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Influence of the cytoprotective drug meldonium on diastolic dysfunction of the myocardium

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Abstract

Background: The use of myocardial cytoprotectors (meldonium) in patients with exertional angina is a scientific-practical dilemma.
Material and methods: An open randomized clinical trial was conducted involving 160 patients with chronic heart faliure (117 men and 43 women) aged 37 to 81 years. Of them, 142 patients had angina pectoris of stable effort from different functional classes, and 21 – unstable angina pectoris. Study groups were comparable according to the frequency of indication of background drugs and meldonium.
Results: The number of patients with normal diastolic function in both groups, but with a net superiority to meldonium combination administration, has considerably increased: 41 patients (91.11%) in group II vs 33 (58.93%) in the group treated with basic treatment after 9 months of medication; 43 patients (95.56%) group II vs 36 (64.29%) in group I at 12 months of medication. During this period, no patients with pseudonormal type of diastolic dysfunction were registered, these passing into a "more" favorable category – delayed relaxation.
Conclusions: The data obtained confirmed the benefit of using cardiocitoprotection in inducing the reverse-remodelling of the myocardium of left ventricle regardless of the initial ventricular geometric pattern, but the administration of mildronate combination demonstrated a significantly superior efficiency to the basic treatment in hypertrophy of left ventricle regression, an event notable towards the end of the research period.
Key words: cardiocitoprotector, cardiac metabolism, ischemic heart disease.

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Introduction

Contemporary guidelines for the management of patients with stable exertion angina include several classes of recommendations and levels of evidence, which allow the clinician to objectively assess the benefit and effectiveness of various diagnostic and treatment measures. The contemporary standard of treatment of patients with angina pectoris of stable effort is presented according to the recommendations of the National Clinical Protocol. When determining the priorities of drug treatment, experts have focused on the principles of evidence-based medicine, and in the absence of high veracity data on one or another problem, the consensus of opinions of several experts is taken into account.

In case of development of heart disease – ischemic heart disease (IHD), hypertension, heart failure, the substrate of cardiac energy intake changes, which is reflected by the process of cardiac adaptation to the conditions of energy deficiency [1-3]. Thus, in case of sufficient oxygen intake, the basic energy substrate in the heart of an adult person are fatty acids. In case of myocardial ischemia, all metabolic adaptive mechanisms are oriented towards the transition of energy metabolism from the use of fatty acids to the use of carbohydrates [4-7]. In conditions of insufficient oxygen, the cell is more convenient to oxidize glucose than fatty acids, since this process requires a smaller amount of oxygen [2, 5, 8].

Meldonium in contrast to trimetazidine stimulates not only the oxidation of glucose, but also other hexoses, does not lead to the accumulation in tissues of lactate and activated fatty acids, enhances the effect of angiotensin converting ensyme inhibitors, acts independently of the concentration of fatty acids, prolongs by 30% the life span of animals with myocardial infarction, improves the survival index in experimental chronic heart failure, stimulates calcium-dependent ATP-aza of the sarcoplasmatic reticulum, which improves myocardial contractility, induces nitric oxide biosynthesis and decreases the peripheral resistance of blood vessels [1, 3, 6, 9]. Meldonium blocks carnitine biosynthesis from gamma-butyrobetaine. The decrease in the concentration of carnitine, the transporter of fatty acids through the mitochondrial membranes, determines the oxygen-saving effects of the preparation. Increasing the concentration of gamma-butyrobetain leads to acetylcholine receptor excitation and stimulation of nitric oxyd biosynthesis [9-12]. The ability of mildronate to correct mitochondrial dysfunction was detected, mainly by limiting the oxidation to free radicals of membrane lipids

[10-12]. As a rule, meldonium is not a remedy used in chronic heart failure (CHF) and CHF monotherapy, but it is used in the composition of the widely accepted complex treatment of these diseases, contributing to the increase of its effectiveness [13-15]. The efficacy and harmlessness of meldonium in patients with moderate heart failure on CHF phonon were confirmed in a randomized, double blind, placebo-controlled clinical trial, which was conducted in four medical centers [2, 5, 6, 9]. The positive effects of the use of meldonium in patients with myocardial infarction and manifestations of heart failure have also been achieved in the research described in the litarature of speciality [3, 5, 11]. In the clinical trials of other authors, the positive coronarodilatatory and inotropic effects of this preparation have also been demonstrated [11, 15], the ability of meldonium to increase patients' tolerance to physical exertion [7, 9, 10, 13], to reduce the electrical instability of the heart [5, 8], to increase the antioxidant protection activity of the myocardium [2, 4, 6], to potentiate the effects of hypotensive preparations [2, 4], to improve the quality of life of the sick [11, 14]. The insulin-like action of meldonium [1, 3, 7], its ability to produce a direct beta-2-adrenosensitizing action on smooth muscle [7, 8] and stimulate endothelial acetylcholine receptors [1, 3, 5] expands the possibilities of application of this drug.

Thus, the use of myocardial cytoprotectors (meldonium) in patients with exertion angina pectoris in the preoperative and postoperative periods of coronary bypass is a scientific-practical dilemma [9-11]. The results of scientific research consist in the study of the influence of meldonium on the clinical course, general and local contractility of the left ventricle myocardium, cardiac arrhythmias, biochemical lesions of the myocardium and lipid peroxidation indices in patients with stable angina pectoris in preoperative and postoperative periods of coronary bypass [5, 15]. It is shown the decrease in the number of angina pectoris on the phonon of cytoprotector administration in the preoperative period and their absence in the postoperative period, the reduction of the number of ventricular rhythm disorders, the increase of the ejection fraction of the left ventricle, the decrease of the local contractility index of the myocardium [1, 13, 14]. Evidence of an increased efficacy of meldonium compared to trimetazidine with respect to all the parameters mentioned above is provided [6, 12].

Despite the pathogenetic argumentation of the use of preparations of the metabolic group in the complex treatment of ischemic heart disease, interest in cardiocitoprotectors is more characteristic for scientists of post-Soviet space countries. In European countries, preparations that have not demonstrated their effect on life expectancy, with a ‚dubious' or ‚philosophical' mechanism of action, do not cause particular confidence, as evidenced by the low frequency (no more than 1 %) of the indication of metabolic remedies for the treatment of angina pectoris in the countries of Europe [1-3]. Lately, scientists have also begun to notice the ambiguous efficacy of this group of preparations, papers have appeared that indicate the limited effectiveness of metabolic preparations. Since a "panacea" was not obtained, the attitude towards these preparations became a skeptical one. But real science begins precisely where there is ambiguity.

The purpose of the study: increasing the effectiveness and harmlessness of the pharmacotherapy of ischemic heart disease by developing personalized approaches for indicating drug of metabolic order – meldonium.

Material and methods

An open randomized clinical trial was conducted that included 160 patients with chronic heart failure (CHF), 117 men and 43 women, aged 37 to 81 years. Of them, 142 patients had angina pectoris of stable effort from different functional classes, and 21 – unstable angina pectoris. In most patients angina pectoris was associated with hypertension (HTA) (143 [89.4%]), rhythm disturbances (39 [24.4%]), postinfarct cardiosclerosis (CSPI) (78 [48.8%]), CHF (151 [94.4 %]), some with diabetes mellitus (DM) type II (37 [23.1 %]). The average age of patients was 59.26±0.74 years. The control group involved 30 practically healthy people. The patients were on inpatient treatment in the cardiology department in the years 2011-2015, they continued the outpatient treatment. The observation period was 6 weeks. Each participant was introduced to the research program and signed an informed agreement.

Meldonium was administered at a dose of 0.5 g/24 hours for a period of 6 weeks: in the first 10 days the preparation was administered intravenously in the stationary, after which the outpatient drug was continued as capsules. Study groups were compared according to the frequency of indication of background drugs.

Results

At the stage of enrollment in the study, the groups were homogeneous according to the E/A parameter, which was reduced compared to normal in both groups of patients: in the first group the E/A ratio was 0.67±0.16, and in group II – 0.69±0.29, $p>0.05$. When analyzing the cohort, this index recorded statistically significant improvement in both groups of patients throughout the treatment period: at 3 months in group I E/A increased by +7.46% compared to the initially compared to group II, where this index increased by +10.14%, $p<0.001$ from the initial; at 6 months – +13.43% in group I and +21.64% in group II, $p<0.001$; at 9 months – +29.85% and +44.93% in groups I and II, respectively, $p<0.001$; at 12 months – +67.16% and +75.36%, respectively, $p<0.001$. At the comparative analysis between groups, the dynamics of the E/A ratio was as follows: at 3 months there was no statistically significant difference in the improvement of the E/A ratio between lots (group I – 0.72±0.13 vs 0.76±0.23 in group II, $p>0.05$); but starting with the 6th month of treatment, it was noted a progressive and continuous improvement of this parameter in both groups, but consistently more

obvious in the group treated with meldonium association: at 6 months in the first group the E/A ratio was 0.76±0.09 vs 0.84±0.15, in lot II, p<0.05; at 9 months – 0.87±0.16 vs 1.00±0.19 in groups I and II, respectively, p<0.05; at 12 months – 1.12±0.22 in group I vs 1.21±0.27 in group II, p<0.001). At the same time, the normalization of the E/A ratio occurred earlier in the group, under medication with meldonium association, a phenomenon already observed at the 3rd month of treatment (0.76±0.23 in group II vs 0.72±0.13 in group I), (tab.1).

Table 1. Evolution of the E/A ratio according to medication

E/A ratio					
	Initial	3 months	6 months	9 months	12 months
Group I	0.67±0.16	0.72±0.13* (+7.46%)	0.76±0.09* (+13.43%)	0.87±0.16* (+29.85%)	1.12±0.22* (+67.16%)
Group II	0.69±0.29	0.76±0.23* (+10.14%)	0.84±0.15* (+21.64%)	1.00±0.19* (+44.93%)	1.21±0.27* (+75.36%)
P-value between groups					
	p>0.05	p>0.05	p<0.05	p<0.05	p<0.001

Note: * – p<0.001 from the initial

At the initial stage of recruitment to the study, the lots were homogeneous after telediastolic (TDE) – in the first group it was 253.03±27.59 ms vs 257.78±33.09 ms in group II (p>0.05). When analyzing the cohort at 3, 6, 9 and 12 months of treatment, the statistically true reduction of this parameter in both groups was assessed. The comparative analysis between the batches did not note statistically authentic differences at 3 months of medication (in the first group with -7.23% at 3 months of uninterrupted medication vs -7.67% in group II, p>0.05), but starting with the 6th month of treatment, the medication with meldonium association proved to be more beneficial in reducing this parameter, obtaining also statistical significance, constituting -12.92% in group I vs -17.67% in group II, p<0.05; at 9 months the reduction of the TDE duration was -19.37% in group I vs -23.31% in group II, p<0.05; at 12 months -23.89% in group I vs -28.88% in group II and constituted 192.59±18.61 ms in group I vs 183.33±14.78 ms in group II, p<0.05. It is worth mentioning that the recovery to the values considered normal for TDE was achieved towards the 6th month of uninterrupted medication in both batches of patients (tab. 2).

According to the E/A and TDE ratio, the distribution of patients by type of diastolic dysfunction was as follows: at the initial stage out of 56 patients in the first group, 53 (94.64%) had diastolic dysfunction LV delayed relaxation type, and 3 patients (5.36%) – pseudonormal type of ventricular filling. In the second group, out of 45 patients enrolled to the study, 43 (95.56%) presented at the initial stage with diastolic dysfunction type delayed relaxation, and 2 patients (4.44%) – pseudonormal type of transmittral pattern. After 3 months of treatment, the share of patients with the pathological pattern of ventricular filling type delayed relaxation was reduced in both groups: group I – 42 patients (75%), group II – 33 patients (73.33%), and the number of patients with pseudonormal impairment of diastolic function remained unchanged (3 patients in group I and 2 patients in group II). Already after 3 months of medication, it was noted the normalization of ventricular filling in 11 patients (19.64%) of the first group and in 10 patients (22.23%) in group II. After 6 months of medication, the process of „migration" of patients from a (more) pathological pattern of ventricular filling into a (more) physiological one continued. Thus, the number of patients with echocardiographic presentation typical of delayed relaxation has decreased both in the first group (32 patients), but especially in group II (10 patients). At the same time, the share of patients with pseudonormal type of diastolic vs dysfunction in both groups was reduced: 2 patients (3.57%) in group I and 1 patient (2.22%) in group II. After 6 months of continuous medication, the number of patients with normal ventricular filling pattern increased dramatically: 22 patients (39.29%) in the group treated with basic treatment compared to 34 (75.56%) in the group treated with meldonium association. The same beneficial trend was maintained at 9 and 12 months of uninterrupted medication, with a major gap in favor of medication with meldonium association. Thus, the number of patients with normal diastolic function in both groups, but with a net superiority to meldonium combination administration, has considerably increased: 41 patients (91.11%) in group II vs 33 (58.93%) in the group treated with basic treatment after 9 months of medication; 43 patients (95.56%) group II vs 36 (64.29%) group I at 12 months of medication. During this period, no patients with pseudonormal type of diastolic dysfunction were registered, these passing into a "more" favorable category – delayed relaxation. This last ventricular filling pattern marked a continuous decrease

Table 2. Evolution of TD index depending on medication

TD, ms					
	Initial	**3 months**	**6 months**	**9 months**	**12 months**
Group I	253.03±27.5	234.73±6.9* (-7.23%)	220.35±25.3* (-12.92%)	204.02±22.4* (-19.37%)	192.59±18.6* (-23.89%)
Group II	257.78±33.0	238.0±8.7** (-7.67%)	212,22±23,7* (-17.67%)	197.67±16.1* (-23.31%)	183.33±14.7* (-28.88%)
	P-value between groups				
	p>0.05	p>0.05	p<0.05	p<0.05	p<0.05

Note: * – p<0.001 compared to the initial, ** – p<0.05 compared to the initial

Table 3. Distribution of patients by type of diastolic left ventricle dysfunction

	Initial		3 months		6 months	
	Gr. I	Gr. II	Gr. I	Gr. II	Gr. I	Gr. II
Delayed relaxation	53 (94.64%)	43 (95.56%)	42 (75%)	33 (73.33%)	32 (57.14%)	10 (22.22%)
Normal pseudo-damage	3 (5.36%)	2 (4.44%)	3 (5.36%)	2 (4.44%)	2 (3.57%)	1 (2.22%)
Normal diastolic function	0	0	11 (19.64%)	10 (22.23%)	22 (39.29%)	34 (75.56%)

	9 months		12 months	
	Gr. I	Gr. II	Gr. I	Gr. II
Delayed relaxation	23 (41.07%)	4 (8.89%)	20 (35.71%)	2 (4.44%)
Normal pseudo-damage	0	0	0	0
Normal diastolic function	33 (58.93%)	41 (91.11%)	36 (64.29%)	43 (95.56%)

in both groups, reaching its peak towards the end of the study and more evident in the group, in which meldonium association was administered: 20 patients (35.71%) in group I vs 2 (4.44%) in group II (tab. 3)

Thus, both types of medication have beneficially influenced the compromised lusitropia of left ventricle, but a clearly superior potency in the restoration of physiological parameters of diastolic function was demonstrated in the group treated with mildronate association.

Discussion

The analysis of the peculiarities of the influence of meldonium on the metabolism of cardiomyocytes in myocardial ischemia in young and old patients leads to the idea of an amazing harmony of changes. The explanation of the results obtained may be the mechanism of action of meldonium. This drug preparation blocks carnitine biosynthesis from the butyrobetain range, causing a double positive effect [3-6]. First of all, it reduces the concentration of carnitine, a transporter of fatty acids through the mitochondrial membrane, which causes energy-saving effects [2, 4, 10]. Secondly, it increases the concentration of gamma-butyrobetaine, which excites acetylcholine receptors and stimulates nitric oxide biosynthesis – the mediator of the stress-limiting NO-ergical system, universal regulator of the adaptation process [2, 5, 9]. In the conducted clinical trials, the ability of meldonium to provide an adaptogenic effect by regulating the biosynthesis of NO has been demonstrated [1-8]. Probably this mechanism has a certain contribution to the realization of such a harmonious influence of the preparation on the metabolism of cardiomyocytes in the conditions of myocardial ischemia in both young and old patients.

In the result of the study, the distribution of patients after the geometry of the myocardium LV remodeling was as follows: at the initiation of the study, all types of left ventricular remodeling had a comparable incidence between the groups. In this context, it was noted the prevalence of patients with ventricular geometry type concentric hypertrophy: 52 patients (92.85%) in group I vs 42 (93.33%) in group II. The maladaptation of the left ventricle myocardium to the pressure overload determined the development of concentric remodeling in 1 patient each in the first group (1.79%) and II (2.22%), eccentric hypertrophy – 3 patients (5.36%) in group I and 2 patients (4.45%) in group II. After 3 months of treatment, it was noted the reduction of the share of patients with the pathological pattern of remodeling LV concentric LVH type in both groups: group I – 50 patients (89.28%) vs 39 (86.66%) in group II. At the same time, it was noted the absence of patients with concentric remodeling LV in both groups, as well as the presence of reverse reshaping of the LV myocardium with the achievement of the values considered normal. After 6 months of continuous medication, the trend of reducing the number of patients with the geometric pattern of myocardial remodeling LV concentric LVH type (group I – 78.58% vs 75.56% in group II) was maintained, with the increase in the number of people expressing normal ultrasound phenotype of LV in both batches of patients (10.71% in group I vs 13.33% in group II), but with a slight prevalence in the group treated with meldonium combination. At the same time, it was noted the presence of patients with concentric remodeling LV in both groups (1 patient each), and the eccentric LVH was designated in equal proportions in both groups: 5 patients (8.93%) in group I and 4 patients (8.89%) in group II. Towards the 9th month of treatment, the share of patients with normal aspect of the LV in both groups increased considerably, but with a major gap between the groups in favor of meldonium association: in 12 patients of group I (21.43%) vs 20 patients (44.44%) in group II. At the same time, the number of patients with pathological remodeling of the concentric type LV was reduced to 37 (66.07%) in the first group and 21 patients (46.67%) in group II, but the share of patients with concentric remodeling left ventricle from the account of migration from a (more) pathological category to a (more)

physiological one (3 patients in group I and 4 in group II) increased numerically. Towards the end of the treatment period, only 33.93% (19 patients) of the group treated with basic treatment presented at the normal-looking left ventricle echocardiographic examination, compared to the group treated with meldonium association, where 60% of the patients (27 patients) demonstrated a normal geometric pattern of the left ventricle.

Thus, the data obtained confirmed the benefit of using cardiocitoprotection in inducing reverse-remodeling of the myocardium LV regardless of the initial ventricular geometric pattern, but the administration of mildronate association demonstrated a much superior efficiency compared to the basic treatment in the regression of LVH, a notable event towards the end of the research period.

Conclusions

1. The molecular structure of the pharmacologically studied drug of the metabolic series – meldonium, has a duality of action; under certain conditions, the metabolic corrector is able to exhibit complex pharmacodynamic effects.
2. The data obtained confirmed the benefit of using cardiocitoprotection in inducing reverse reshaping of myocardium left ventricle regardless of the initial ventricular geometric pattern, but meldonium combination administration demonstrated a significantly superior efficiency compared to the basic treatment in hypertrophy of left ventricle regression, a notable event towards the end of the research period.
3. A general concept of personalization of the metabolic pharmacotherapy of meldonium has been developed, according to which it is able to present a cytoprotective effect depending on the initial state of the functional adaptation system.

References

1. Dambrova M, Makrecka-Kuka M, Vilskersts R, et al. Pharmacological effects of meldonium: biochemical mechanisms and biomarkers of cardiometabolic activity. Pharmacol Res. 2016;113:771-780. doi: 10.1016/j.phrs.2016.01.019.
2. Greenblatt HK, Greenblatt DJ. Meldonium (mildronate): a performance-enhancing drug? Clin Pharmacol Drug Dev. 2016;5(3):167-169. doi: 10.1002/cpdd.264.
3. Schobersberger W, Dünnwald T, Gmeiner G, et al. Story behind meldonium – from pharmacology to performance enhancement: a narrative review. Br J Sports Med. 2017;51(1):22-25. doi: 10.1136/bjsports-2016-096357.
4. Dzerve V, Matiosone D, Kukulis I, et al. Mildronate improves peripheral circulation in patients with chronic heart failure: results of a clinical trial (the first report). Semin Card. 2005;11(2):56-64.
5. Dambrova M, Liepinsh E, Kalvinsh I. Mildronate cardioprotective action through carnitine-lowering effect. Trends Cardiovasc Med. 2002;12(6):275-279. doi: 10.1016/s1050-1738(02)00175-5.
6. Zhao Z, Chen J, Peng W, et al. Single- and multiple-dose pharmacokinetic, safety and tolerability study of mildronate injection in healthy Chinese subjects pharmacokinetic of mildronate injection. Drug Res. 2015;66(5):251-256. doi: 10.1055/s-0035-1569297.
7. Panchaud A, Csajka C. Outbreak in meldonium positive laboratory tests: are we missing something? Br J Sports Med. 2016;50(22):1422-1423. doi: 10.1136/bjsports-2016-096253.
8. Liepinsh E, Skapare E, Kuka J, et al. Activated peroxisomal fatty acid metabolism improves cardiac recovery in ischemia-reperfusion. Naunyn Schmiedebergs Arch Pharmacol. 2013;386(6):541-550. doi: 10.1007/s00210-013-0849-0.
9. Liepinsh E, Vilskersts R, Skapare E, et al. Mildronate decreases carnitine availability and up-regulates glucose uptake and related gene expression in the mouse heart. Life Sci. 2008;83(17-18):613-619. doi: 10.1016/j.lfs.2008.08.008.
10. Klusa V, Pupure J, Isajevs S, et al. Protection of azidothymidine-induced cardiopathology in mice by mildronate, a mitochondria-targeted drug. Basic Clin Pharmacol Toxicol. 2006;99(4):323-328. doi: 10.1111/j.1742-7843.2006.pto_543.x.
11. Sjakste N, Kalvinsh I. Mildronate: an anti-ischemic drug with multiple indications. PharmacologyOnLine. 2006;1:1-18.
12. Sesti C, Simkhovich BZ, Kalvinsh I, et al. Mildronate, a novel fatty acid oxidation inhibitor and antianginal agent, reduces myocardial infarct size without affecting hemodynamics. J Cardiovasc Pharmacol. 2006;47(3):493-499. doi: 10.1097/01.fjc.0000211732.76668.d2.
13. Šlampová A, Kubáň P. Rapid determination of meldonium in urine samples by capillary electrophoresis with capacitively coupled contactless conductivity detection. J Chromatogr A. 2016;1468:236-240. doi: 10.1016/j.chroma.2016.09.027.
14. Görgens C, Guddat S, Dib J, et al. Mildronate (meldonium) in professional sports – monitoring doping control urine samples using hydrophilic interaction liquid chromatography – high resolution/high accuracy mass spectrometry. Drug Test Anal. 2015;7(11-12):973-979. doi: 10.1002/dta.1788.
15. Liepinsh E, Vilskersts R, Loca D, et al. Mildronate, an inhibitor of carnitine biosynthesis, induces an increase in gamma-butyrobetaine contents and cardioprotection in isolated rat heart infarction. J Cardiovasc Pharmacol. 2006;48(6):314-319. doi: 10.1097/01.fjc.0000250077.07702.23.

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