ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Mildronate in Patients with the Stable Angina Pectoris: Influence on Mitochondrial Activity.

**Olesya Viktorovna Romaschenko*.**

Belgorod State National Research University, 308015 Belgorod, Pobedy Street, 85, Russia

**ABSTRACT**

At the research of individual sensitivity of leukocytes' mitochondria of 56 patients with a stable angina pectoris to introduction of mildronate in tests in vitro have found out two variants of reaction - in the form of activation (in 46 % of patients) or oppressions of mitochondria functional activity (at 54 % of patients). Sensitivity of mitochondria to introduction of mildronate has appeared dependent on an individual condition of bodies-targets, neurohumoral regulation, tissue hypoxia degree, initial mitochondrial activity and level of power supply of an organism of patients that testifies the necessity of personification of mildronate appointment in complex treatment of patients with a stable angina pectoris.

**Keywords:** Mildronate, a stable angina pectoris, mitochondria

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

The urgency of the present research is caused by wide prevalence, death rate and the high social importance of ischemic heart disease (IHD) [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].

Attempt it is essential to raise efficiency of complex treatment of ischemic heart disease is introduction in clinical practice of metabolic pharmacotherapy for the purpose of maintenance cardiocytoprotection [3]. A point of the action of such preparations are mitochondria [4]. In countries of Western Europe the preparations which have not proved the influence on life expectancy, do not cause special trust, to what testifies low (no more than 1 %) frequency of appointment of metabolic means for treatment of stable angina pectoris, unlike Russia where them use widely enough [5]. In the limited efficiency of the given group of preparations specify works of some authors [6,7], in clinical medicine to them there was an ambiguous relation - 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 mildronate 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. At the majority of patients the angina pectoris was combined with arterial hypertension (89,4 %), rhythm disturbance (24,4 %), cardiosclerosis after myocardial infarction (48,8 %), chronic heart failure (94,4 %), at some - with a diabetes of II type (23,1 %); middle age of patients was 59,26±0,74 years.

Carried out the general clinical methods of research, tool and laboratory, for statement of the diagnosis and treatment of a stable angina pectoris according to recommendations of the European scientific organisation of cardiologists (2008) [2]. Carried out also specific methods of the research of neurohumoral profile of patients by definition of cortisol, insulin, cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) concentration in blood and level of endothelial nitric oxide synthase (eNOS) in erythrocytes by the method of immunoassay on device BioRad by means of sets of reactants of firm "BiochemMac". The metabolic status of patients estimated on concentration of adenosine triphosphate (ATP) and adenosine diphosphate (ADP) in erythrocytes, degree of tissue hypoxia estimated on the basis of an indicator 2,3-diphosphoglycerate (2,3-DPG), which level defined in erythrocytes by biochemical methods [8]. Research of individual influence of mildronate on mitochondria of the person carried out by the technique developed by us in tests in vitro [9] with the help of confocal laser scanning microscopy on device Nikon Eclipse Ti under special program Nikon C1 [10].

The obtained data were statistically analyzed. Differences were analyzed by Student and Mann-Whitney test. $P<0.05$ was considered statistically significant. Character of interrelation between factors estimated by regression analyze and graphic three-dimensional plotting method.

## RESULTS AND DISCUSSION

During the research of individual sensitivity of leukocytes' mitochondria of patients with a stable angina pectoris to mildronate introduction have found out two variants of reaction - in the form of activation (at 26 [46 %] patients) or oppressions of mitochondrial functional activity (at 30 [54 %] patients).

Carried out the comparative analysis between groups of patients with various variants of mitochondrial reaction on mildronate introduction, have received a number of authentic differences (Table 1 and 2.).

ISSN: 0975-8585

**Table 1: Influence of mildronate on the person's mitochondria**

| Indicator | Mitochondrial activation, n=26 | Mitochondrial oppression, n=30 |
|---|---|---|
| Microalbuminuria, g/l | 0,015±0,006 *** | 0,175±0,015 *** |
| Creatinine of blood, mmol/l | 85,48±3,06 | 95,29±4,34 |
| Aspartataminotransferasa of blood, international unit (IU) | 23,60±1,22 ** | 31,00±1,21 ** |
| Total voltage of tooth R in standard assignments of electrocardiogram, mm | 19,42±1,51 | 15,40±1,69 |
| Coronaroangiography: level of anterior interventricular artery stenosis, % | 35,83±9,49* | 70,00±9,89* |
| Cortisol/insulin in serum of blood | 0,37±0,11 | 0,99±0,23 |
| cAMP / cGMP in serum of blood | 3,11±1,56 | 14,91±6,20 |
| level of endothelial nitric oxide synthase (eNOS) in erythrocytes, pg/ml | 1876,92±726,56 | 440,01±94,59 |
| ADP in erythrocytes, mkmol/l | 312,83±4,95 | 302,25±4,44 |
| 2,3-DPG in erythrocytes, mkmol/ml | 6,17±0,14* | 6,55±0,11* |

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

**Table 2: Influence of mildronate on the person's mitochondria**

| Indicator | Mitochondrial activation, n=26 | Mitochondrial oppression, n=30 |
|---|---|---|
| Stage of chronic heart failure | 1,52/<br>2,00 (1,00; 2,00) | 1,11/<br>1,50 (0,00; 2,00) |
| Functional class of chronic heart failure (NYHA) | 2,12/<br>3,00 (1,50; 3,00) ** | 1,32/<br>2,00 (0,00; 2,00) ** |
| Intensity of mitochondrial fluorescence (colouring – pyren), relative unit | 286,32/<br>268,85 (180,25; 379,93) *** | 441,16/<br>435,55 (333,13; 517,18) *** |

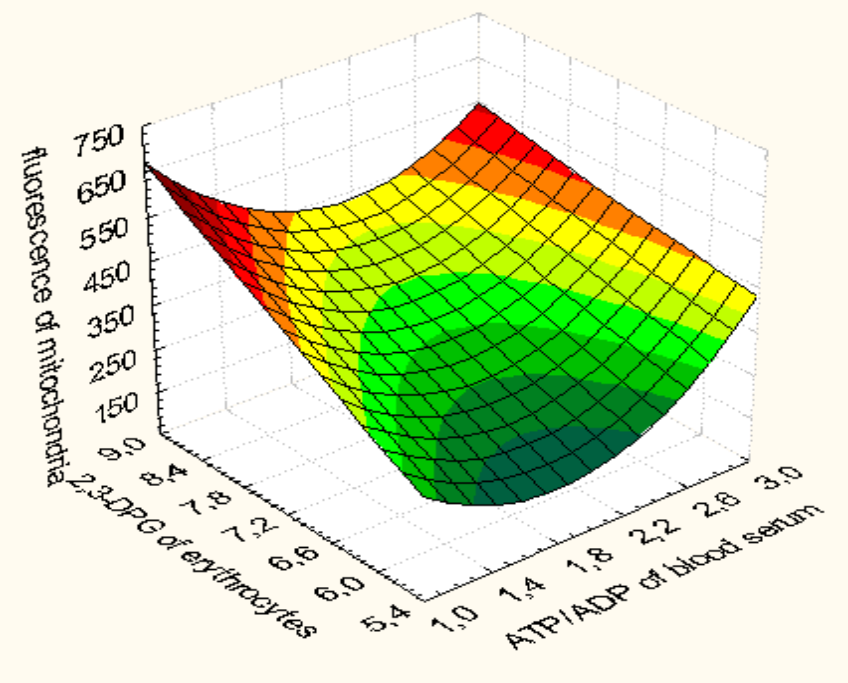
The note. Numerator - an average arithmetic, a denominator - a median, 25 % and 75 % quartile. Differences were analyzed by Mann-Whitney test: ** p <0.01; *** p <0.001.

Have found out following conditions of mitochondrial activation under action of mildronate: presence of progressing chronic heart failure (CHF), normal function of liver and kidneys, normal voltage on an electrocardiogram (absence of myocardiodystrophia), small degree of coronary arteries stenosis, low values of parities cortisol/insulin and cAMP/cGMP in blood serum, high level of endothelial nitric oxide synthase (eNOS) in erythrocytes, initially low mitochondrial activity, presence of hypoergosis signs at enough of power substrata (ADP), small degree of tissue hypoxia according to level of 2,3-DPG in erythrocytes. Mildronate is capable to oppress mitochondria in tests in vitro in the presence of following conditions: initial stages of CHF, the tendency to infringement of liver and kidneys function, the tendency to myocardiodystrophia development according to an electrocardiogram, marked degree of coronary arteries stenosis, high indicators of parities cortisol/insulin and cAMP/cGMP in blood serum, low level of endothelial nitric oxide synthase (eNOS) in erythrocytes, initially high mitochondrial activity with absence of hypoergosis signs, expressed tissue hypoxia according to level of 2,3-DPG in erythrocytes.

Mildronate is considered as active metabolic preparation with proved cardioprotective (and neuroprotective) effect which is realised by regulation of mitochondrial processes [11]. It might be suggested that the molecular conformation of mildronate can facilitate its easy binding to mitochondria, and regulate the expression of different signal molecules, hence maintaining cellular signaling and survival [12].

Between indicators of mitochondrial activity, tissue hypoxia and intensity of cellular breath we have found out indirect interrelations (Drawing 1.).

ISSN: 0975-8585




**Drawing 1: Interrelation between intensity of mitochondrial fluorescence (colouring - pyren, relative unit), level of 2,3diphosphoglycerate (2,3-DPG) in erythrocytes (mkmol/l) and a parity of concentration ATP/ADP of blood serum in patients with angina pectoris**

In drawing 1 it is visible, that there are two peaks of mitochondrial maximum activity (instead of one): the first associates with high parity ATP/ADP in blood serum and with low level of 2,3-DPG in erythrocytes, i.e. with high level of power supply of a cage and low degree of tissue hypoxia, and the second peak of mitochondrial activity is connected with low parity ATP/ADP in blood serum and with high level of 2,3-DPG in erythrocytes, i.e. with a condition of hypoergosis and tissue hypoxia. Character of interrelations is nonlinear, in a two-dimensional plane is described by polynomial schedule of regression curve: between mitochondrial fluorescence and 2,3-DPG the regress equation has the following appearance $y = -1E\text{-}14x^6 + 3E\text{-}11x^5 - 3E\text{-}08x^4 + 1E\text{-}05x^3 - 0{,}0035x^2 + 0{,}4379x - 14{,}751$, factor of determination $R^2 = 0{,}2602$; between 2,3-DPG and parity ATP/ADP of blood serum - $y = -2{,}8955x^6 + 125{,}44x^5 - 2255{,}7x^4 + 21547x^3 - 115297x^2 + 327657x - 386289$, $R^2 = 0{,}4046$; between mitochondrial fluorescence and parity ATP/ADP of blood serum - $y = -2E\text{-}15x^6 + 4E\text{-}12x^5 - 4E\text{-}09x^4 + 2E\text{-}06x^3 - 0{,}0005x^2 + 0{,}0599x - 0{,}4661$, $R^2 = 0{,}1299$.

Change of mitochondrial activity can have both positive, and negative influence on cell viability. So, normal activation of mitochondria is accompanied by activation of cellular breath, and superfluous activation - generation of peroxide of hydrogen [13]. It was found out, that mildronate has the ability to restore mitochondrial function mainly by reducing hydrogen peroxide generation [14]. Oppression of ATP-dependent kalium channels of mitochondrial membranes can be the possible mechanism of cell destruction in case of superfluous activation of mitochondria and ATP surplus, that lead to accumulation of hydrogen ions and water inside cell and its lysis [15]. Destruction of mitochondria is accompanied by sharp deficiency of endocellular energy and the subsequent destruction of cell on type oncosis or apoptosis [16]. Among researches it is shown, that the inhibition of ATP-dependent kalium channels of mitochondrial membranes can lead to cellular apoptosis by opening $Ca^{2+}$-activated mitochondrial pore, and, on the contrary, activation of ATP-dependent kalium channels prevents apoptosis, so that plays an essential role in protection of a myocardium against ischemic damages [15,17,18]. Possibility of mitochondrial oppression under the influence of energy stimulators is caused by the physiological phenomenon of Krebs cycle key enzymes braking at increase of parity ATP/ADP in cells.

The found out ability of mildronate 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 mildronate appointment in complex treatment of patients with a stable angina pectoris.

ISSN: 0975-8585

## RESOLUTION

Mildronate is capable to make various impact on mitochondrial activity in patients depending on an individual condition of their bodies-targets, neurohumoral regulation, tissue hypoxia degree, initial mitochondrial activity and level of power supply of an organism of patients that testifies the necessity of personification of mildronate appointment in complex treatment of patients with a stable angina pectoris.

## CONCLUSIONS

- Mildronate in tests in vitro has shown the ability both to activate, and to oppress mitochondria in patients with a stable angina pectoris.
- Conditions of mitochondrial activation under influence of mildronate are: presence of progressing chronic heart failure, normal function of liver and kidneys, normal voltage on an electrocardiogram (absence of myocardiodystrophia), small degree of coronary arteries stenosis, low values of parities cortisol/insulin and cAMP/cGMP in blood serum, high level of endothelial nitric oxide synthase in erythrocytes, initially low mitochondrial activity, presence of hypoergosis signs at enough of power substrata (ADP), small degree of tissue hypoxia according to level of 2,3-DPG in erythrocytes.
- Conditions of mitochondrial oppression under influence of mildronate are: initial stages of chronic heart failure, the tendency to infringement of liver and kidneys function, the tendency to myocardiodystrophia development according to an electrocardiogram, marked degree of coronary arteries stenosis, high indicators of parities cortisol/insulin and cAMP/cGMP in blood serum, low level of endothelial nitric oxide synthase in erythrocytes, initially high mitochondrial activity with absence of hypoergosis signs, expressed tissue hypoxia according to level of 2,3-DPG in erythrocytes.

## ACKNOWLEDGEMENT

I express gratitude for the help in statistical data processing to PhD Assistant Professor of chair mathematical and the software of information systems of Belgorod state national research university Vadim Valerevich Rumbesht.

## REFERENCES

[1] Ratmanova A. Med Rev 2009; 1(6): 6-12. (In Russian)
[2] Eur Heart J 2006; 27: 1334-1381.
[3] Astashkin EI. MG Glezer. 2009. Metabolicheskie cytoprotektori I mechanizmi ih deistviya. Moskva, 13s. (In Russian)
[4] Morin D, R Assaly, S Paradis and A Berdeaux. Curr Med Chem 2009;16(33): 4382-4398.
[5] Mazur NA. Klinicheskaya Medicina 2007;8: 19-25. (In Russian)
[6] Turikov PU. 2005. Vliyanie mildronata i preduktala v sochetanii s kompleksnoj antianginal'noj terapiej na uroven' produktov perekisnogo okisleniya lipidov, sostoyanie endotelial'noj disfunktsii i fizicheskuu tolerantnost' bol'nih IBS: avtoref. Diskand.med.nauk. Kursk, 22s. (In Russian)
[7] Hobot VV. 2005. Dlitel'noe primenenie trimetazidina u bol'nih v postinfarktnom periode: avtoref. diskand.med.nauk. Moskva, 25s. (In Russian)
[8] Mranova IS. Laboratornoe delo 1975;7: 652-654. (In Russian)
[9] Romaschenko OV. 2013. Sposob opredeleniya individual'noj reaktivnosti mitochondrij cheloveka pod dejstviem preparatov metabolicheskogo ryada v probah in vitro: svidetel'stvo o nou-hau, Belgorodskij gosudarstvennij universitet, Belgorod, # 125. (In Russian)
[10] Pawley JB. 1995. Handbook of biological confocal microscopy. New York: Plenum Press, pp. 632.
[11] Klusa V, U Beitnere, J Pupure, S Isajevs, J Rumaks, S Svirskis, Z Dzinkale and I Kalvinsh. Medicina (Kaunas) 2013;49(7): 301-309.
[12] Pupure J, S Isajevs E, Scapare J Rumaks, S Svirskis, D Svirina, I Kalvinsh and V Klusa. Neurosci Lett 2010; 470(2):100-105.
[13] Boveris A, N Oshino and B Chance. Biochem J 1972;128: 617-630.
[14] Pupure J, et al. Cell Biochem Funct 2008;26(5): 620-631.
[15] Mironova GD, EV Kachaeva, IB Krilova I DR. Vestnik Rossijskoj AMN 2007; 2: 44-50. (In Russian)
[16] Skulachev VP. Sorosovskij obrazovatel'niy zhurnal 2001;7(6): 4-10. (In Russian)
[17] Takashi E, Y Wang and M Ashraf. Circ Res 1999;85:1146-1153.
[18] Akao M, BO Rourke, H Kusuoka et al. Circ Res 2003; 92: 195-202.