КАРДИОЛОГИЯ
CARDIOLOGY

LEUKOCYTE AS AN ADEQUATE MODEL FOR STUDYING CHANGES IN ENERGY METABOLISM IN HEART CELLS UNDER THE INFLUENCE OF CARDIOCYTOPROTECTORS IN MYOCARDIAL ISCHEMIA

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ABSTRACT

The aim of this study *was to determine the possibility of studying the nature of the influence of cardiocytoprotectors on energy metabolism in cardiomyocytes using a model of human peripheral blood leukocytes.*

Materials and methods. *Sixty Wistar rats were divided into groups: 1) intact rats; 2) rats with experimental myocardial ischemia; 3) rats with myocardial ischemia, which were injected with cardiocytoprotector – trimetazidine, 4) meldonium, 5) cytoflavin and 6) ethoxydol.*

Animals were taken out of the experiment 10 days after the administration of drugs by decapitation. The activities of pyruvate dehydrogenase and citrate synthase were determined in mitochondria of myocardial homogenates and in mitochondria of leukocytes by spectrophotometric methods.

Results. *The decrease in pyruvate dehydrogenase and citrate synthase activity in cardiomyocytes and in leukocytes were revealed in case of myocardial ischemia modeling. The introduction of cardiocytoprotectors led to the activation of these enzymes both in heart cells and in blood leukocytes. Direct positive correlations were obtained between the activity of pyruvate dehydrogenase in the mitochondria of cardiomyocytes and in the mitochondria of leukocytes ($r = 0.811$; $p < 0.0001$); between citrate synthase activity in the mitochondria of cardiomyocytes and in the mitochondria of leukocytes ($r = 0.909$; $p < 0.0001$).*

Conclusion. *Changes in energy metabolism in blood leukocytes under the influence of cytoprotectors reflect similar changes occurring in heart cells.*

Key words: *leukocyte, cardiomyocyte, energy metabolism, cytoprotectors, myocardial ischemia*

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ЛЕЙКОЦИТ КАК АДЕКВАТНАЯ МОДЕЛЬ ИЗУЧЕНИЯ ХАРАКТЕРА ИЗМЕНЕНИЙ ЭНЕРГЕТИЧЕСКОГО ОБМЕНА В КЛЕТКАХ СЕРДЦА ПОД ВЛИЯНИЕМ КАРДИОЦИТОПРОТЕКТОРОВ ПРИ ИШЕМИИ МИОКАРДА

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РЕЗЮМЕ

Целью данного исследования *явилось определение возможности изучения характера влияния кардиоцитопротекторов на энергетический обмен в кардиомиоцитах на модели лейкоцитов периферической крови человека.*
Материалы и методы. *Шестьдесят крыс линии Вистар были разделены на группы: 1) интактные крысы; 2) крысы с экспериментальной ишемией миокарда; 3) крысы с ишемией миокарда, которым вводили кардиоцитопротектор – триметазидин, 4) мельдоний, 5) цитофлавин и 6) этоксидол. Животных выводили из эксперимента через 10 суток после введения препаратов методом декапитации. Активность пируватдегидрогеназы и цитратсинтазы определяли в митохондриях гомогенатов миокарда и в митохондриях лейкоцитов спектрофотометрическими методами.*
Результаты. *При моделировании ишемии миокарда выявлено снижение активности пируватдегидрогеназы и цитратсинтазы в кардиомиоцитах и в лейкоцитах. Введение кардиоцитопротекторов приводило к активации этих ферментов как в клетках сердца, так и в лейкоцитах крови. Получены прямые положительные корреляции между активностью пируватдегидрогеназы в митохондриях кардиомиоцитов и в митохондриях лейкоцитов ($r = 0,811$; $p < 0,0001$); между активностью цитратсинтазы в митохондриях кардиомиоцитов и в митохондриях лейкоцитов ($r = 0,909$; $p < 0,0001$).*
Заключение*. Изменения энергетического обмена в лейкоцитах крови под влиянием цитопротекторов отражают аналогичные изменения, происходящие в клетках сердца.*

Ключевые слова: *лейкоцит, кардиомиоцит, энергетический обмен, цитопротекторы, ишемия миокарда*

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INTRODUCTION

Cytoprotectors that affect energy metabolism in cardiomyocytes are included in the treatment standards for a number of diseases of the cardiovascular system: coronary heart disease, chronic heart failure [1, 2]. However, it is not possible to evaluate their direct influence on the energy processes in the mitochondria of myocardial cells in routine outpatient or inpatient practice without the use of invasive surgical methods for the purpose of myocardial biopsy. In this regard, we decided to study the possibility of assessing the effect of cardiocytoprotectors on energy processes in the mitochondria of myocardial cells according to the state of energy metabolism in peripheral blood leukocytes under experimental conditions.

Leukocytes are highly specialized, functionally different blood cells that implement specific and nonspecific cellular immunity in the body. According to a number of studies, the quantitative and qualitative composition of leukocytes in the blood can reflect, as in a mirror, the state of various organs and systems, including the myocardium [3, 4].

The energy status of the cell is provided by mitochondria, specialized organelles present in almost all cells of the body. The synthesis of high-energy adenosine triphosphate (ATP) molecules by oxidative phosphorylation is associated with many metabolic processes occurring in the mitochondrial matrix and on its membranes: transport of electrons and protons in the respiratory chain, pyruvate decarboxylation, citric acid cycle, beta-oxidation of fatty acids, etc., and, of course, associated with oxygen consumption [5].

An analysis of the literature data has shown that disorders of cellular energy, which is based on mitochondrial deficiency, lead to a wide range of diseases that are not limited to hereditary syndromes caused by mutations in mitochondrial DNA genes, but also include structural, biochemical defects in mitochondria, disorders of tissue respiration and decreased synthesis of ATP [6–8].

The most energy-intensive tissues include: nervous, muscular, cardiac [9]. In particular, cardiomyocytes contract throughout life and are absolute champions among cells of other tissues both in terms of the amount of ATP produced and the volume of oxygen consumed [10]. For effective work the heart muscle needs a full and timely delivery of oxygen and energy substrates [11]. Obviously under conditions of ischemia, there is a lack of oxygen supply and energy substrates, which justifies the need for the use of drugs that affect the energy metabolism in cardiomyocytes [11]. At the same time, in real clinical practice, there is no technical possibility to assess those changes in energy metabolism that occur under the influence of metabolic correctors inside cardiomyocytes. Therefore, it becomes necessary to study the indicators of energy exchange in a tissue that is easily accessible to people – blood. At the same time, the question of extrapolation of data obtained from blood tests to the myocardium, remains open.

In recent years, an increasing number of researchers, as a result of experiments, have come to unequivocal conclusions that the activity of enzymes of lymphocytes, as migrating cells, can reflect the state of the enzymatic status of all cell populations of the body [12–14]. In this regard, it is great scientific interest to study the nature of changes in the activity of key mitochondrial enzymes under the influence of cardiocytoprotectors both in cardiomyocytes and in peripheral blood leukocytes under conditions of experimental myocardial ischemia in order to determine the potential possibility of assessing the state of energy exchange in the heart in terms of energy exchange in blood leukocytes, which is the subject of this study.

The aim of this study was to assess the adequacy of the leukocyte model for studying the nature of changes in energy metabolism under the influence of cardiocytoprotectors in myocardial ischemia.

MATERIALS AND METHODS

The object of our study were 60 male Wistar rats aged 10 months, which were kept under standard vivarium conditions. The age of rats was chosen based on its correspondence to the average age of humans [15], when, according to the latest data, coronary heart disease, the subject of our study, most often manifests [16]. The following groups of animals were used: 1) intact rats (n = 10); 2) rats with experimental myocardial ischemia (n = 10); 3) rats with myocardial ischemia treated with 1-[(2,3,4-trimethoxyphenyl)methyl]piperazine (trimetazidine) (n = 10); 4) rats with myocardial ischemia, which were injected with trimethylhydrazinium propionate dihydrate (meldonium) (n = 10); 5) rats with myocardial ischemia treated with inosine + nicotinamide + riboflavin + succinic acid (cytoflavin) (n = 10); 6) rats with myocardial ischemia treated with ethylmethylhydroxypyridine malate (ethoxidol) (n = 10).

Chronic myocardial ischemia was modeled in animals using the method described by D.V. Gaman (2011): 0.1 ml of 0.1 % adrenaline solution was administered subcutaneously to rats daily for 7 days at a dose of 0.1 ml per 100 g of animal's weight. After completing the course of adrenaline, against the background of already formed myocardial ischemia, medications were administered. The dose of drugs, administered for therapeutic purposes, was calculated according to the formula of Yu.R. Rybolovlev, it was 1) for trimetazidine (pure dry matter from Sigma Aldrich, USA) – 0.5 mg/100 g of rat body weight in 2 ml of 0.9 % NaCl solution intragastrically 2 times a day, which is equivalent to the recommended dose of trimetazidine for human (35 mg 2 times a day orally); 2) for meldonium (Grindex, Latvia) – 0.03 ml/100 g of rat body weight in 1.5 ml of 0.9 % NaCl solution intravenously 1 time per day, which is equivalent to the recommended dose of meldonium for humans (5 ml intravenously 1 time per day); 3) for cytoflavin (Polysan, Russia) – 0.07 ml/100 g of rat body weight in 1.5 ml of 0.9 % NaCl solution intravenously 1 time per day, which is equivalent to the recommended dose for humans (10 ml intravenously by drop infusion in a dilution of 200 ml of 0.9 % NaCl solution 1 time per day); 4) for ethoxidol (Sintez, Russia) – 1.2 mg/100 g of rat body weight in 1.5 ml of 0.9 % NaCl solution intravenously 1 time per day, which is equiv-

alent to the recommended dose for humans (2 ml intravenously 1 time per day).

Animals were taken out of the experiment 10 days after the administration of drugs by decapitation. The heart was perfused with chilled 0.9 % NaCl solution. The preparation of myocardial homogenates and the isolation of mitochondria were carried out according to the method described by N.P. Meshkova, S.E. Severin (1979).

Cranial blood (10–12 ml) was used for research. Heparinized blood (20 IU of heparin per 1 ml) was mixed with a 0.3 % gelatin solution in a ratio of 1:5. The mixture in the test tube was defended for 40 minutes at an angle of 45° in a thermostat at a temperature of 37 °C until the erythrocytes precipitated. Leukocyte-rich plasma was collected. The isolated cells were washed by 2-fold centrifugation in Hank's solution at 1000 rpm for 5 minutes. Mitochondria were isolated from leukocytes by differential centrifugation (standard methods). In the mitochondria of the myocardial homogenate and leukocytes, the activities of citrate synthase and pyruvate dehydrogenase were determined by biochemical spectrophotometric analysis using proprietary methods described by F.E. Putilina and N.D. Yeshchenko [17, 18].

The work complied with bioethical standards for conducting experiments on animals for scientific purposes in accordance with Directive 2010/63/EU of the European Parliament and of the Council of the European Union of September 22, 2010, on the protection of animals used for scientific purposes, as well as in accordance with Guide for the care and use of laboratory animals, National academy (Washington, D.C., 1996). The work received approval from the Ethical Committee of Yaroslavl State Medical University (Protocol No. 58 dated November 11, 2022).

The materials were processed statistically. The belonging of the studied parameters to the normal distribution law was checked using the Shapiro – Wilk test. For all parameters, the values of the arithmetic mean, median, the 1st and the 3rd quartiles (Me (Q1; Q3)) were calculated. To compare groups of animals, the nonparametric Mann – Whitney U-test was used. The relationship between the factors was assessed by the methods of correlation analysis with the calculation of Spearman correlation coefficients, as well as by the method of multiple regression analysis with the calculation of determination coefficients. A 3D plotting method was used to depict a regression relationship between three variables. The software "Statistica" (StatSoft Inc., USA) was used.

RESULTS AND DISCUSSION

Modeling myocardial ischemia in rats led to a significant decrease in the activity of the main enzymes of energy metabolism – pyruvate dehydrogenase and citrate synthase, both in cardiomyocytes and in peripheral blood leukocytes, as shown in Table 1.

When modeling myocardial ischemia, a significant decrease in the activity of mitochondrial enzymes is observed both in cardiomyocytes and leukocytes. Pyruvate dehydrogenase activity decreased from 29.98/29.75 (29.16; 30.84) to 19.89/19.77 (19.10; 20.61) μmol NAD/min/mg protein ($p < 0.0001$) in cardiomyocytes and from 17.22/17.00 (16.91; 17.49) to 12.26/12.17 (11.88; 12.81) μmol NAD/min/mg protein ($p < 0.0001$) in leukocytes. Citrate synthase activity decreased from 4.06/4.03 (3.87; 4.21) to 2.06/2.07 (1.89; 2.21) nmol/min/mg protein ($p < 0.0001$) in cardiomyocytes and from 2.09/2.10 (1.95; 2.17) to 1.1/1.01 (0.97; 1.07) nmol/min/mg protein ($p < 0.0001$) in leukocytes.

The results obtained can be explained by the following biochemical features of the regulation of energy metabolism in cells.

Pyruvate dehydrogenase and citrate synthase are among the most important regulatory enzymes at the final stage of catabolism [5]. Coordinated control of the activity of these enzymes is carried out in various ways: by the availability of substrates, inhibition by reaction products, allosterically and by covalent modification [5]. The ratio of NADH/NAD and ATP/ADP also plays an important role in the regulation of the activity of these enzymes. With an increase in the concentration of NADH, the activity of regulatory enzymes decreases. This, in turn, leads to allosteric inactivation of pyruvate dehydrogenase and blocking of other NAD-dependent enzymes of the tricarboxylic acid cycle [19, 20]. Those, the rate of the Krebs cycle (tricarboxylic acids) under conditions of hypoxia and the inability to use reduced NADH equivalents decreases, which leads to the accumulation of acetyl-CoA in the mitochondrial matrix of cardiomyocytes, which stimulates pyruvate dehydrogenase protein kinase, converting it into an inactive form and, thereby limiting the formation of the itself from pyruvate and citrate, which allosterically inhibits the key enzyme of glycolysis – phosphofructokinase [19].

Citrate synthase is a regulatory enzyme of the Krebs cycle, catalyzes the key reaction of the cycle – the formation of citrate (citric acid). The regulation of the activity of this enzyme is also influenced by the ratio of the main modulators of ATP/ADP and NADH/NADH and intermediate metabolites of the common pathway of catabolism of substances: acetyl-CoA, which is a substrate of citrate synthase and palmitoyl-CoA [21]. During ischemia (hypoxia), there is a deficiency of the first, as a result of blocking the pyruvate dehydrogenase complex, and the accumulation of the second, as a result of a decrease in the process of beta-oxidation. This leads to a decrease in activity due to substrate deficiency and specific inhibition of citrate synthase by activated palmitic acid [11].

When analyzing the data in Table 1, attention is drawn to the difference in the activity of pyruvate dehydrogenase and citrate synthase in cardiomyocytes and leukocytes: in cardiomyocytes it is significantly higher. The most likely explanation for this fact is the direct dependence of enzyme activity on the concentration of the enzyme itself: with an increase in the number of enzyme molecules, the reaction rate increases continuously and in direct proportion to the amount of enzyme, because more enzyme molecules produce more product molecules. Therefore, the differences in the activity of pyruvate dehydrogenase and citrate synthase found by us in cardiomyocytes and pe-

TABLE 1

THE ACTIVITY OF MITOCHONDRIAL ENZYMES – PYRUVATE DEHYDROGENASE AND CITRATE SYNTHASE – IN CARDIOMYOCYTES AND IN BLOOD LEUKOCYTES DURING ADMINISTRATION OF CARDIOCYTOPROTECTORS IN RATS WITH SIMULATED MYOCARDIAL ISCHEMIA

Observation groups	Object of study			
	Cardiomyocytes		Blood leukocytes	
	PDH, μmol NAD/min/mg protein	CS, nmol/min/mg protein	PDH, μmol NAD/min/mg protein	CS, nmol/min/mg protein
Intact rats	29.98/29.75* (29.16; 30.84)	4.06/4.03* (3.87; 4.21)	17.22/17.00* (16.91 ;17.49)	2.09/2.10* (1.95; 2.17)
Rats with myocardial ischemia	19.89/19.77* (19.10; 20.61)	2.06/2.07* (1.89; 2.21)	12.26/12.17* (11.88; 12.81)	1.1/1.01* (0.97; 1.07)
Rats with myocardial ischemia + trimetazidine	23.09/23.05* (22.56; 23.85)	3.28/3.24* (3.13; 3.42)	14.10/13.99* (13.67; 14.53)	1.41/1.40* (1.33; 1.49)
Rats with myocardial ischemia + meldonium	26.31/26.42* (25.65; 26.88)	3.57/3.58* (3.46; 3.65)	15.25/15.29* (14.83; 15.66)	1.72/1.72* (1.66; 1.77)
Rats with myocardial ischemia + ethoxidol	24.37/27.13* (25.87; 28.15)	2.18/3.78* (3.64; 4.05)	13.49/16.40* (15.80; 17.50)	1.22/1.78* (1.65; 1.89)
Rats with myocardial ischemia + cytoflavin	33.24/33.66* (32.11; 34.10)	3.91/3.90* (3.78; 4.06)	15.91/15.83* (15.23; 16.63)	2.03/2.03* (1.93; 2.12)

Note. PDH – pyruvate dehydrogenase; CS – citrate synthase. Numerator – arithmetic mean, denominator – median (25%; 75% quartile). The significance of differences was assessed using the Mann – Whitney U test.
* – $p < 0.0001$, the significance of differences between the group with myocardial ischemia and other groups of animals.

ripheral blood leukocytes (according to Table 1) are probably due to the fact that mitochondria in heart cells occupy up to a third of the cytoplasm volume, while they are present in a smaller volume in leukocytes [6, 22].

When cardiocytoprotectors are administered to rats with simulated myocardial ischemia, their corrective effect on energy metabolism parameters is observed in the form of activation of pyruvate dehydrogenase and citrate synthase, however, without reaching the level of PDH and CS activity of intact rats (see Table 1). Moreover, the nature of changes in the activity of mitochondrial enzymes in heart cells and in blood cells turned out to be the same, despite the fact that cytoprotectors with different mechanisms of action were used.

So, trimetazidine (a derivative of piperazine-1-(2,3,4-trimethoxybenzyl) piperazine dihydrochloride) blocks the oxidation of free fatty acids, inhibiting the activity of 3-ketoacyl-CoA-thiolase (3-CAT), thereby reducing the formation of acetyl-CoA and NADH, promotes the unblocking of pyruvate dehydrogenase and switching of myocardial energy metabolism to glucose utilization through more "profitable" energy production – glycolysis (anaerobic breakdown of glucose to lactate) and oxidative decarboxylation (aerobic oxidation in the Krebs cycle) [23]. The excess of free fatty acids entering cardiomyocytes under these conditions is directed to the synthesis of phospholipids, which determines the membrane-protective properties of trimetazidine [24–26].

Meldonium (3-(2,2,2-trimethylhydrazinium) propionate (monohydrate)) is a structural synthetic analogue of gamma-butyrobetaine. It reduces the synthesis of carnitine and the transport of long-chain fatty acids through cell membranes [27, 28]. The mediated effect of meldonium is similar to the action of trimetazidine: by preventing long-chain fatty acids from entering the mitochondria, meldonium thereby switch myocardial energy metabolism to glucose utilization through glycolysis and oxidative decarboxylation [27, 28].

Ethoxidol (2-ethyl-6-methyl-3-hydroxypyridinium hydroxybutanedioate) is a metabolically active drug belonging to the group of antioxidants. Due to the presence of unpaired electrons in its molecule, ethoxidol is able to capture electrons of reactive oxygen species and neutralize them, thus preventing damage to cell membranes and other structures – mitochondria, DNA molecules, RNA, maintaining normal ATP production and cell viability [29–31].

Cytoflavin is a combined drug and contains four components: inosine, nicotinamide, riboflavin and succinic acid [32]. Due to the active substances included in the composition, cytoflavin activates the redox enzymes of the mitochondrial respiratory chain, stimulates respiration and energy production in cells [33].

When conducting a correlation analysis, direct positive correlations were obtained between the activity of pyruvate dehydrogenase in the mitochondria of cardiomyocytes and in the mitochondria of leukocytes ($r = 0.811$; $p < 0.0001$); between the activity of citrate synthase in mitochondria of cardiomyocytes and mitochondria of leukocytes ($r = 0.909$; $p < 0.0001$). The data are highly reliable and indicate the potential possibility of considering the leukocyte as an adequate model for studying the nature of the effect of cardiocytoprotectors on the activity of energy metabolism enzymes in heart cells.

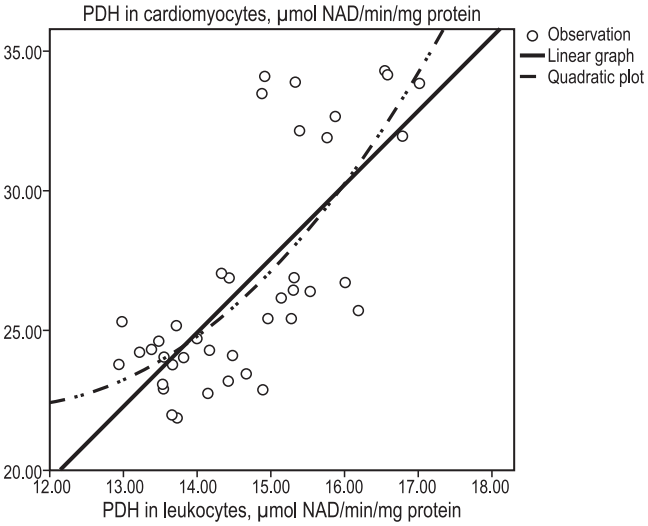

FIG. 1.

Correlation between the activity of pyruvate dehydrogenase in the mitochondria of cardiomyocytes and the activity of pyruvate dehydrogenase in the mitochondria of peripheral blood leukocytes (correlation coefficient r = 0.838; p < 0.0001)

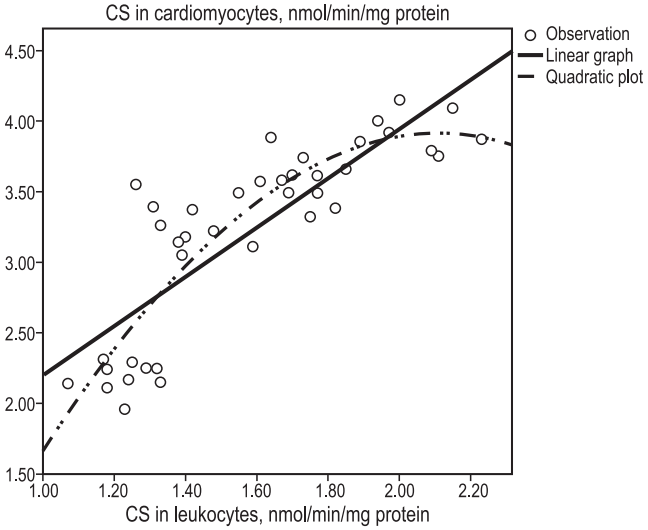

FIG. 2.

Correlation between the activity of citrate synthase in the mitochondria of cardiomyocytes and the activity of citrate synthase in the mitochondria of peripheral blood leukocytes (correlation coefficient r = 0.909; p < 0.0001)

The method of multiple regression analysis made it possible to construct three-dimensional graphs of the dependences of pyruvate dehydrogenase activity in cardiomyocytes on the activity of PDH and CS in leukocytes (Fig. 3) and the dependence of citrate synthase activity in cardiomyocytes on the activity of PDH and CS in leukocytes (Fig. 4) with the calculation of the corresponding regression equations (1) and (2).

The regression equation for the dependence of the level of activity of PDH in cardiomyocytes from the level of activity of PDH and CS in leukocytes has the following form (1):

$$Z = 0.552x^2 - 15.990x + 11.174y^2 - 26.679y + 154.941 \quad (1),$$

where Z – is the level of PDH activity in cardiomyocytes, x – the level of PDH activity in leukocytes, y – the level of CS activity in leukocytes.

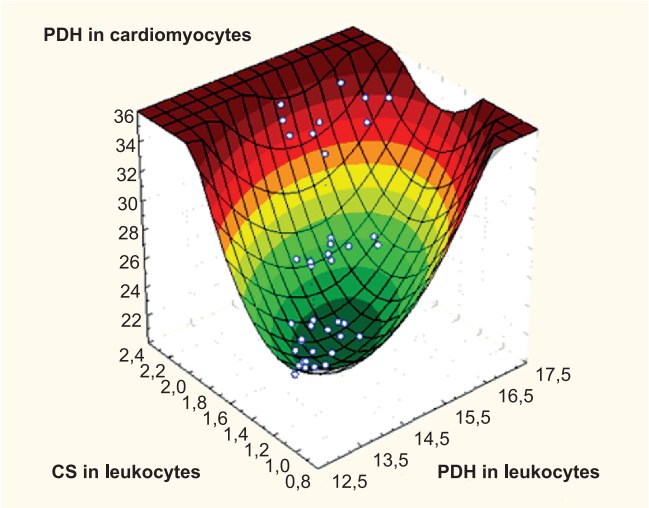

FIG. 3.

Three-dimensional image of the regression dependence of the level of pyruvate dehydrogenase activity in cardiomyocytes from the level of activity of pyruvate dehydrogenase and citrate synthase in leukocytes

The coefficient of determination of this model R^2 was 0.859, which indicates a high reliability of the described dependence.

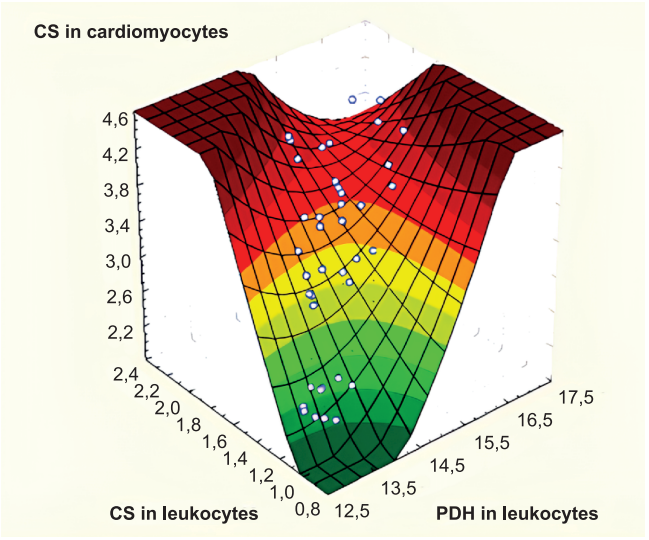

FIG. 4.

Three-dimensional image of the regression dependence of the citrate synthase level in cardiomyocytes from the level of pyruvate dehydrogenase and citrate synthase in leukocytes

The regression equation for the dependence of the level of CS in cardiomyocytes from the level of PDH and CS in leukocytes has the following form (2):

$Z = -0.022x^2 + 0.802x - 1.452y^2 + 6.085y - 9.598$ (2),
where Z – the level of CS in cardiomyocytes, x – the level of PDG in leukocytes, y – the level of CS in leukocytes.

The coefficient of determination of this model R^2 was 0.785, which indicates a high reliability of the described dependence.

Based on the described regression models, it can be said that the level of activity of pyruvate dehydrogenase in cardiomyocytes during the introduction of cardiocytoprotectors, by 86 % depends upon the level of activity of pyruvate dehydrogenase and citrate synthase in peripheral blood leukocytes, and by 14 % – from other unaccounted factors. The level of activity of citrate synthase in cardiomyocytes during the introduction of cardiocytoprotectors, by 79 % depends upon the level of activity of pyruvate dehydrogenase and citrate synthase in peripheral blood leukocytes, and by 21 % – from the other unaccounted factors. Thus, knowing the level of activity of CS and PDH in blood leukocytes, it is possible to judge with a high degree of certainty the level of activity of these mitochondrial enzymes in cardiomyocytes during the administration of cardiocytoprotectors. The above regression equations (1) and (2) make it possible to make an accurate calculation of these indicators.

CONCLUSION

The data obtained indicate the potential possibility of studying the nature of changes in energy metabolism in cardiomyocytes under the influence of cardiocytoprotectors by studying biochemical markers in peripheral blood leukocytes.

Conflict of interest

The authors declare that there is no conflict of interest.

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