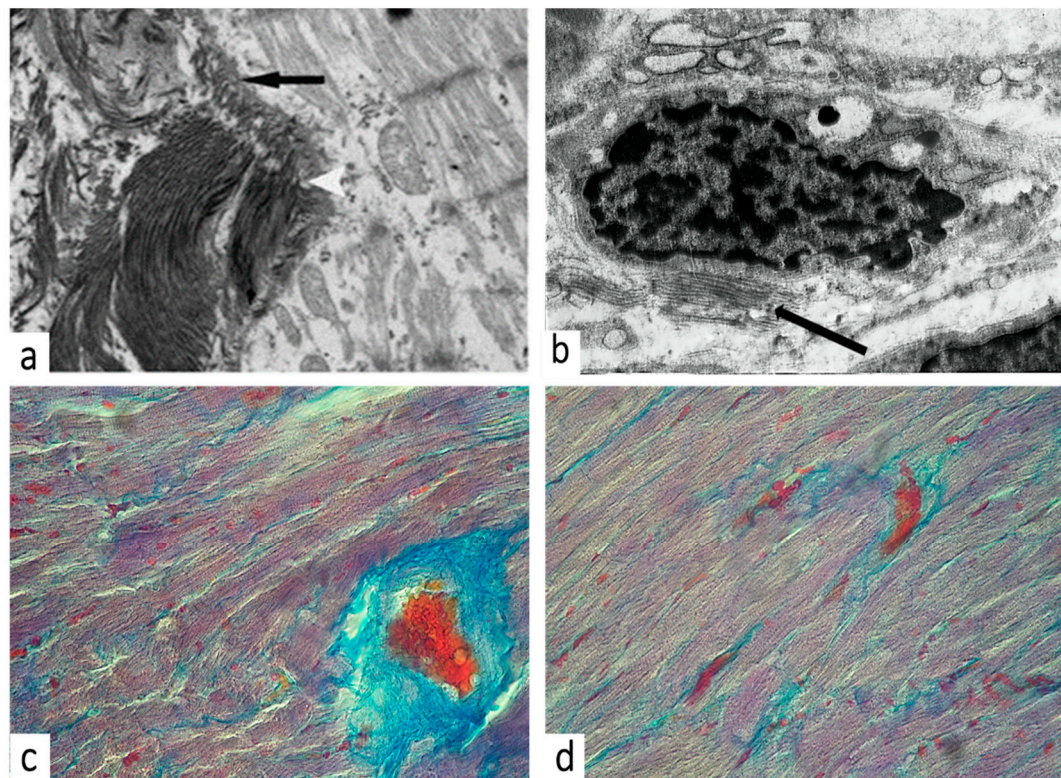
Fig. 2. Collagen deposition in the cardiac interstitium. a – collagen synthesis replaces lesioned cardiomyocyte (arrows). TEM, x 12000. **b** – fibroblast with low amount of collagen fibers (arrows) interconnected with sarcolemma. TEM, x12000. **c** – blue connective tissue increases the distance required for diffusion of oxygen between cardiomyocytes and arteriole. Mallory staining, x 400. **d** – blue connective tissue between cardiomyocytes and capillaries. Mallory staining, x 400.

Markers of the initial myocardial remodeling

The vast majority of cells of the connective tissue component of the myocardium are fibroblasts (90-95%). They are the main producers of matrix macromolecules, including collagen and major structural proteins. The remaining cell forms are mast cells, macrophages, lymphoid cells. The main mass of connective tissue myocardial cells are resident, however, in pathological conditions, an increase in the proportion of cells migrating into the myocardium from the outside is noted [17]. The cell population of myocardial connective tissue is distinguished by polymorphism, reflecting its functional heterogeneity. Many cells have different degrees of differentiation and degradation: poorly differentiated, young and mature fibroblasts, fibrocytes; monocytoid, activated and decaying macrophages; immature, mature and degranulated mast cells; lymphoid and plasma cells. In addition, there is a structural and functional specialization of mature cells [18]. Thus, among fibroblasts, activated fibroblasts (collagenoblasts), fibroblasts, and myofibroblasts are isolated.

Myofibroblasts combine the qualities of a fibroblast (collagen synthesis, mainly of the III type) and a smooth myocyte (the presence of myofilament, the ability to contract). Myofibroblasts are attributed to the ability of dynamic changes in the volume of the intercellular substance, as well as the effect on the orientation of its fibrous elements [16]. The switching of fibroblast phenotype to myofibroblasts is due to a transforming growth factor beta-1 (TGF-pi)

and is associated with the beginning of their expression of smooth muscle alpha actinin (a-SMA) and desmin [5].

Appreciating the dual roles of cardiac myofibroblasts in the myocardial remodelling process is important, as they can be perceived to be both beneficial and detrimental according to the amount and their temporal and spatial location. An increase in the number of myofibroblasts which was observed in the hypoxic rat heart in the second group can affect the ability of fibroblasts to conduct polarization from one cardiomyocyte to another (Fig. 2a). However, myofibroblasts can maintain dynamical features of myocardium by maintaining myocardial integrity against a background of continuous mechanical forces associated with the pumping of the heart. That effect was probably observed in the third group because fibroblast were located mainly nearby hypertrophied cardiomyocytes integrating their contractions with another myocardial elements (Fig. 2b). Still even moderate activation of fibroblasts can lead to progressive fibrogenesis.

Fibroblasts secrete the proteins of the myocardial extracellular matrix and matrix metalloproteinases, thereby performing central role in maintaining the structure of the stroma. Collagen degrading enzymes belong to the group of matrix metalloproteinases that can cleave a large number of proteins of the extracellular matrix [19]. Matrix metalloproteinases have some similar properties: they share common regions of the amino acid sequence, are synthesized as inactive pro-enzymes, and require zinc as a cofactor [20]. The balance between the synthesis of matrix proteins and their degradation plays an essential role in maintaining the integrity of the myocardium. They are secreted in an inactive form into the extracellular space, where they are activated under the action of other proteases and function under the physiological conditions of tissue reorganization. It is known that the progressive activation of matrix metalloproteinases in ischemia promotes dilatation and deterioration of left ventricular function that leads to the development of heart failure [21].

Prolonged hypobaric exposure perivascular and in the interstices appeared intensely colored connective tissue cells (neutrophils, macrophages and fibroblasts) expressing the matrix metalloproteinase-9 (MPP-9), the intensity of staining was high, i.e. there was a complete uniform staining of the cytoplasm [Fig. 3c].

Rats of the third group comparison of single MMP-9-positive cells in the specimens were detected at separate sites in the myocardium, the severity of expression of the marker is weakly positive, indicating that the low intensity of the processes of myocardial remodeling (Fig. 3d). It is known that the continued sustained high concentrations of MMP-9 testify to the permanence of the destructive processes in the extracellular matrix of the myocardium and are predictors of poor prognostic outcome of the disease.
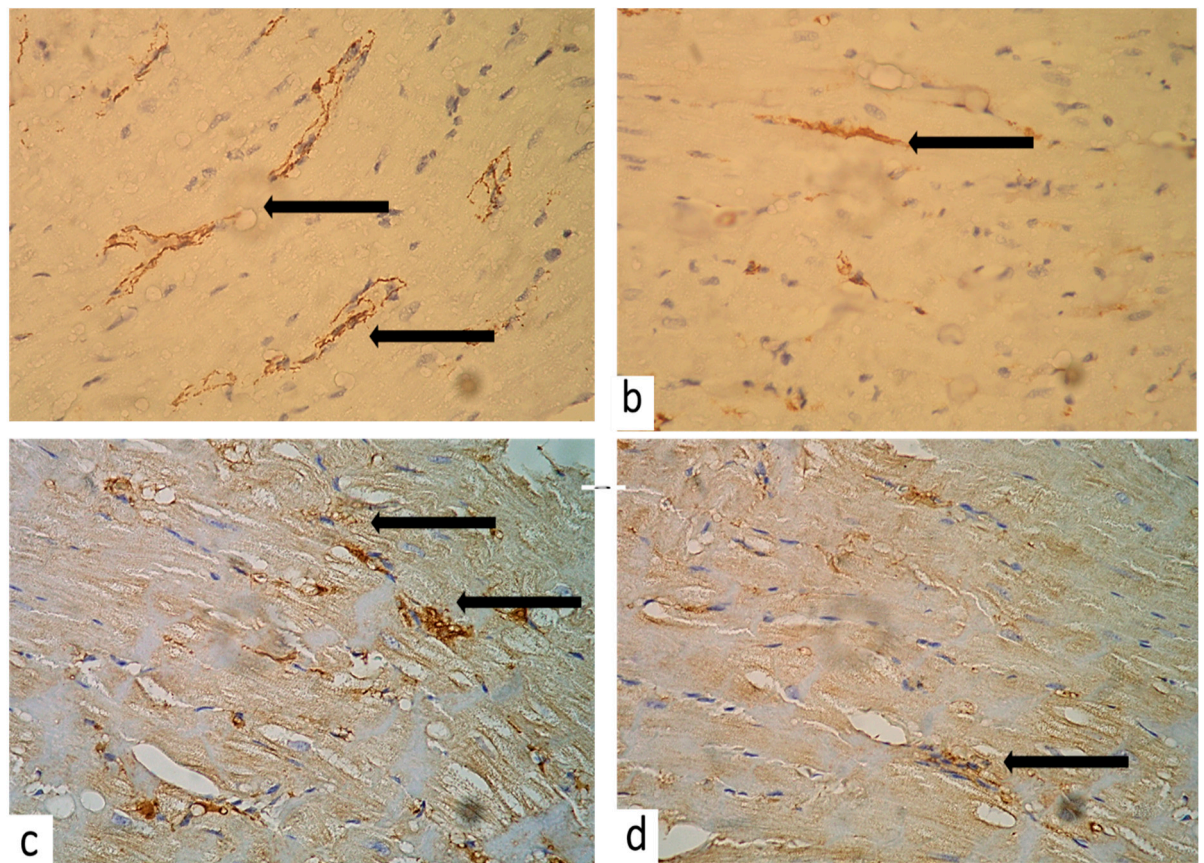
Fig. 3. Identification of the main trigger proteins of myocardial remodeling in rats exposed to hypobaric hypoxia. a - the presence myofibroblasts in the expanded perivascular and interstitial spaces, x400 **b** – few myofibroblasts adjacent to enlarged cardiomyocytes, x400. **c** - myocardium of male rats exposed to histotoxic hypoxia. High cytoplasmic expression of MPP-9 by interstitial cells, x 400. **d** - myocardium of male rats exposed to hypobaric hypoxia with subsequent correction. Slightly positive expression of MPP-9 by interstitial cells, x 400.

Conclusions

The connective tissue extracellular matrix contains a network of macromolecular fibers supporting cardiomyocytes. It has a complex spatial organization that largely determines the structural and functional integrity of the heart muscle. The qualitative and quantitative composition of the components of the myocardium, their relationship and interactions are regulated quite tightly at all levels of the organization of the heart muscle. The analysis of the current reviews allows us to state that activated fibrosis is an essential component of myocardial remodeling in a wide variety of pathological conditions. Violations of this balance affect the functionality of the myocardium in normal conditions, as well as determine the pathomorphosis of heart disease during hypobaric hypoxia. Understanding the alterations in cardiac metabolism and function associated with sustained exposure to high altitude advances our understanding of hypoxia-related disease. Fibrosis in the remote myocardium inevitably leads to increased myocardial stiffness, resulting in systolic and diastolic dysfunction, neurohormonal activation and, ultimately, heart failure

Changes in cardiac highenergy phosphate metabolism may underlie the myocardial dysfunction caused by hypobaric hypoxia. Reduced oxygen delivery due coronary microvascular damage and increased perivascular fibrosis associated with reduced cellular oxygen availability

may later contribute to contractile failure. Succinic acid combined with inosine acts as a high-energy phosphate reserve, to maintain adenosine triphosphate (ATP) at levels sufficient to support contractile function. Cyto- and angioprotective effects in rats after medication with succinic acid combined with inosine, riboflavin and nicotinamide in complex therapy are associated, in our opinion, with the drug composition where active components have mutually potentiating effects and work as inducers of the main metabolic pathways in cells, and activators of key energy-forming processes. These changes are probably associated with the positive effect of succinic acid on the oxidative phosphorilation in the cell with the prevention of excessive production of reactive oxygen species, which improves the state of the endothelium and, consequently, the state of hemodynamics of the myocardium.

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