MYOCARDIUM TISSUE ALTERATIONS IN ACUTE CARDIAC ATTACK AND RESPIRATORY ARREST EVALUATED BY POLARIZED LIGHT

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ABSTRACT

Many pathologies leading to the death of the organism are tied to the cessation of cardiac or respiratory activity. In the present study, we studied the microscopic structure of the myocardium of male Wistar rats under conditions of acute cardiac and respiratory arrest. Unstained histological sections of the myocardium are examined with a polarizing microscope for the length of the sarcomere, A- and I-discs measurements. The results of the study showed that the length of the sarcomere during respiratory arrest and cardiac attack significantly decreases due to the length of the I-disc, especially in acute cardiac attack. Correlation-regression analysis showed sharp decrease of correlation in acute respiratory arrest, reaching extremely low values in cardiac attack. The study allows assessing ultrastructural changes in the myocardium and forms the basis for diagnosing acute heart pathology with the machine learning and automated analysis.

Keywords: polarization, hypercontraction, cardiomyocyte ischemia, cardiac attack, respiratory arrest

1. INTRODUCTION

The normal heart functioning requires a large amount of energy, which cannot be obtained without oxygen. When oxygenated blood supply to the heart decreases as a result of coronary spasm or thrombosis, respiratory disorders in the organ lead to ischemia, the consequences of which are most pronounced in myocardium and manifest, in particular, in changes of cardiomyocyte structure. These changes may occur in the initial stages of ischemia, when the heart is still functioning normally and no significant homeostasis disturbances occur. It is not always possible to detect early morphological changes in ischemic heart on histological sections stained by routine methods. Staining of histological sections according to the Lee or Sellier methods is more informative and equivalent in results; they use a set of dyes to detect damaged cardiomyocytes in the early stages. The cytoplasm of ischemic cells acquires a bright purplish-red color. One of the early manifestations of myocardial ischemia is cardiomyocyte hyper decrease associated with ATP depletion [1,3], which can be identified by conventional optical microscopy. The method of direct polarization microscopy makes it possible to visualize myofibril sarcomeres due to the ability of A-disks to double ray refraction [2], and assess the state of cardiomyocyte contractile apparatus in stained and unstained myocardial slices. It is established that pathological changes in organs and tissues under conditions of respiratory or cardiac arrest occur differently [4]. Therefore, the aim of this study is to assess myocardial condition in two fundamentally different acute conditions associated with insufficient oxygen supply to the heart.

2. MATERIAL AND METHODS

In order to provide comparative analysis of polarized and non-polarized images we used Zeiss Axio Lab.A1 (Carl Zeiss, Germany) microscope with and without polarization system applied. The polarizing microscope system includes a polarizer and an analyzer. As a result of the anisotropy phenomenon presence in the myocardium, such an optical property as birefringence is observed. This allows to visualize the components of the sarcomere, since the disks have

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heterogeneity and unique optical properties. In our experiment we used lambda (phase) plate to improve the quality of image for the analysis. Figure 1 shows the scheme of the microscope used in the study.

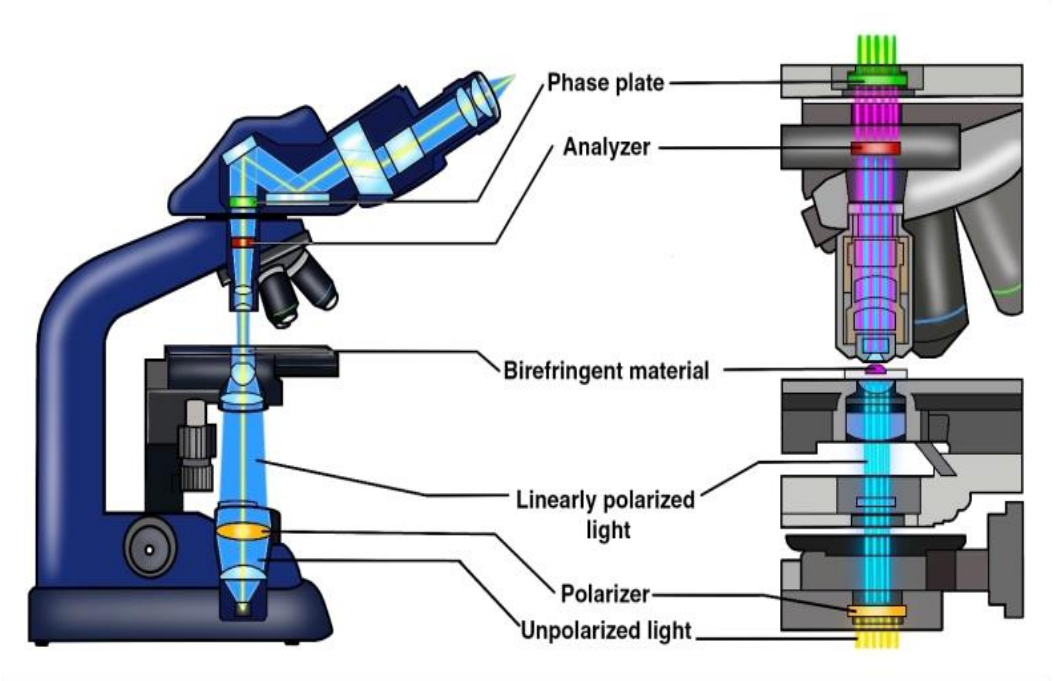

Figure 1. Schematic presentation of the experimental polarization microscopy system.

In male Wistar rats (weight - 150 g) the state of cardiomyocytes after acute cardiac arrest or after respiratory arrest, as well as in intact animals (n=3 in each group) is analyzed. After anesthesia, animals are catheterized in the right external jugular vein and acute cardiac arrest is induced by injecting 1 ml of 2% lidocaine solution (experimental group №1), or respiratory arrest by injecting 1 ml of 2% cisatracurium besylate solution (experimental group №2). At the end of the experiment, the animals are fixed intravital by perfusion into the great circle of the circulation through the left ventricle with buffered formalin, then a dissection is performed, the heart is extracted and additionally fixed in formalin by immersion. To study cardiac cell anisotropy, we used serial deparaffinized 5-μm-thick myocardial sections, one of which is left unstained for polarization microscope examination, and the other is stained using standard histological methods.

We counted the length of whole sarcomeres, A and I disks, and then performed statistical analysis of the data using Origin Pro software (OriginLab, USA). Measurement results are assessed after checking the normality of the distribution by nonparametric analysis using the ANOVA criterion. On the basis of this data, we conducted correlation and regression analysis with the determination of correlation and determination coefficients, as well as the construction of polynomial models of order 3 with the derivation of the equations of dependence describing the obtained data.

3. RESULTS

The study of polarization properties of cardiomyocytes showed that the sarcomere length decreased significantly during respiratory arrest and cardiac arrest on average. We studied polarized and non-polarized images of unstained cardiac sections as well as sections stained with hematoxylin and eosin, basic fuchsin and Lee staining method (Fig 2).

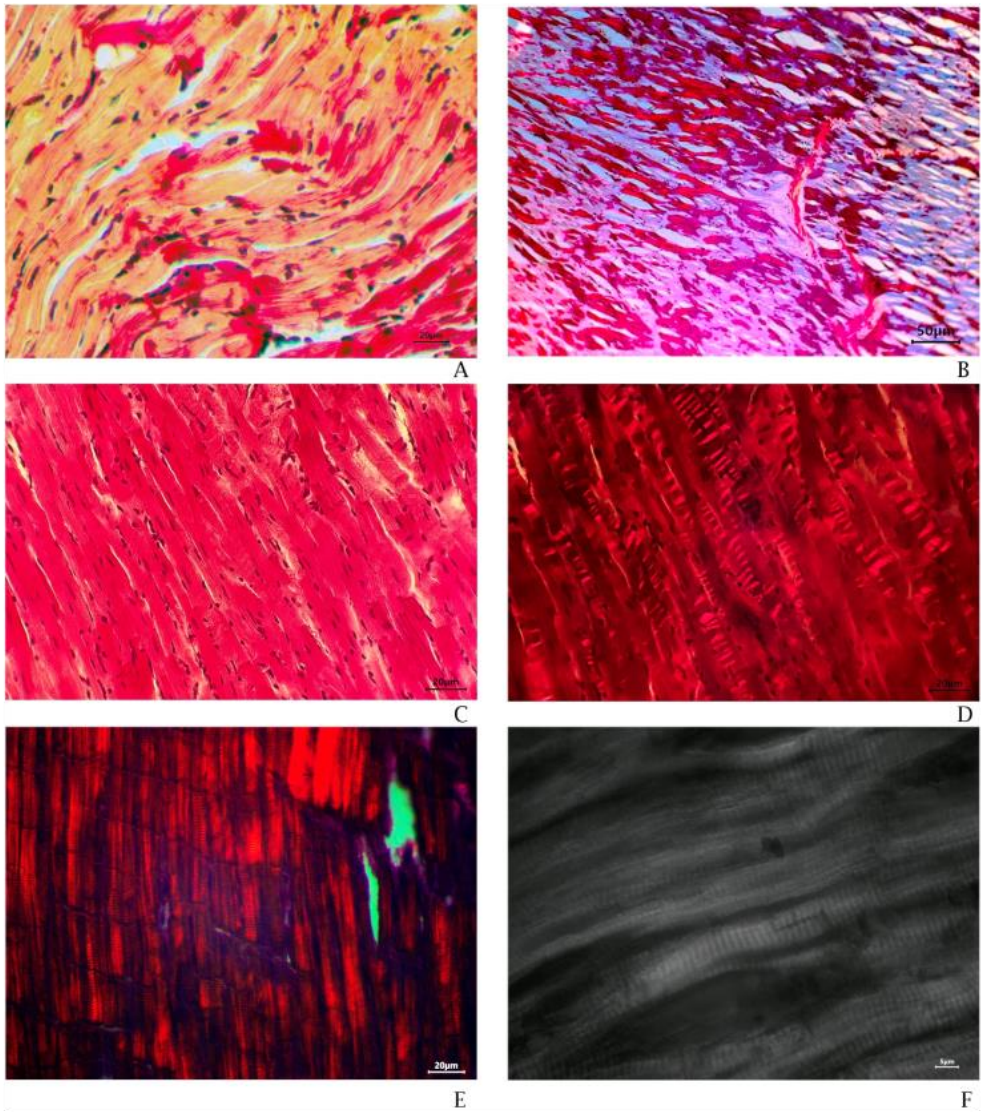

Figure 2. Myocardial micrographs under ischemic conditions: Lee staining, ob. 40x, oc. 20x, light microscopy (A), light microscopy with polarization, ob. 40x, oc. 20x (B), myocardial areas subjected to ischemia are stained red; basic fuchsin staining, light microscopy, ob. 40x, oc. 20x (C), light microscopy with polarization, ob. 40x, oc. 20x (D), sarcomeric hypercontraction seen; areas of intense anisortopia in individual cardiomyocytes, ob. 40x, oc. 20x (E), individual cardiomyocytes in shiemia, ob. 100x, oc. 20x (F)

The median value of sarcomer length is 1.86 (1.79; 1.92) μm in normal, 1.77 (1.66; 1.82) μm in respiratory arrest, and 1.55 (1.43; 1.67) μm in cardiac arrest. The size of the I-discs also decreased in the experimental groups. The median isotropic disc length for controls is 0.56 (0.45; 0.65) μm, and 0.44 (0.38; 0.57) μm for respiratory arrest, whereas it fell to 0.25 (0.22; 0.26) μm for cardiac arrest. At the same time, A-disks in median values for all groups are not characterized by the presence of significant differences.

4. CONCLUSION

The study showed that the degree of correlation between A-disk and I-disk sarcomere parameters strongly decreases during the development of various pathological processes associated with hypoxia. The relationship between disc lengths is significantly lower in the experiment with acute cardiac arrest, which can be characterized as a faster process of

myocardial damage compared to acute respiratory arrest, which may be associated with circulatory arrest, rapid blood deoxygenation and pronounced development of myocardial ischemia. The study of the above parameters of cardiomyocytes is of great importance for evaluation of ultrastructural changes of myocardium and can form the basis for evaluation of the degree of cardiac tissue damage by methods of machine learning and automated analysis using neural networks.

REFERENCES

[1] Schaper, J., "Ultrastructural changes of the myocardium in regional ischaemia and infarction," Eur. Heart J. 7, Suppl B:3-9 (1986).

[2] Berry, C. L., van der Walt, J., Wyse, R., "Sarcomere relaxation and ischaemic myocardial injur," Virchows Arch. A Pathol. Anat. Histol. 390(2), 205-10 (1981).

[3] Gerdes, A. M., Capasso, J. M., "Structural remodeling and mechanical dysfunction of cardiac myocytes in heart failure," J. Mol. Cell Cardiol. 27(3), 849-56 (1995).

[4] Piavchenko, G., Kozlov, I., Dremin, V., Stavtsev, D., Seryogina, E., Kandurova, K., Shupletsov, V., Lapin, K., Alekseyev, A., Kuznetsov, S., Bykov, A., Dunaev, A., Meglinski, I., "Impairments of cerebral blood flow microcirculation in rats brought on by cardiac cessation and respiratory arrest," J. Biophotonics 14 (12), e202100216 (2021).