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Myocardial fibrillar collagen network: response to ischemia

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Both myocardial extracellular matrix as a whole and the fibrillar collagen network can alter in response to environmental stimuli such as ischemia.

The aim of the study was to evaluate changes of the fibrillar collagen network in chronic ischemic myocardium and in cases of its regional losses. The percentage volume, perimeter, number of fiber foci per field of the myocardial fibrillar collagen network were estimated employing the computerized Quantimet 520 image analysis system (Cambridge Instruments, United Kingdom) in 152 autopsied males who had died suddenly (within 6 h) of ischemic heart disease (IHD). The decedents were divided into three groups according to ischemic injury: (1) chronic ischemia – 71 males; (2) group of acute regional losses – 21 males; (3) group of formed myocardial scars – 60 males. Thirty-two males who had died within the same 6 hours following an accident served as controls.

All parameters of fibrillar collagen in patients with chronic ischemia were significantly higher than those in the control group, indicating diffuse interstitial myocardial fibrosis. In patients with acute myocardial regional losses the percentage volume and the collagen-to-cardiomyocyte ratio of fibrillar collagen in the intact (remote from infarction) zone were the same as in patients with chronic ischemia alone, while the collagen fibers were found to be larger. In patients with formed scars, all fibrillar collagen parameters in the intact myocardial zone were significantly higher as compared with the corresponding indices of chronic ischemia and acute regional losses groups showing the progression of myocardial interstitial fibrosis. The relationship between the abundance of fibrillar collagen and total stenotic coronary artery lesions was determined.

The occurrence and progression of myocardial fibrillar collagen accumulation together with eccentric left ventricular hypertrophy indicate a cardiac compensatory process in ischemic conditions.

Key words: fibrillar collagen, myocardium, interstitial fibrosis, ischemia

INTRODUCTION

The extracellular matrix (ECM) provides the physical scaffolding for the three-dimensional organization of cells and determines the physical properties of biological tissues. The ECM in most tissues, including myocardium, is composed of a complex arrangement of fibrillar collagen, elastin, microfibrillar proteins, proteoglycans and the adhesive proteins laminin and fibronectin. The fibrillar collagen network, and ECM as a whole, can respond to environmental stimuli such as ischemia and tissue injury by altering its abundance, composition, and spatial organization, with profound implications to the structure and function of the tissues [1–5].

The myocardial fibrillar collagen network is described by a specific organization of three layers. The outer layer of the epimysium surrounds all the cardiomyocyte bundles; the perimysium describes the sheath of connective tissue that surrounds and interconnects cardiomyocyte groups in a particular orientation; the inner layer of the endomysium surrounds individual cardiomyocytes within each bundle and connects them among themselves and with capillars [6]. The fibrillar collagen network maintains the architecture of the myocardium and the arrangement of the cardiomyocyte during a cardiac cycle. The integrity of the fibrillar collagen network plays a critical role in the coordination of the overall myocardial contraction. The structure of the fibrillar collagen network is believed to be responsible for the maintenance of the shape and distensibility of the cardiac chambers [1, 5–7].

There are not many data on the quantitative changes of the myocardial fibrillar collagen network in various ischemic situations. The aim of this study was to evaluate

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the response of left ventricular fibrillar collagen to chronic ischemia and regional losses of the myocardium in various stages of IHD course.

MATERIALS AND METHODS

Materials. Autopsied 152 males (average age 48.6 ± 8.2 yrs) who had died suddenly (within 6 h of the onset of symptoms of the terminal heart attack) due to the first (myocardial scars absent) or repetead (myocardial scars present) acute IHD events were studied. They had no other factors such as systemic hypertension, myocarditis, cardiomyopathy, diabetes, etc., except ischemia predisposing to myocardial overload.

The decedents were divided into three groups according to IHD stages: (1) chronic ischemia (preinfarction IHD group) – 71 males; (2) group of acute regional losses – myocardial infarction (acute MI) – 21 males; (3) group of formed scars (postinfarction IHD) – 60 males. Thirty-two males (average age 46.0 ± 12.8 yrs) who had died within the same 6 hours following an accident served as controls. Acute ischemic myocardial lesions in the decedents of the first and third groups had the duration of not more than 12 hours. This fact permitted to distinguish strictly the preexisting structural changes from those occurring during an acute event.

Methods. Transverse left ventricular (LV) and septal paraffin-embedded histotopograms were sliced 4 μ thick and stained with H–E and Picrosirius Red. The strong aniline stain Sirius Red (F3BA) in saturated picric acid solution has a great affinity to collagen fibers while other structures remain unstained. This pattern is employed in quantitative determination of fibrillar collagen.

The fibrillar collagen network area and percentage volume, the number of fiber foci per field and their perimeter were assessed by LV and septal myocardium intramural part area away from acute ischemic injury or postinfarction scars, approximately in 100 fields of each slice, magnification 700×, one field 35578 μ^2 with the computerized Quantimet 520 image analysis system (Cambridge Instruments, United Kingdom). Foci of replacement fibrosis determined by polarized light and intramural vessels larger than 20 μ in diameter were omitted. The least necessary number of measurements was obtained after evaluation of pilot study parameter means

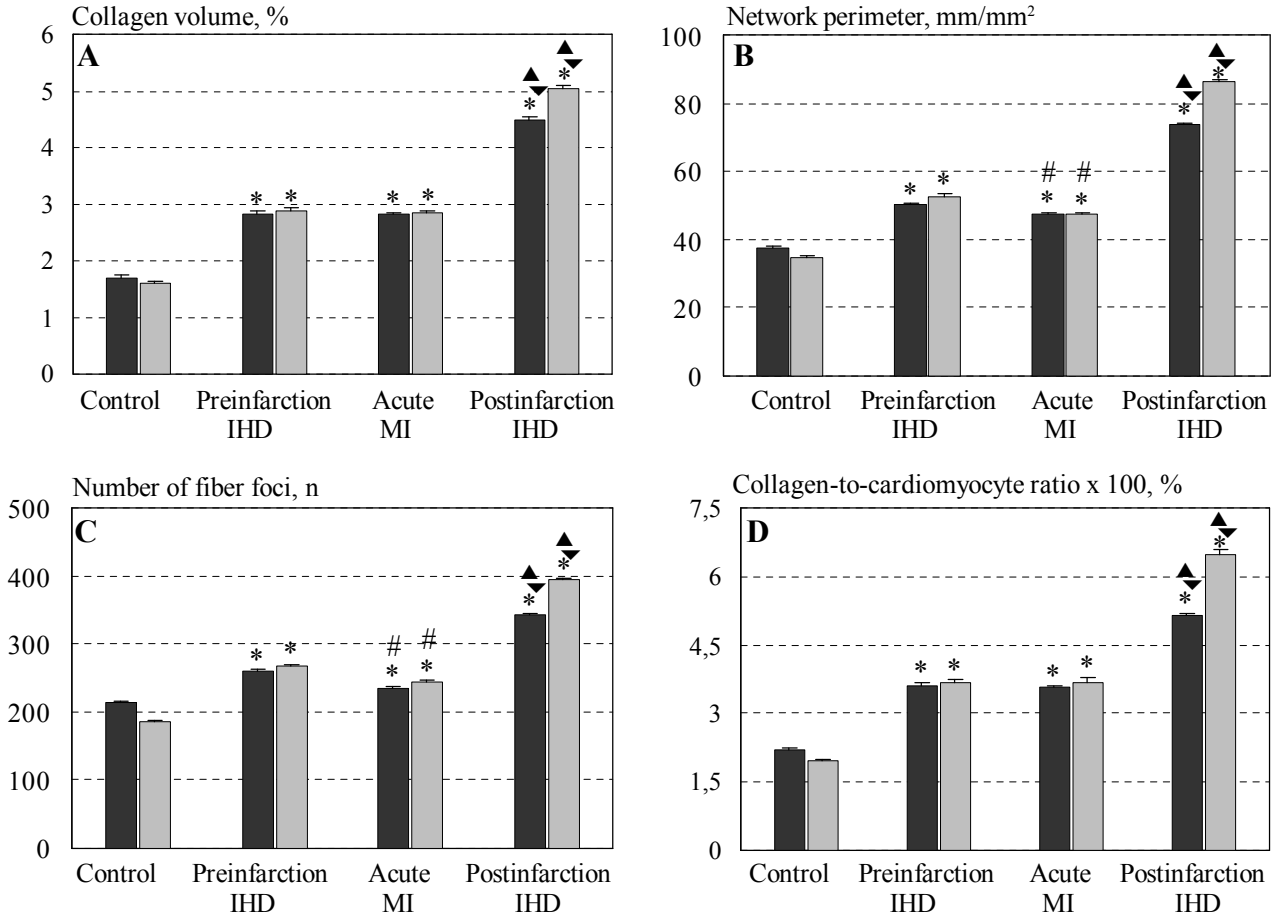

Fig. 1. Left ventricular (left) and interventricular septal (right) percentage volume (A), perimeter (B), number of fiber foci (C) of fibrillar collagen network and collagen-to-cardiomyocyte ratio (D) (means ±SE)

* – significant difference between research group and control group;

\# – significant difference between acute MI group and preinfarction IHD group;

▼ – significant difference between postinfarction MI group and preinfarction MI group;

▲ – significant difference between postinfarction MI group and acute MI group.

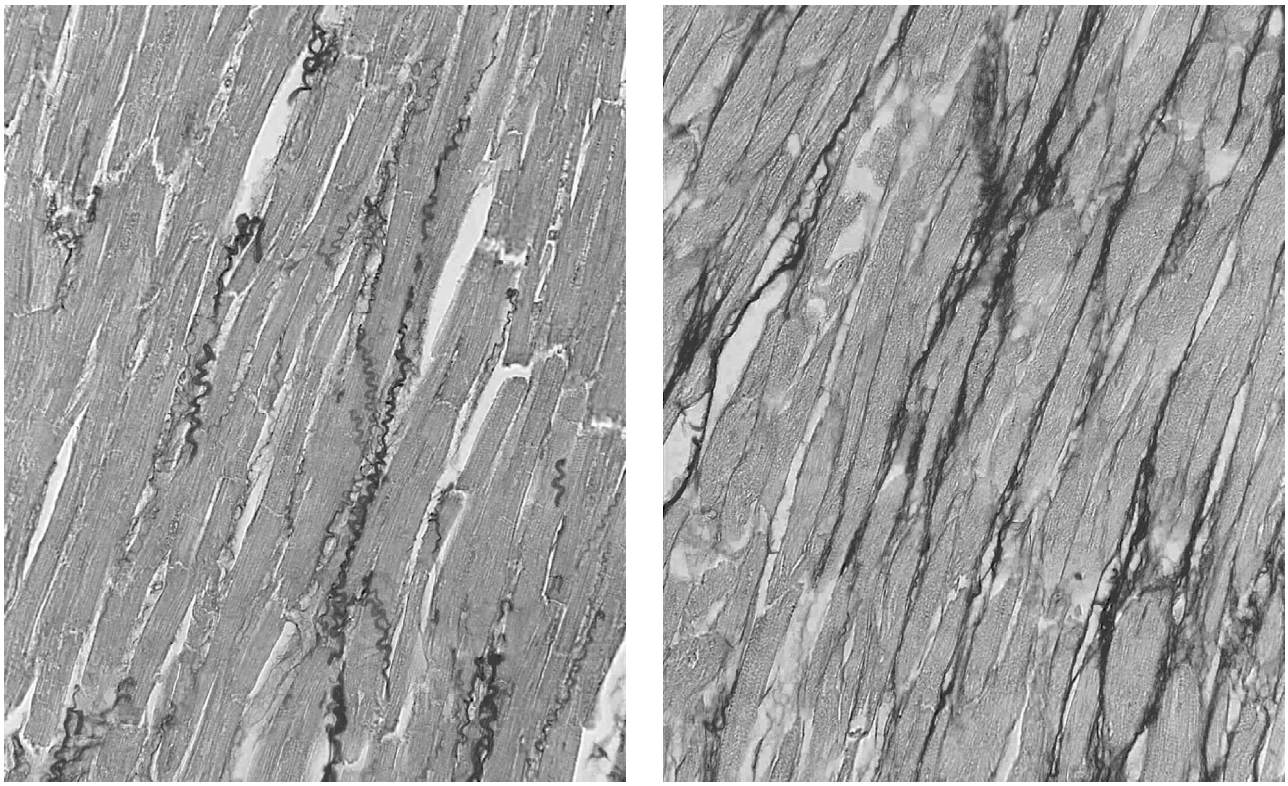

Fig. 2. Myocardial collagen network: left – normal structure, right – interstitial fibrosis (Picrosirius Red)

and dispersions. A pilot study was employed for standardization of histomorphometry evaluating the results of two investigators by their deviations during blinded repeated measurements. The difference was less than 5%. The total number of 14397 fields of IHD groups and 5551 fields of the controls were investigated.

The stenosis index, i.e. the extensiveness of total coronary artery atherosclerotic stenotic lesions, was obtained as described previously [9].

Statistical analysis. The normal distribution of parametrical variables of every case was tested according to χ^2 and Kolmogorov–Smirnov criteria. Multifactor analysis of variance (ANOVA) was applied to test differences in means (for groups and variables) of statistical significance (nested design). Regression analysis was used to determine relations among different variables. Values of $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Fibrillar collagen network parameters in chronic ischemic myocardium. The results of this study show that during chronic ischemia, i.e. before the first myocardial infarction – preinfarction IHD group, all parameters of myocardium fibrillar collagen are significantly larger than the same indices in the controls (Fig. 1). The percentage volume of collagen network of the LV was 1.5 times and of the interventricular septum 1.8 times larger than in the control group, the number of fiber foci being 1.2 and 1.4 times, network perimeter 1.3 and 1.6 times, and the collagen-to-cardiomyocyte ratio 1.6 and 1.9 times, larger than in the controls ($p < 0.01$), indicating an imbalanced fibrillar collagen accumulation, i.e. diffuse myocardial interstitial fibrosis (Fig. 2).

Fibrillar collagen network percentage volume in the chronic ischemia group depended upon the coronary artery (CA) stenosis index: Pearl–Reed's limited augmentation curve of regression was stated between the fibrillar collagen percentage volume and the stenosis index (Fig. 3). A similar relationship was noted between the CA stenosis index and the other collagen parameters, such as the number of fiber foci and the network

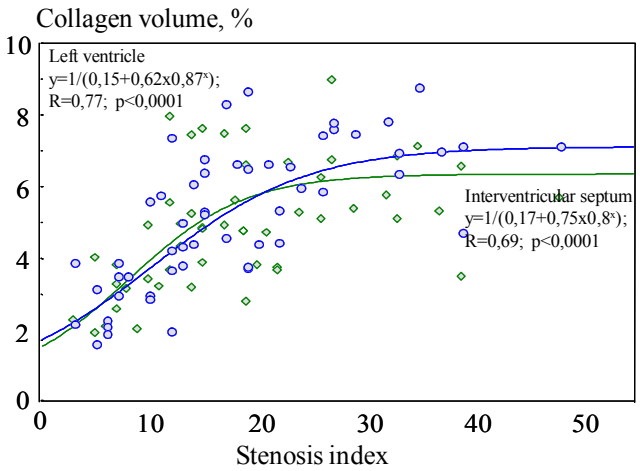

Fig. 3. Left ventricular (left) and interventricular septal (right) distribution of percentage volume of fibrillar collagen network according to stenosis index of coronary arteries in chronic ischemia group

perimeter. Thus, the more extensive CA lesions, the higher interstitial collagen accumulation.

Fibrillar collagen network parameters in the intact zone with acute regional losses of myocardium. It was found that although in the intact (remote from acute ischemic injury) zone the myocardial collagen-to-cardiomyocyte volume ratio and the volume percentage of collagen of the acute MI group did not differ from those of the chronic ischemia group, the number of fiber foci per field and the network perimeter were reduced by 10% and 6% in the LV myocardium and by 9% and 7% in the septal myocardium, respectively, $p < 0.001$ (see Fig. 1). Thus, a prevalence of large perimysial fibers was found.

Fibrillar collagen network parameters in intact myocardium zone of formed scar. When a connective tissue scar is replacing myocardial necrosis, fibrillar collagen network volume percentage in the intact zone of LV and septum is by 2.2 and 3.1 times higher than in the control group, by 1.4 and 1.8 times higher than in the chronic ischemia and the acute MI groups, respectively ($p < 0.05$) (see Fig. 1). The number of fiber foci was respectively 1.5 and 1.6 times higher than in the acute MI group and by 1.3 and 1.5 times higher than in the chronic ischemia (preinfarction IHD) group; the LV and septal myocardium fibrillar collagen network perimeter were 1.5 and 1.7 times higher than in the acute MI group, 1.4 and 1.6 times higher than in the chronic ischemia group, and 1.9 and 2.6 times higher than in the control group ($p < 0.001$). These figures indicate a further accumulation of endomysial collagen struts and perimysial fibers in both LV and septal intact myocardium.

Human ischemic myocardium fibrillar collagen network quantitative studies are scarce and because of different methods of investigation applied cannot be compared [3, 10–13]. According to our data, chronic myocardial ischemia due to IHD is associated with reactive interstitial fibrosis in LV and septal myocardium. These data are consistent with M. Ishijima's data indicating that myocardial ischemia is associated with fibrosis when the stenosis of all three CA is ≥ 75% [11]. Interstitial fibrosis is a result of increased collagen production when its degradation is normal or reduced [13]. However, the exact mechanisms are not yet clear.

After both experimental MI [14–16] and infarction in humans [3, 6, 8], a possible intact zone interstitial fibrosis formation is reported. We were able to provide evidence of progressing interstitial fibrosis of an intact myocardial zone in the postinfarction period.

Because our method is able to describe not only the percentage volume but also other collagen network parameters, we have managed to prove that during the first two weeks after infarction, although the collagen-to-cardiomyocyte volume ratio is the same as in chronic ischemic group, the larger perimysial fibers prevail. This may be associated with enzymatic disruption and digestion of small endomysial fibers with a concomitant increase in the synthesis of larger perimysial weaves, which is induced during the losses of myocardium [17].

Myocardial fibrillar collagen, like the entire ECM, is constantly changing and adapts to functional needs of the tissue. During ischemia, systemic and locally produced neurohumoral factors such as rennin–aldosterone–angiotensin system hormones, norepinephrine, growth factors (GF), e.g., basic fibroblast GF, insulin-like GF, and β-transforming GF, as well as their modulator volume and/or pressure overload (which is present during myocardial tissue losses) activate fibroblast proliferation and collagen synthesis [18–21]. A parallel hypertrophy of cardiomyocytes is found [6, 7]. The increase in fibrillar collagen network is an adaptive process at the outset: it helps the myocardium a more effective contraction, and the increase in wall stiffness prevents dilation of the ventricle. Ultimately, however, the increasingly growing stiffness results in diastolic dysfunction.

CONCLUSIONS

Our investigation proves that myocardial ischemia is associated with quantitative changes of fibrillar collagen. In chronic myocardial ischemia, left ventricular and septal reactive interstitial fibrosis continuing to progress following myocardial losses is found. The biological message of interstitial fibrosis is to maintain ventricular function and to prevent dilatation.

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MIOKARDO FIBRILINIO KOLAGENO TINKLAS: ATSAKAS Į ISCHEMIJĄ

S a n t r a u k a

Neląstelinio miokardo matrikso ir jo komponento – fibrilinio kolageno tinklo – kiekis ir sandara kinta reaguodami į aplinkos stimulus, pvz., ischemiją.

Darbo tikslas buvo nustatyti kiekybinius miokardo fibrilinio kolageno tinklo pokyčius, kai yra chroninė miokardo ischemija, taip pat išlikusio intaktinio miokardo netekus jo dalies, t. y. įvykus miokardo infarktui bei susiformavus poinfarktiniam jungiamojo audinio randui.

Kompiuterine vaizdo analizės sistema „Quantimet 520“ (Cambridge Instruments, Jungtinė Karalystė) ištirta 152 vyrų, mirusių staiga (per 6 val.) įvairiu ischeminės širdies ligos raidos tarpsniu, kairiojo skilvelio ir tarpskilvelinės pertvaros fibrilinio kolageno tinklo histomorfometriniai parametrai, taip pat šių širdies dalių masė, endokardo paviršiaus plotas bei bendras stenozinis vainikinių arterijų pažeidimas. Kontrolinę grupę sudarė 32 vyrai, mirusieji per tokį pat laikotarpį nuo nelaimingų atsitikimų.

Esant tik chroninei miokardo ischemijai, t. y. ikiinfarktinės IŠL grupės, visi tirtųjų fibrilinio kolageno tinklo parametrai – procentinis tūris, perimetras, skaidulų plotelių skaičius regėjimo lauke bei kolageno ir kardiomiocitų tūrio santykis – buvo reikšmingai padidėję lyginant su atitinkamais kontrolinės grupės rodikliais. Tai rodo esant difuzinę intersticinę fibrozę. Nustatyta sąsaja tarp fibrilinio kolageno procentinio tūrio padidėjimo ir bendro aterosklerozinio stenozuojamojo vainikinių arterijų pažeidimo. Mirusiųjų nuo dviejų savaičių ūminio miokardo infarkto kolageno procentinis tūris bei kolageno ir kardiomiocitų tūrio santykis buvo tokie patys, kaip ir ikiinfarktinės IŠL grupės, tačiau skaidulų plotelių skaičius ir perimetras mažesni, t. y. skaidulų pluoštai stambesni. Poinfarktinės IŠL grupės tirtųjų visi fibrilinio kolageno tinklo kiekybiniai rodikliai buvo didesni ne tik už kontrolinės bet ir ikiinfarktinės IŠL grupės rodiklius. Tai reiškia, kad intersticinė miokardo fibrozė progresuoja.

Kartu su ekscentrine kairiojo skilvelio hipertrofija didėjantis fibrilinio kolageno tinklo tūris yra kompensacinis atsakas į chroninę miokardo ischemiją ir miokardo struktūrų netektį.

Raktažodžiai: fibrilinis kolagenas, miokardas, intersticinė fibrozė, ischemija