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INFLUENCE OF PHOSPHOCREATINE ON MITOCHONDRIAL ACTIVITY IN PATIENTS WITH ANGINA PECTORIS

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Abstract

The research of individual sensitivity of leukocytes' mitochondria of 56 patients with a stable angina pectoris to introduction of phosphocreatine in tests in vitro have been made. It has been found out two variants of reaction - in the form of activation (in 49 % of patients) or oppressions of mitochondria functional activity (in 51 % of patients). Sensitivity of mitochondria to introduction of phosphocreatine has appeared dependent on an individual condition of bodies-targets, smoking factor, neurohumoral regulation, initial mitochondrial activity and degree of damaged DNA in patients. It testifies about the necessity of personification of phosphocreatine appointment in complex treatment of patients with angina pectoris.

Key words: phosphocreatine, a stable angina pectoris, mitochondria

Introduction.

The high indicators of prevalence and death rate from ischemic heart disease (IHD), despite the accepted standards of pharmacotherapy, cause necessity of modernisation of the accepted schemes of treatment [1]. The accepted standards of a stable angina pectoris treatment by preparations from groups of antiagregants, anticoagulants, beta-adrenoblokers, statins, inhibitors of angiotenzin converting enzyme, nitrates, calcium antagonists have high level of proof, however do not provide to the full efficiency of treatment [2].

One of the promising directions of improving the pharmacotherapy of IHD is considered cardiocytoprotection [3]. A point of the action of such preparations are mitochondria [4]. Metabolic-line drugs are widely used by doctors of the countries in post-Soviet space as ones that enhance the patients' life quality, whereas in Europe they are administered

not more than in 1% of cases due to the lack of evidence of their impact on the patients' life expectancy [5]. Works of some authors specify about the limited efficiency of the given group of preparations [6, 7]. There was an ambiguous relation to them in clinical medicine - from enthusiastic acceptance to absolute nonacceptance. Personification of appointment of preparations of a metabolic number in complex treatment of patients with IHD can become the possible decision of the specified problem.

The purpose of the present research was definition of individual mitochondrial sensitivity to introduction of phosphocreatine in patients with a stable angina pectoris.

Methods.

It has been spent randomized open controlled clinical research of 56 patients with a stable angina pectoris of various functional classes at the age from 37 to 81 years. The majority of patients with angina pectoris had combined arterial hypertension (89.4 %), rhythm disturbance (24.4 %), atherosclerosis after myocardial infarction (48.8 %), chronic heart failure (94.4 %), and some of them had diabetes of II type (23.1 %). Middle age of patients was 59.26 ± 0.74 years.

It had been carried out the general clinical methods of research, tool and laboratory, for the statement of the diagnosis and treatment of a stable angina pectoris according to recommendations of the European Society of Cardiology (2013) [2]. Morphofunctional state of the myocardium and coronary vessels was assessed by Doppler echocardiography methods, coronary angiography and electrocardiography. Availability of smoking factor in each patient was evaluated. Kidney function was evaluated on the base of creatinine determination in blood and microalbuminuria using biochemical methods. Condition of DNA of patients' white blood cells was assessed using the method of DNA comets in terms of the index of DNA comets and the number of damaged cells [8]. Specific methods of the research of neurohumoral profile of patients were carried out also by definition of the level of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase (iNOS) in erythrocytes by the method of immunoassay on BioRad device by means of sets of reactants of "BiochemMac" firm. Research of individual influence of phosphocreatine on mitochondria of each person were carried out by the technique developed by us in tests in vitro [9] with the help of confocal laser scanning microscopy on Nikon Eclipse Ti device under special Nikon C1 program [10].

The obtained data were statistically analyzed. Differences were analyzed by Student and Mann-Whitney test. P<0.05

was considered as statistically significant. The character of interrelation between factors was estimated by regression analyze and graphic three-dimensional plotting method.

Basic part (results and discussion)

During the research of individual sensitivity of leukocytes' mitochondria of patients with a stable angina pectoris to phosphocreatine introduction it has been found out two variants of reaction - in the form of activation (at 27 [49 %] patients) or oppressions of mitochondrial functional activity (at 29 [51 %] patients).

It was carried out the comparative analysis between groups of patients with various variants of mitochondrial reaction on phosphocreatine introduction, and it was received a number of authentic differences (Table 1 and 2.).

Table 1. Influence of phosphocreatine on the person's mitochondria

| Indicator | Mitochondrial activation, n=27 | Mitochondrial oppression, n=29 |
|--|--------------------------------|--------------------------------|
| Smoking: the number of cigarettes per day | 1.90±0.90** | 5.48±1.86** |
| Microalbuminuria, g/l | 0.05±0.03 | 0.16±0.03 |
| Creatinine of blood, mmol/l | 81.64±3.64** | 97.17±3.44** |
| Echocardiography: end-systolic volume of the left ventricle, ml | 49.18±7.49* | 78.31±10.83* |
| Echocardiography: end-diastolic volume of the left ventricle, ml | 120.40±8.31 | 146.60±11.84 |
| Echocardiography: left ventricular ejection fraction, % | 62.28±2.45* | 53.22±3.22* |
| Electrocardiogram: the displacement of the sigment ST on ...mm | 0.83±0.15* | 1.57±0.32* |
| Coronaroangiography: level of anterior interventricular artery stenosis, % | 32.00±8.41* | 67.86±9.93* |
| Coronaroangiography: the average degree of stenosis, % | 20.22±5.99 | 37.05±6.79 |
| level of endothelial nitric oxide synthase (eNOS) in erythrocytes, pg/ml | 1486.58±554.96 | 454.51±104.04 |
| level of inducible nitric oxide synthase (iNOS) in erythrocytes, ng/ml | 8.94±6.30* | 30.83±3.38* |
| DNA: negative control, the number of damaged cells,% | 2.83±1.14* | 11.33±2.96**% |

The note. Differences were analyzed by Student test: *p <0.05; ** p <0.01; *** p <0.001.

Table 2. Influence of phosphocreatine on the person's mitochondria.

| Indicator | Mitochondrial activation, n=27 | Mitochondrial oppression, n=29 |
|--|---------------------------------------|---------------------------------------|
| Echocardiography: a violation of local contractility, points | 0.26/ 0.00 (0.00; 0.00)** | 1.13/ 1.00 (0.00; 2.75)** |
| Intensity of mitochondrial fluorescence (colouring – pyren), relative unit | 281.86/ 261.80 (181.10; 372.80)*** | 450.64/ 434.10 (340.45; 517.65)*** |

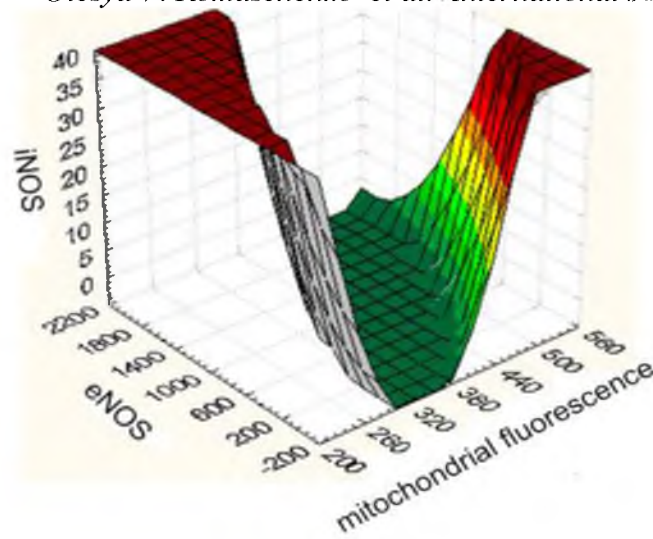
| | | |
|--|-----------------------------|-----------------------------|
| DNA: negative control, DNA comet index | 0.04/ 0.06 (0.02; 0.46)* | 0.15/ 0.10 (0.03; 0.40)* |
|--|-----------------------------|-----------------------------|

The note. Numerator - an average arithmetic, a denominator - a median, 25 % and 75 % quartile. The differences were analyzed by Mann-Whitney test: ** p <0.01; *** p <0.001.

It has found out following conditions of mitochondrial activation under action of phosphocreatine: the absence of smoking factor, normal renal function, intact morpho-functional state of the myocardium according to echocardiography, a small degree of myocardial ischemia, high level of endothelial nitric oxide synthase and low level of inducible nitric oxide synthase in the lysate of cells, initially low activity of mitochondria and the low degree of damaged DNA. Phosphocreatine is capable to oppress mitochondria in tests in vitro in the presence of the following conditions: presence of smoking factor, tendency to renal dysfunction, enlargement of the heart cavities, the tendency to reduce left ventricular ejection fraction, more severe myocardial ischemia, low level of endothelial nitric oxide synthase and high level of inducible nitric oxide synthase in the cell lysate, initially high activity of mitochondria and high degree of damaged DNA.

Phosphocreatine is the key component in the intracellular system of energy buffering and transport from the site of energy production to the site of energy utilization to ensure that supply meets the high and dynamic demands of the heart [11, 12]. Particularly, phosphocreatine makes energy of phosphoryl bonds of adenosinetriphosphate available at the myofibrillar creatine kinase that allows myocardium contraction [11, 12]. A consistent experimental evidence was found that in chronic heart failure and acute ischaemia, there are both gradual loss of component of the energy transport system and reduction in its activity [13, 14, 15]. Supplementation with phosphocreatine was, therefore, suggested as potentially beneficial in patients with acute and chronic myocardial ischaemic injury [16, 17]. Several studies demonstrated that phosphocreatine use has protective effects in different clinical settings, including cardiac surgery, myocardial infarction, chronic heart failure, skeletal muscle hypotrophy and cerebral ischaemia [16]. Meta-analysis of recent publications showed the possibility of phosphocreatine influence at the endpoints - patient mortality and survival rates [18].

However, in our study the possibility of multidirectional effect of phosphocreatine on mitochondrial activity was revealed, which depends on the initial state of energy exchange, neurohumoral regulation and target organs' state. In particular, according to our data, the functional state of neurohormonal stress-limiting nitric oxide system is reflected in the functional activity of mitochondria (Drawing 1.).



Drawing 1. The relationship between level of endothelial nitric oxide synthase (eNOS) in erythrocytes (pg/ml), level of inducible nitric oxide synthase (iNOS) in erythrocytes (ng/ml) and intensity of mitochondrial fluorescence (colouring - pyren, relative unit).

In drawing 1 it is visible, that the highest tension of neurohumoral stress limiting system of nitric oxide as high concentration of endothelial NO synthase provides efficient functioning of active mitochondria. Reducing the concentration of endothelial NO synthase and increased concentration of inducible NO synthase is accompanied by a breach of mitochondrial function either towards reducing their activity or to the side of excessive activation. The nature of the relationship is non-linear, periodic, described by a polynomial line of 6th degree: between the iNOS and eNOS regression equation is $y = 4E-16x^6 - 3E-12x^5 + 6E-09x^4 - 6E-06x^3 + 0.0026x^2 - 0.2811x + 9.4157$, $R^2 = 0.8869$; between eNOS and the magnitude of mitochondria fluorescence is $y = 1E-11x^6 - 3E-08x^5 + 3E-05x^4 - 0.0146x^3 + 3.2412x^2 - 300.74x + 7576$, $R^2 = 0.1016$; between the iNOS and fluorescence of mitochondria is $y = 3E-11x^6 - 8E-08x^5 + 9E-05x^4 - 0.0477x^3 + 14.495x^2 - 2303.7x + 149493$, $R^2 = 0.787$. The degree of reliability of relationships is very high, as evidenced by the coefficients of determination.

The found out ability of phosphocreatine to make various impact on mitochondrial activity in patients, in our opinion, explains the possibility of ambiguous efficiency of these drug and testifies the necessity of personification of phosphocreatine appointment in complex treatment of patients with angina pectoris.

Resolution.

Phosphocreatine is capable to make various impact on mitochondrial activity in patients depending on an individual condition of their bodies-targets, smoking factor, neurohumoral regulation, initial mitochondrial activity and degree of damaged DNA, that testifies the necessity of personification of phosphocreatine appointment in complex treatment of patients with a stable angina pectoris.

Conclusions:

1. Phosphocreatine in tests in vitro has shown the ability both to activate, and to oppress mitochondria in patients with a stable angina pectoris.
2. Conditions of mitochondrial activation under influence of phosphocreatine are: the absence of smoking factor, normal renal function, intact morpho-functional state of the myocardium according to echocardiography, a small degree of myocardial ischemia, high level of endothelial nitric oxide synthase and low level of inducible nitric oxide synthase in the lysate of cells, initially low activity of mitochondria and the low degree of damaged DNA.
3. Conditions of mitochondrial oppression under influence of phosphocreatine are: presence of smoking factor, tendency to renal dysfunction, enlargement of the heart cavities, the tendency to reduce left ventricular ejection fraction, more severe myocardial ischemia, low level of endothelial nitric oxide synthase and high level of inducible nitric oxide synthase in the cell lysate, initially high activity of mitochondria and high degree of damaged DNA.

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