Research Article

Analysis of the influence of chronic heart failure type on changes in the microelement composition of myocardial tissue in patients of senile age

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ABSTRACT

Aim: Conduct a comparative analysis of the content and distribution of macroelements (Ca2+, K+, Mg2+, and Na+) in myocardial tissue according to autopsy data in elder patients with CHF, depending on the LV EF. **Materials and Methods:** Methods of optical plasma emission spectrometry of parallel action with inductively coupled plasma-9000 and scanning electron microscopy (FEI Nova Nano scanning electron microscope) were used to conduct a comparative analysis of the content of macronutrients in the tissue of myocardial autopsy of the patients of senile age with chronic heart failure depending on the degree of myocardial contractility - the ejection fraction of the left ventricle. **Results:** According to the created profiles of maps of macronutrient tissue composition and calculations of heterogeneity, it was found that changes in myocardial tissue of senile patients with systolic dysfunction were represented by significant differences in the heterogeneity of macronutrient distribution (Ca2+, K+, Mg2+, and Na+), while statistically significant differences in the absolute values of macronutrient content in cardiomyocytes were absent. **Conclusion:** Thus, areas of increased Ca2+ content of Group U1 indicate a local calcium overload of CMC, which results in local contractile dysfunction of myocardial tissue. This statement is reasoned by the presence of dyssynchrony confirmed by the EchocG method in patients of this group.

KEY WORDS: Chronic heart failure, Heart the content of macroelements, Mapping of the trace element composition of heart tissue, Scanning electron microscopy

INTRODUCTION

At present, cardiovascular diseases (CVD) play a crucial role in the evolution of general mortality in Russia. Despite significant therapeutic advances in the treatment of patients with chronic heart failure (CHF), the prevalence and mortality from this syndrome remain high. CHF is characterized by a gradual loss of inotropic left ventricular function (LV) and ejection fraction (EF), dilatation and thinning of the LV wall, increased peripheral vascular resistance, dysregulation of fluid homeostasis, neurohumoral and cytokine activation, and an increase in electrophysiological instability of myocardium.[1] There is ample evidence that CHF develops as two different syndromes: Systolic and diastolic heart failure. To date, according to the degree of reduction of the contractile function of the myocardium of the LV, CHF is classified into heart failure with low EF (LEF) (<40%) (LCHFEF), CHF with intermediate EF (IEF) (from 40% to 49%) (iCHFEF), and CHF with preserved EF (PEF) (50 % and more) (pCHFEF), while in healthy individuals at physiological rest the normal value of EF is considered to be 50–75%, with increase up to 80–85% during physical exercises in healthy people.[2]

Cardiomyocytes (CMC) in diastolic and systolic heart failure are fundamentally different at both the structural and functional levels. This is observed in complex multifactor processes that represent the mobilization of a whole set of compensatory mechanisms.[3,4] However, the concept of the formation of pathophysiological and pathological mechanisms of progression of CHF remains poorly understood. Disruption of the electrical and mechanical functions of the myocardium is one of the reasons for the decrease in LV inotropic ability and, as a consequence, the decrease in EF. LV synchronism has an important effect on its effectiveness. Delayed electrical activation and a weak link between excitation and contraction leads to a dispersion of regional mechanical activation, known as intraventricular dyssynchrony,

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which creates conditions for a progressive course of stretching of the contracture segments, a change in the heart geometry and dilatation of the LV cavity, which is one of the leading mechanisms of CHF progression.[5] The cascade of these mechanisms has an additional negative effect not only on the extracellular matrix but also on the intracellular elemental composition of CMC, causing changes in its elemental composition.[6-8] Calcium overload is recognized as one of the important factors of lowering the mechanical activity of CMC. It is known that reducing the speed of the Na^+-K^+ pump can cause calcium overload, since it contributes to the accumulation of sodium ions in CMC and, as a consequence, an increase in the exchange of Na^+-Ca^{2+}, which, in turn, leads to the accumulation of calcium ions.[9] The studies of mathematical modeling of rhythm disturbances showed that the modes of Na^+-K^+, Na^+-Ca^{2+}, и Ca^{2+}-Mg^{2+} pumps play an important role, which makes significant and relevant the task of comparative analysis of experimental mapping data on the content and distribution of macronutrients in myocardial tissue in patients with CHF depending on the state LV contractile function.

Objective

Conduct a comparative analysis of the content and distribution of macroelements (Ca^{2+}, K^+, Mg^{2+}, and Na^+) in myocardial tissue according to autopsy data in elder patients with CHF, depending on the LV EF.

MATERIALS AND METHODS

The research was carried out on the basis of the scientific educational and innovation center "Nanostructured Materials and Nanotechnologies," Belgorod State National Research University, the resource center "Development of Molecular and Cellular Technologies," St. Petersburg State University, St. Joasaph Belgorod Regional Clinical Hospital, Pathoanatomical Department of Prof. A. I. Meshchaninov Municipal Clinical Hospital of Emergency Medicine, and Kharkiv Medical Academy of Postgraduate Education.

To study the concentration and distribution of the macroelements (Ca, K, Mg, and Na) in the myocardial tissue, autopsies of 27 samples were taken. The resulting material was divided into three groups: The first group (U1) consisted of patients with LCHFEF (included in the "heart transplantation" protocol and died of terminal heart failure), whose mean EF was 27.87 ± 7.6 (27.9 [18.04–35.13]) (n = 10). The second group (U2) included patients with pCHFEF (died from a hemorrhagic stroke on the background of arterial hypertension) whose mean EF was 61.54 ± 6.3 (61.55 [50.04–67.24]) (n = 10). Group U3 (control group) is represented by autopsy of the myocardial tissue of healthy individuals without CVD, who died in road traffic accidents (n = 7). The study of the macroelement composition of the LV myocardium tissues was carried out using an optical plasma emission spectrometer of parallel action with inductively coupled plasma (ISP-OES) inductively coupled plasma-9000. Macroelement composition was mapped using FEI Quanta Nova Nano scanning electron microscope.[10-12] Statistical processing of mapping of the macroelements was carried out using the MATLAB 2017R (Mathworks) and ImageJ (National Institutes of Health) software package. To determine the integral value of heterogeneity, a map profile was used. The graph on the abscissa axis is represented by the "pixel" coordinate, on the ordinate axis by the vertical averaged intensity of the macroelement. The magnitude of the heterogeneity (v) was calculated as the ratio of the maximum peak of the intensity of the macroelement to the width at half maximum of the peak (FWHM - full width half maximum) on the profile of the distribution of macroelements in the myocardial tissue. The localization of the heterogeneity of the content of macroelements was studied using mutual correlation. For statistical processing of the obtained clinical data, Statistica 6.0 software package was used with the median test. The indicators are presented as a median (Me) with an interquartile range of 25% and a 75% percentile (Me [Me(n)–Me(i)]). A comparative analysis was performed using the non-parametric Mann–Whitney U-test. Differences were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

The results of measuring the concentration of the macroelements in the LV myocardial tissue in Groups U1, U2, and U3 using the ISP-OES method are presented in Tables 1 and 2, respectively. Statistically significant differences of calcium and magnesium were determined in both groups of pathology (U1 and U2) compared with the control group (U3); however, no significant differences in the content of Ca^{2+}, K^+, Mg^{2+}, and Na^+ were found between the Groups U1 and U2. The mapping of the myocardial tissue showed that the distribution of macronutrients is conditionally homogeneous in the patients of Group U2. Localization of calcium in the lower part of the map is a crystal of $CaCl_2$ salt, placed in the field of view for the purpose of positive control and assessment of the resolution of the method. The fluctuation of the intensities was within the standard error of measurement of 15 units. When mapping the macroelement composition of myocardial tissues in a patient of Group U1, an uneven distribution of Ca^{2+}, K^+, Mg^{2+}, and Na^+ was found. Regions of heterogeneity have a complex shape, and their number does not exceed ten.

To characterize the heterogeneity of distribution, profiles of maps of the macroelemental composition of myocardial tissues were constructed, and the heterogeneity values were calculated. Thus, using the example of the case of a patient of Group U2, the profile was easily approximated by a horizontal line, which indicates a uniform distribution. The value v was 0.03.

Table 1: A comparative analysis of the concentration of macronutrients in the Group U1, U2 with the control group

Indicator	U1			U2			U3			Level of significance $P<0.05$	
	1			2			3			1–3	2–3
	Minimum	Maximum	Median	Minimum	Maximum	Median	Minimum	Maximum	Median		
Calcium	0.6120	2.0	1.235	0.920000	1.70000	1.22000	1.63000	3.12000	1.86000	0.012049	0.005946
Magnesium	0.8350	1.4	1.310	1.130000	1.58000	1.32000	1.56000	2.70000	2.15000	0.002694	0.003619
Iron	0.6050	0.8	0.692	0.459000	0.82100	0.68200	0.56900	1.06000	0.72800	0.500560	0.381733
Potassium	7.2400	13.2	11.700	8.450000	16.2000	12.70000	10.2000	15.70000	11.30000	1.000000	0.736585
Sodium	7.7000	12.6	10.505	8.720000	14.6000	11.50000	4.71000	13.50000	8.43000	0.244624	0.117898

A patient of Group U1 with a low LV EF had pronounced peaks in the profile. Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+} had 2 peaks, which were on the coordinate interval (300, 600) and (800, 1000). In Mg and Na, the peaks were located on the interval of coordinates (800, 1000) and a gap was noted in the interval (600, 800). It can be argued that this indicates a high heterogeneity of the distribution of macroelements in the LV myocardium tissue.

When mapping the content of macronutrients, there is a need to compare maps of the content of macronutrients. We created the maps of macronutrient contents as two-dimensional brightness functions (discrete two-dimensional signal intensity matrices). Representing one map as a function (multidimensional vector) F and the second map as a function (multidimensional vector G) the correlation value was obtained (F, G) using the formula:

$$(F,G)=\sum F(i)\times G(i)$$

This value is the scalar product of two functions. The following value was used as a measure between the maps:

$$m(F,G)=\frac{(F,G)}{|F|\times|G|}$$

When dealing with large data arrays (the dimension of the map is 1000 × 1000 elements on average), a convolution operation is used. According to the definition, the convolution of two functions F and G is the function FxG:

$$F\otimes G(t)=\sum_{t=-\infty}^{\infty}F(i)\times G(t-i)$$

Then, under the condition G ‘(t) = G (-t), an expression was obtained for the correlation of two images:

$$m(F,G)=F(t)\otimes G'(t)$$

Next, applying the Fourier transform (F), the following was defined:

$$F\otimes G'(t)=BFT(FFT(F)\otimes FFT(G'))$$

Where FFT is the operation of the direct Fourier transform, BFT is the operation of the inverse Fourier transform. The expression obtained above allows us to calculate the mutual correlation of two maps since the cross-correlation function is related to the convolution function by the relation:

$$F(t)*G(t)=F(t)\otimes G'(-t)$$

Using the mathematical method described above allowed us to measure the local overload areas of the CMC by a specific macroelement and localize this area. Hence, when mapping Ca^{2+} in the field of

Table 2: A comparative analysis of the concentration of macronutrients in the U1 and U2 Groups

Indicator	U1			U2			Level of significance $P<0.05$
	Median	Minimum	Maximum	Median	Minimum	Maximum	1–2
Calcium	1.235	0.6120	2.0	1.2200	0.9200	1.700	0.845252
Iron	0.692	0.6050	0.8	0.6820	0.4590	0.821	0.807250
Potassium	11.700	7.2400	13.2	12.7000	8.4500	16.200	0.204560
Magnesium	1.310	0.8350	1.4	1.3200	1.1300	1.580	0.329115
Sodium	10.505	7.7000	12.6	11.5000	8.7200	14.600	0.379776

measurement of the tissue of a patient of the Group U2, this approach made it possible to detect and determine the degree of heterogeneity on a scale from 0 to 1 with different criteria for specifying a heterogeneity search. It should also be noted that the degree of heterogeneity ν measured by different methods has the same values and if the first provides an integral indicator of heterogeneity, then the second provides its localization.

The presence of areas of the high content of Ca^{2+}, K^{+} and low content of Mg^{2+}, Na^{+} on the map confirms the hypothesis of a strong uneven distribution of macroelements in the LV myocardium tissue as a result of local calcium overload and impaired operation of ion pumps in patients with low EF and as a result of local violation of the electromechanical conjugation in the myocardium, which is the cause of the development of contractile dysfunction of the LV myocardium.

CONCLUSION

All cells in the human body are similar in chemical composition; they include both inorganic and organic substances.[6,7] Patients with CHF undergo significant changes in their membrane processes. Since the content of K^{+} decreases with the progression of CHF, it affects the permeability of the membrane. Ca^{2+} accumulates in the tissue, which is reflected in the contractile function of the LV myocardium. Calcium and potassium dysmetabolism with a subsequent lengthening of the action potential and an increase in the dispersion of the repolarization process leads to disruption of rhythm and conductivity.[10,11] An increase in intracellular Ca^{2+} concentration is not balanced by an increase in the activity of the Na^{+}/Ca^{2+} pump, an overload of Ca^{2+} in diastole can lead to postdepolarization, trigger activity and ultimately to the development of life-threatening ventricular arrhythmias.[12-15] Magnesium is a universal regulator of energy, plastic, electrolyte exchanges, and a natural calcium antagonist. It contributes to the fixation of K in the cell and provides polarization of the cell membranes, thereby controlling the normal functioning of the myocardial cell at all levels, including regulating the contractility of the myocardium. The nature of the action of potassium ions on the heart is similar to the effect of excitation of the vagus nerves, and the action of calcium ions - with the effect of stimulation of sympathetic nerves.[16-18]

Thus, our study found that in both cases the integral content of macroelements Ca^{2+}, K^{+}, Mg^{2+}, and Na^{+} is within the standard deviation $\sigma = 0.61$, which is confirmed by the absence of statistically significant differences between the Groups U1 and U2 ($P > 0.05$). The mapping of the content of macronutrients showed significant differences in the homogeneity of the distribution of macronutrients in the LV myocardium tissue. Thus, areas of increased Ca^{2+} content of Group U1 indicate a local calcium overload of CMC, which results in local contractile dysfunction of myocardial tissue. This statement is reasoned by the presence of dyssynchrony confirmed by the EchocG method in patients of this group. In other words, in the absence of statistically significant differences in the absolute values of the content of macroelements, the mapping of the macroelemental composition of myocardial tissues makes it possible to identify and interpret the existing differences in the local uneven content of chemical elements.

REFERENCES

1. Dorn GW, Molkentin JD. Manipulating cardiac contractility in HF: Data from mice and men. Circulation 2004;109:150-8.
2. Meta-analysis Global Group in Chronic Heart Failure (MAGGIC). The survival of patients with heart failure with preserved or reduced left ventricular ejection fraction: An individual patient data meta-analysis. Eur Heart J 2012;33:1750-7.
3. Kaliuzhin VV, Tepliakov AT, Vecherskii II, Riazantseva NV. Pathogenesis of chronic heart failure: Changing the dominant paradigm. Bull Siberian Med 2007;4. Available from: http://www.cyberleninka.ru/article/n/patogenez-hronicheskoy-serdechnoy-nedostatochnosti-izmenenie-dominiruyuschey-paradigmy. [Last accessed on 2017 Nov 23].
4. Liu L, Wu J, Kennedy DJ. Regulation of cardiac remodeling by cardiac Na(+)/K(+)-Atpase isoforms. Front Physiol 2016;9:382.
5. Spragg DD, Kass DA. Pathobiology of left ventricular dyssynchrony and resynchronization. Prog Cardiovasc Dis 2006;9:26-41.
6. Osipova OA, Nagibina AI, Komisov AA, Petrova GD, Shekhovtsova LV, Vlasenko MA, *et al*. Pathomorphological mechanisms of the regulation of the formation of myocardial fibrosis in patients with chronic heart failure against the background of coronary heart disease. J Heart Fail 2016;17:357-64.
7. Cherniavskiĭ AM, Levicheva EN, Loginova II, Kamenskaia OV, Okuneva GN, Volkov AM, *et al*. Heart failure and imbalance of chemical elements in myocardium of patients with ishemic heart disease. Kardiologiia 2011;51:15-21.
8. Despa S, Bers DM. Functional analysis of Na+/K+-ATPase isoform distribution in rat ventricular myocytes. Am J Physiol Cell Physiol 2007;293:321-7.
9. Bers DM. Excitation-Contraction Coupling and Cardiac

Contractile Force. 2nd ed. Dordrecht Boston London: Kluwer Academic Publishers; 2001. p. 427.
10. Nagata T. X-ray microanalysis of biological specimens by high voltage electron microscopy. Prog Histochem Cytochem 2004;39:185-319.
11. Karaskov AM, Kamenskaia OV, Levicheva EN. Chemical imbalances in the progression of heart failure in patients with ischemic heart disease. Heart Fail J 2011;2:86-90.
12. Komisov AA, Osipova OA, Shepel RN, Drapkina OM, Osipov PG, Plaksina KG. Development of quantitative mapping methodology for macroelement composition of the heart tissue using the nanotechnology approaches, namely, a scanning transmission microscope. Russ J Cardiol 2016;12:18-22.
13. Zaugg CE, Buser PT. When calcium turns arrhythmogenic: Intracellular calcium handling during the development of hypertrophy and heart failure. Croat Med J 2001;42:24-32.
14. Shattock MJ, Ottolia M, Bers DM, Blaustein MP. Na+/Ca2+ exchange and Na+/K+-ATPase in the heart. J Physiol 2015;593:1361-82.
15. Goch A. Concentration of elements in plasma of patients with essential hypertension. Pol Arch Med Wewn 2005;114:947-52.
16. Palmer BM, Vogt S, Chen Z. Intracellular distributions of essential elements in cardiomyocytes. J Struct Biol 2006;155:12-21.
17. da Silva F, Williams RJ. The Biological Chemistry of the Elements: The Inorganic Chemistry of Life. Oxford: Clarendon; 1991. p. 561.
18. Zang WJ, Yu XJ, Zang YM. Ca2+ sparks evoked by depolarization of rat ventricular myocytes involve multiple release sites. Acta Pharmacol Sin 2003;24:555-62.

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