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Personalized Approaches To The Use Of Meldonium As A Cytoprotector In Patients With Coronary Heart Disease

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Abstract

Introduction: The personalized approach to prescribing medications to patients with stable angina pectoris has been designated as promising, “diamond”. How this is true for meldonium is to be sanctified in this article.

Researchtasks: to develop a personalized approach to the use of meldonium in patients with coronary heart disease (CHD) based on the definition of criteria for predicting the cytoprotective properties of this drug when tested in vitro.

Materialand Methods: We examined 30 patients with CHD: stable angina pectoris I-III functional classes with concomitant arterial hypertension. The patients underwent echocardiography, coronary angiography, biochemical blood test with determination of the cholesterol profile. To determine the cytoprotective activity of meldonium, blood leukocytes of patients were examined in vitro by fluorescence microscopy using an Eclipse Ti-U inverted fluorescence microscope (Nikon, Japan). By staining leukocytes with fluorescent dyes (Calcein AM, Ethidium bromide), living and dead cells were determined, and the cell viability index (VI_{cells}) was calculated. The materials were processed statistically, the criteria for predicting the cytoprotective effect of meldonium were determined using Wald's prognostic analysis.

Results: with the introduction of meldonium into a sample with a leukocyte suspension, two variants of changes in cell viability were observed: in 40% of patients, VI_{cells} increased, on average, by 34% (from 28% to 62%, $p < 0.001$) and in 60% of patients, VI_{cells} decreased, on average , by 26% (from 52% to 26%, $p < 0.05$).

A number of conditions of the initial state of a patient with CHD were identified for the manifestation of the cytoprotective activity of meldonium: the initial stages of concomitant arterial hypertension (1-2), a pressure gradient on the pulmonary artery valve up to 3 mm Hg, the presence of violations of local myocardial contractility according to echocardiography, the presence of stenosis of coronary arteries (more than 60% in diagonal branch of the left coronary artery and more than 50% in the right coronary artery), normal (non-atherogenic) cholesterol profile with serum cholesterol levels less than 5.3 mmol / L and low density lipoproteins less than 2 mmol / L.

Conclusion: the metabolic drug meldonium exhibits cytoprotective activity only in some patients with coronary heart disease (40%). At the same time, there are a number of conditions for the initial somatic status of the patient, which determine the advisability of using meldonium as a cytoprotector.

Keywords: meldonium, leukocytes, viability, coronary heart disease, patients, microscopy, in vitro research, personalized pharmacotherapy.

INTRODUCTION

The personalized approach to the choice of drugs in the treatment of patients with coronary heart disease is designated by the ex-president of the European Society of Cardiology Robertro Ferrary as "diamond" [1]. The widespread prevalence and high mortality from CHD are aimed at finding rational combinations of drugs in the treatment of patients [2,3,4]. Rationalization of pharmacotherapy is important in clinical medicine [5,6,7,8]. The accepted standards for the treatment of stable angina pectoris with drugs from the groups of antiplatelet agents, anticoagulants, beta-blockers, statins, angiotensin-converting enzyme inhibitors, nitrates, calcium antagonists have a high level of evidence, but do not fully ensure the effectiveness of treatment [9]. Currently, the standard of treatment for stable angina includes a number of metabolic drugs that provide a cardiocytoprotective effect [9].The direction of cytoprotective pharmacotherapy is traditionally considered to be of secondary importance in the treatment of hypoxic conditions associated with tissue ischemia, as well as immuno-inflammatory diseases, including stable angina pectoris [9,10,11]. The ambiguous efficacy of cytoprotectors discovered by a number of authors may indicate the need for a personalized approach to prescribing this group of drugs [12,13,14]. Currently, a whole area of personalized pharmacotherapy is actively developing [15].In this regard, it is of particular interest to study the effect of the metabolic drug meldonium on cell viability in patients with coronary heart disease. What determines the manifestation of the cytoprotective properties of meldonium, is a personalized approach to the use of this drug required in patients with CHD? This work is devoted to finding answers to all these questions.

The experimental model for studying the viability of cells was the blood leukocytes of patients, since they can reflect the internal state of the human body and are readily available material for research. These immune cells are considered as a kind of "mirror of homeostasis", which can be used to determine the nature of the process underlying the disease, its severity, prognosis and effectiveness of therapy [16]. Moreover, W. Jin, G. Deng-Feng, W. Hao et al, based on a number of their own studies, argue that the nature of mitochondrial damage in cardiomyocytes and peripheral blood leukocytes is identical, leukocytes reflect changes in cardiomyocytes, as in a mirror [17].

PURPOSE

To develop a personalized approach to the use of meldonium in patients with coronary heart disease based on the definition of criteria for predicting the cytoprotective properties of this drug when tested in vitro.

MATERIALS AND METHODS

We examined 30 patients with coronary heart disease: stable angina pectoris of I-III functional classes (acute coronary syndrome was excluded from the study, concomitant hypertension was allowed), who were admitted to the Department of Cardiology No. 1 of the Belgorod Regional Clinical Hospital of St. Joasaph from January to June 2019. The study group included 20 women and 10 men aged from 49 to 81 years, the average age of patients was 66.0 ± 2.0 years old.

The patients underwent echocardiography, coronary angiography, biochemical blood test with determination of the cholesterol profile.

Blood sampling was performed in the morning on an empty stomach in a vacuum tube with ethylenediamine tetraacetic acid (EDTA). A prerequisite for the selection of patients for the study was the absence of X-rays for at least 21 days before blood sampling due to the well-known destructive effect of X-rays on human leukocytes and the ability of white blood cells to completely renew the composition within 21 days with an average life expectancy of leukocytes of 7-9 days [18].

To determine the viability of blood cells, leukocytes (0.5 ml) were collected manually with a micropipette under aseptic conditions, mixed with 2 ml of RPMI-1640 culture medium with glutamine (PanEko, Russia), then placed into the wells of a 24-well plate, 20 µl of leukocyte suspension in each well. The culture medium and the drug were added in an amount necessary to create a therapeutic concentration of the drug in the well, equivalent to the introduction of 5 ml (500 mg) of meldonium intravenously into a person. We were guided by the official instructions for the medical use of the drug "Mildronate" (meldonium). Then the samples were incubated for 3 hours (time sufficient for the drug to interact with cells) in an incubator with 5% CO_2 content at a temperature of 37°C (conditions of the human internal environment). After 3 hours of incubation, 500 µl of the supernatant was taken from each well and fluorescent dyes were added to the remaining 500 µl at a final concentration of 1nM/µl for Calcein AM (Invitrogen , USA), which stains only viable cells and at a final concentration of 2nM/µL for Ethidium bromide (Sigma-Aldrich, USA), which stains only dead cells [19]. The samples were again placed in a thermostat under the same conditions for another 30 minutes (time sufficient for staining the cells). When developing the scheme of the experiment, we were guided by the tutorial ofMitroshina E.V. et al. (2015) [20].

The results were evaluated by fluorescence microscopy using an Eclipse Ti-U inverted microscope (Nikon, Japan). The data were processed using the specialized software EZ-C1 FreeViewer Ver3.90 (Nikon).

The number of living and dead cells was counted, the cells viability index was calculated using the formula:

$$VI_{cells} = (Z_{living\ cells} - Z_{dead\ cells}) / (Z_{dead\ cells}) * 100, (1)$$

where VI_{cells} is the cells viability index (in%),

$Z_{living\ cells}$ - the number of living cells in 10 fields of view

$Z_{dead\ cells}$ - the number of dead cells in 10 fields of view

By the nature of the change in the cells viability index under the influence of the drug administered in vitro, the presence of the cytoprotective properties of meldonium was judged according to the method we developed [21].

A total of 12,000 cells were analyzed. The materials were processed statistically with the calculation of the arithmetic mean, error of the mean, assessment of the significance of differences by Student's t-test.

Wald's predictive analysis was also performed.

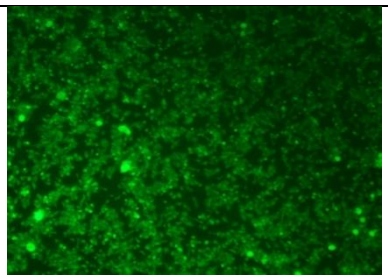
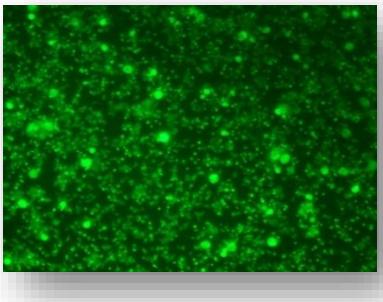
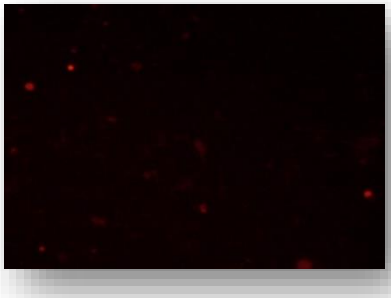
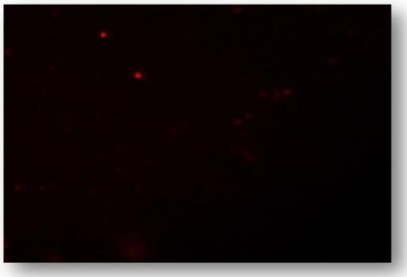
The study was carried out on the basis of the laboratory of cell technologies of the Research Institute of Pharmacology of Living Systems, Belgorod State University.

RESULTS AND DISCUSSION

Figures 1A and 1B show micrographs of living and dead cells in the fields of view without the addition of meldonium. VI_{cells} in patients with CHD, on average in the group (without the introduction of meldonium), was 42%. Figures 2A and 2B show micrographs of living and dead cells in the field of view of the wells, where meldonium was added at a therapeutic concentration. The index of viability of leukocytes in patients with CHD, on average in the group, after the introduction of meldonium did not change significantly and amounted to 41%.

A more detailed analysis of the dynamics of the VI_{cells} index showed two variants of changes in cell viability under the influence of meldonium: in 40% of patients, VI_{cells} increased, on average, by 34% (from 28% to 62%, $p < 0.001$) and in 60% of patients, VI_{cells} decreased, in on average, by 26% (from 52% to 26%, $p < 0.05$).

	without meldonium	**with meldonium**
Luminescence of		

membrane structures of living white blood cells (dye - Calcein AM). Fluorescence microscopy, magnification X200.	 Figure 1.A.	 Figure 2.A.
Luminescence of dead leukocytes (dye - Ethidium bromide). Fluorescence microscopy, magnification X200	 Figure 1.B.	 Figure 2.B.

To elucidate the reasons for the discovered phenomenon of variability of changes in cell viability after the introduction of meldonium into samples with a leukocyte suspension of patients with CHD and to determine prognostic criteria for the cytoprotective effect of meldonium, we carried out Wald's statistical prognostic analysis. A number of the most significant parameters of the initial state of the patient were obtained, which make it possible to predict the reaction of the cells (blood leukocytes) of the patient to the introduction of meldonium (Table 1.)

Table 1. Predictive model of the manifestation of the cytoprotective properties of meldonium in patients with CHD (according to in vitro drug testing)

No.	Feature	Range	Predictive coefficient	Informativeness ratio (private)	Informativeness ratio (general)
1	The degree of hypertension	1	5	0.33	1.58
		2	2	0.16	
		3	-6	1.09	
2	Pressure gradient across the	<3	3	0.73	2.36

	pulmonary artery valve, mm Hg	from3	-7	1.64	
3	Disorders of local myocardial contractility according to echocardiography	no	-7	1.62	2.43
		yes	3	0.81	
4	The degree of stenosis of the diagonal branch of the left coronary artery according to coronary angiography, %	<60	-2	0.42	1.39
		from60	5	0.97	
5	The degree of stenosis of the right coronary artery according to coronary angiography,%	<50	-4	1.00	2.50
		from50	6	1.50	
6	Blood cholesterol, mmol / l	<5.3	3	0.71	1.68
		>=5.3	-5	0.97	
7	The level of low density lipoproteins in the blood, mmol / l	<2	6	1.58	3.13
		>=2	-5	1.55	

Note. A positive predictive coefficient indicates the prediction of the manifestation of the cytoprotective properties of meldonium in a patient, a negative predictive coefficient indicates the prediction of the absence of the manifestation of the cytoprotective effect of meldonium.

According to the results of our study, the cytoprotective activity of meldonium depends on a number of parameters of the patient's initial state:

1. The degree of concomitant hypertension: the cytoprotective effect is observed only in the initial stages of concomitant hypertension (1-2).
2. Some indicators of echocardiography: pressure gradient on the pulmonary artery valve (cytoprotective activity is observed only with a gradient of up to 3 mm Hg), and in the presence of violations of local myocardial contractility.

3. Some indicators of coronary angiography: the cytoprotective activity of meldonium is observed in the presence of signs of coronary artery stenosis (more than 60% of the diagonal branch of the left coronary artery and more than 50% of the right coronary artery).

4. Indicators of the lipid profile: the cytoprotective activity of meldonium is observed with an

initially normal (non-atherogenic) cholesterol profile with serum cholesterol levels less than 5.3 mmol / l and low density lipoproteins less than 2 mmol / l.

The pathogenetic substantiation of the validity of the results and conclusions obtained by us is the following theoretical provisions ...

According to the official instructions for medical use and literature data, meldonium belongs to the class of partial inhibitors of β-oxidation of fatty acids, to the group of cytoprotectors - antihypoxants that provide protection and energy supply to various cells of the body under conditions of ischemia and increased stress [22,23,24,25] ... This drug blocks the biosynthesis of carnitine from gamma-butyrobetaine, resulting in a double positive effect.First, the concentration of carnitine, a carrier of fatty acids across the mitochondrial membranes, decreases, which determines the oxygen-saving effects of the drug. Secondly, the concentration of gamma-butyrobetaine increases, which irritates acetylcholine receptors and stimulates the biosynthesis of nitric oxide (NO), an endothelial vasodilation factor [22], which leads to an improvement in endothelial function and the vasodilating effect of meldonium [22,23,24,25]. Many research works are devoted to the pharmacological correction of endothelial dysfunction; endothelioprotection is an important pharmacodynamic target [26,27,28,29,30].

According to our data, meldonium exhibits its cytoprotective activity, improving cell viability, in those clinical cases when there is an individual "pharmacodynamic target" for it - myocardial ischemia zones (according to echocardiography), coronary angiosclerosis is more than 50% (according to coronary angiography), mild or moderate systemic arterial hypertension, a small pressure gradient across the pulmonary valve.Meldonium does not show its cytoprotective activity in cases of the absence of an individual pharmacodynamic target for it - no signs of myocardial ischemia and coronary sclerosis, and in the presence of signs of adaptation reserves depletion - severe arterial hypertension, high blood cholesterol levels, and proatherogenity of plasma.

The fact is that nitric oxide is not only an endothelial factor of vasodilation, this highly reactive molecule is considered today as a mediator of the NO-ergic stress-limiting system [31]. Meldonium is able to increase the amount of nitric oxide in the blood and thereby exhibit an adaptive effect [22]. However, as our previous studies have shown, this property of meldonium is manifested only if reserves for adaptation are preserved [32]. The results of this study are consistent with the data of our previous studies [32, 33, 34], and indicate the need for a personalized approach to the use of this drug as a cytoprotector in patients with coronary heart disease.

CONCLUSION

Thus, according to the results of our in vitro study, the metabolic drug meldonium exhibits cytoprotective activity only in a part of patients with coronary heart disease (40%). At the same time, there are a number of conditions for the initial somatic status of the patient, which determine the

presence of an individual pharmacodynamic target for the action of the drug and, accordingly, the appropriateness of its use as a cytoprotector.

1. During the introduction of meldonium into a sample with a leukocyte suspension, isolated from the blood of patients with coronary heart disease, two variants of changes in cell viability were observed: in 40% of patients, the VI_{cells} increased, on average, by 34% (from 28% to 62%, $p < 0.001$) and in 60% of patients, VI_{cells} decreased, on average, by 26% (from 52% to 26%, $p < 0.05$).
2. A number of conditions of the initial state of a patient with CHD were identified for the manifestation of the cytoprotective activity of meldonium: the initial stages of concomitant arterial hypertension (1-2), a pressure gradient on the pulmonary artery valve up to 3 mm Hg, the presence of violations of local myocardial contractility according to echocardiography, the presence of stenosis of coronary arteries (more than 60% in diagonal branch of the left coronary artery and more than 50% in the right coronary artery), normal (non-atherogenic) cholesterol profile with serum cholesterol levels less than 5.3 mmol / L and low density lipoproteins less than 2 mmol / L.

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