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## METABOLIC CARDIOPROTECTION: NEW CONCEPTS IN IMPLEMENTATION OF CARDIOPROTECTIVE EFFECTS OF MELDONIUM

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**Abstract.** Recent studies confirm the need to find means to correct ischemic / reperfusion injury due to the hemodynamic medicine, which are already known do not have the proper cardioprotective effects. Key issue is the possibility of drug effects on the mitochondria of cardiomyocytes that controls the aerobic metabolism and maintenance of ATP admission into cardiomyocytes. Moreover mitochondria are the target of ischemia / reperfusion injury and involved in the cardioprotective mechanism ischemia and pharmacologic pre- and postconditioning. Effect of nitric oxide during ischemic reperfusion directed to mitochondria, is considered as the ultimate goal of cardioprotection. Preconditioning effects is begun from sarcolemmal membrane and then is directed to the cytoplasm through a plurality of stages of enzymes, including nitric oxide synthase (NOS), soluble guanylatecyclase (sGC) and protein kinase G (PKG). Thus, the signal is transmitted to the mitochondria, where it occurs cardioprotection. It is proved that the mitochondria provides protection of the heart against ischemia-reperfusion injury by opening the mitochondrial ATP-sensitive K<sup>+</sup> channels and by converting the capacity of mitochondria. Metabolic modulation concept can be used in the development of strategies cardioprotection for treatment in ischemic / reperfusion myocardial injury.

**Keywords:** meldonium, pharmacological preconditioning, ischemic / reperfusion injury, heart.

### A brief review on the mechanisms of meldonium action.

#### 1. Influence on metabolic rate

Over the last few decades in medicine "metabolic" trend has been developing intensively, which aims at the analysis of cell metabolism disorders in cardiovascular disease. Changes in metabolism in ischemic cardiomyocytes can be regarded as the point of application of medical effects, particularly with drugs that can affect the processes occurring in mitochondria directly. By now, it has been created a number of drugs that directly affect the metabolic processes in cardiomyocytes, which is known as "myocardial cytoprotectors" [1, 2, 3, 4, 5, 6].

It is accumulated activated long-chain fatty acids in myocardial cells in ischemic heart disease [7, 8]. These conjugates of fatty acids inhibit additional myocardial oxygenation and intracellular production of ATP. As it has been shown in some studies, the delay of synthesis of ATP as well as the intracellular accumulation of long-chain fatty acids can reduce L-carnitine [9, 10]. In his studies on isolated porcine heart A. J. Liedtke et al. [11] showed that carnitine

blocked toxicity of long-chain fatty acids during hypoxic myocardial injury [12, 13].

However, under normal physiological concentration carnitine stimulates the transport of fatty acids. However, at high concentrations of carnitine it is pumping oxidized fatty acids from cells, which lead to the destruction of the myocardium. The ability to reduce the size of infarct is caused by a decrease of L-carnitine content in the tissues of the heart, and the subsequent inhibition of fatty acid transport and protection of the outer mitochondrial membrane of myocardial mitochondria

Meldonium is a drug, which can reduce the level of carnitine. It blocks the synthesis of carnitine from  $\gamma$ -buterobetaine by reversible competitive inhibition of the enzyme gamma-butyrobetaine hydroxylase, which reduces the carnitine-dependent transport of fatty acids into the mitochondria [14, 15, 16].

Under normal conditions, when there is no shortage of oxygen, energy of the myocardium is mainly formed from fatty acids (FA) and glucose to adenosine triphosphate(ATP).According to the needs, it is occurred the mobilization of FA by the signal the nervous system via carnitine, which activated

transport of FA across the mitochondrial membrane, where is supplied a sufficient amount of oxygen [17].

The result of mitochondrial  $\beta$ -oxidation of FA is formed acetyl-coenzyme A, which enters into the Krebs cycle, where ATP is synthesized. Another source of energy is a way of aerobic or anaerobic oxidation of glucose. However, myocardial metabolism varies in ischemic conditions. Short-chain and long-chain FA enter the mitochondria, but there is not enough oxygen for the oxidation in the cell. Consequently, in the mitochondria of ischemic tissue it is accumulated metabolites unoxidized FA (acylcarnitine and acyl-coenzyme A (acyl-CoA)), which block transport earlier synthesized ATP from mitochondria into the cytosol. It causes a devastating effect on the cell membranes, which can lead cells to ischemic death. In addition, the accumulation of FA blocks the oxidation of glucose (it is studied during reperfusion of ischemic myocardium), and long-chain acylcarnitine contributes to reductions in ischemic myocardium, which leads to a vicious circle [6, 18]. The consequence of the toxic effect of non-oxidized metabolites FA is the blockade of  $\text{Ca}^{2+}$ -ATPase of the sarcoplasmic reticulum – calcium pump required for normal myocardial contractility. Consequently, it is necessary to restrict the flow of long-FA through the mitochondrial membrane for the correction of disturbed metabolism in the ischemic cells, simultaneously activate an alternative oxidation mechanism of the glucose for energy production in the cells [19, 20, 21]. In conditions of an oxygen shortage, it is more advantageous to use an oxidation of glucose than FA, since this process requires less amount oxygen by the cells. The formation of ATP through aerobic glycolysis requires 12% less oxygen than ATP production by the oxidation of FA.

On the one hand, due to oxygen deficiency, the oxidized products of FA (acylcarnitine and acyl-CoA) accumulate in the myocardial ischemia, transport of ATP from mitochondria is blocked, cell membranes are destroyed, ionic composition is changed, and contracture of ischemic myocardium is developed.

On the other hand, in hypoxia, glucose oxidation only occurs to lactate, acidosis and electrical instability of myocardium are developed, and arrhythmias arise. Thus, under ischemic conditions, it is essential to change the process of ATP production from FA oxidation to glycolysis. Thereby the need of the cells in oxygen for ATP production is reduced and it is prevented the accumulation of activated forms FA in the cells mitochondria [22, 23]. Thus, it is important to use the oxygen sparing cytoprotection for the treatment of ischemic heart disease what are partial inhibitors of oxidation, activators of FA transport and glucose oxidation. All the preparations for metabolic therapy in cardiology can be divided into two groups: metabolic therapy preparations and preparations for the correction

of metabolism. According to modern concepts, "ideal" metabolism corrector should prevent the accumulation of a large amount of unoxidized FA in the cells (prevention of cell membrane damage), activate the capture and oxidation of glucose by cells, inhibit the formation of lactate and stimulate the oxidation of pyruvate, as well as being able to prevent the formation of activated oxygen species (prevention oxidative stress). To the greatest degree, the "ideal" metabolism corrector may include well-known meldonium, because it affects all three components [24, 25, 26].

### Cytoprotection mechanism of myocardial ischemia

Meldonium belongs to a group of so-called cytoprotectors–antihypoxants, which provide protection and power of the body cells in conditions of ischemia and increased load. Mildronat is a structural analogue of the gamma-butyrobetaine (GBB), and therefore it is a competitive inhibitor of the GBB-hydroxylase – the last enzyme in the chain of carnitine biosynthesis in the body of humans and animals. Consequently, the drug decreases concentration of carnitine in serum, and in the cytosol, and mitochondrial matrix reversibly. By several researchers it has been proposed another possible way of meldonium influence on the content of carnitine in the body. It has been proved, that meldonium is also an inhibitor of carnitine reabsorption in the kidney, because it reduces renal transport of carnitine [27]. This mechanism provides a rapid decrease concentration of carnitine in the blood, which subsequently affects the gradual decrease of its concentration in the tissues. As a result, carnitine is not reabsorbed in the kidneys and is not metabolized again and excreted from the body immediately. Reduced carnitine levels has a dual effect on the human body. Limiting the availability of carnitine in the cytosol reduces the rate of activation and transport of long-chain FA to the place of their oxidation in mitochondria. In other words, in ischemia meldonium slows the rate of penetration and accumulation of long-chain fatty acids in mitochondria. Thereby it prevents blockage transport ATP from mitochondria to the cytosol and mitochondrial membrane integrity disruption due to the destroying properties of activated FA (acylcarnitine and acyl-CoA). Due to the limited transport and oxidation of fatty acids in the mitochondria, their concentration in the cytosol increases, which is a signal of inclusion of alternative ways of energy production by aerobic glycolysis. It established, that meldonium increases the sensitivity of the insulin receptor to insulin and stimulate the insulin controlled glucose uptake that promotes the availability of glucose for inclusion in the energy production processes [28, 29].

At the same time meldonium activates the two most important enzyme of aerobic glycolysis – hexokinase and pyruvate dehydrogenase, which

involve pyruvate formed from sugars into the Krebs cycle, and it prevents the formation of lactate. Moreover, it is not only increased the activity of these enzymes under the influence of meldonium, as well as it is induced their biosynthesis.

It is found, that the development of atherosclerosis and associated incidents of myocardial infarction, stroke and mortality directly correlated with the concentration of carnitine in the plasma of patients and increased levels trimethylamine oxide, which is a metabolite of carnitine because of its degradation by intestinal microorganisms [30, 31]. The explanation is that trimethylamine oxide, which is generated from carnitine, blocks macrophages capture of excessive amounts of cholesterol at its peak concentration in the blood, and return transport of cholesterol from plasma into the liver and the bile. As a result, the formation of cholesterol lesions can occur even at low levels of average values in plasma cholesterol concentration. This explains the mechanism by which meldonium significantly reduces plaque formation in the aorta in animals predisposed to atherosclerosis [32, 33]. Also, in patients with diabetes mellitus type 2 (DM2) of disease severity is directly correlated with the concentration of carnitine in the blood plasma [34]. This indicates the need to review the use of carnitine or carnitine-containing products, as the degradation of carnitine to trimethylamine oxide significantly increased risk of atherosclerotic cardiovascular disease [30].

The only remedy in the world, which reduces the level of carnitine in humans, is meldonium. A pilot study in healthy volunteers, who received dietary trimethylamine, or its predecessors, has shown, that meldonium not only reduces the level of carnitine, but also reduces the level of formation of pro-atherogenic factor – trimethylamine oxide. Thus, the use of meldonium to reduce to safe levels of carnitine and trimethylamine oxide is pathogenetically justified and purposeful way of preventing the development of atherosclerosis, and possibly the DM2. Consequently, meldonium is not only has unique cytoprotective properties in ischemic lesions of different etiology and localization, but also acts on the molecular mechanisms of atherosclerosis. Possible mechanisms of actions of meldonium is discussed in the publications of several authors [35, 36].

#### **The points of action meldonium: protection of endothelial cells and cardioprotective effect**

Another important pathogenetic part of the defeat of the cardiovascular system is the development of dysfunction of vascular endothelium [37, 38, 39, 40], the consequent remodeling of the vascular wall and formation of angiopathy in arterial hypertension, which creates conditions for stable high blood pressure, reducing the effectiveness of antihypertensive drugs, the development of atherosclerotic lesions of arteries [41, 42, 43, 44].

A special role in the regulation of vascular tone plays nitric oxide. Nitric oxide is the most potent endogenous vasodilator. Nitric oxide, acting via guanylate cyclase, increases the formation of cyclic guanidin monophosphate, accumulation of which is the causes of vascular relaxation. Oxidative stress and the high concentration of free radicals leads to accelerated degradation of nitric oxide [45]. The development of endothelial dysfunction in hypertension accompanied by apoptosis of vascular endothelial cells caused by free radicals and a violation of the processes of intracellular energy transfer, so the correction of free-radical processes and intracellular metabolism in the vascular endothelium is one of the conditions for the effective treatment of hypertension and endothelial dysfunction.

In this regard, meldonium may be the drug for correction dysfunction of the vascular endothelium and inhibition of free radical oxidation processes, as plasma concentration of carnitine precursor – gamma-butyrobetaine increases under the influence of meldonium, which is first and foremost – its esters – promotes NO biosynthesis – nitric oxide, which is the main factor regulating vascular tone, and also affects platelet aggregation and flexibility of red blood cells. It has shown that the  $\gamma$ -butyrobetaine esters have potent acetylcholine-like effect on blood vessel tonus. Meldonium has the same effect [46, 47, 48]. Consequently, there are carnitine-independent effects of meldonium that cause a positive effect on the microcirculation. It should be emphasized, that the characteristic feature of NO is the ability rapidly (in less than 5 seconds) to diffuse through the membrane in its cells synthesize extracellular space and easily (without the receptors) to penetrate into the target cells. Inside the cell, it activates one and inhibits other enzymes participating in the regulation of cellular functions, and in fact acting as a local signaling molecule. NO is a potent vasodilator, which is synthesized in the endothelium of the vascular wall, it quickly penetrates into the subendothelial space, and affects vascular smooth muscle cells. NO molecule reduces intracellular calcium by adenylate cyclase mechanism. This leads to the relaxation of vascular smooth muscle cells, improve microcirculation and endothelial function [49, 50, 51].

#### **New concepts in implementation of cardioprotective effects meldonium**

Another promising direction of studying the mechanisms of action meldonium is a pharmacological preconditioning. One version is the inclusion of compensatory mechanisms by inhibiting the synthesis of carnitine.

In addition, a number of authors attempted to explore a new approach to the possible mechanism of action meldonium [52, 53, 54, 55]. In a number of experimental studies have been proven the ability meldonium activate a cascade of reactions through ATP-dependent potassium channel system which



lead to the implementation of the preconditioning phenomenon. Thus, they provide endothelial- and cardioprotective effect as well as anti-ischemic effect through this mechanism. These properties are

essential for highly efficient drug, which is used for treatment and prevention of diseases associated with endothelial dysfunction Figure 1.

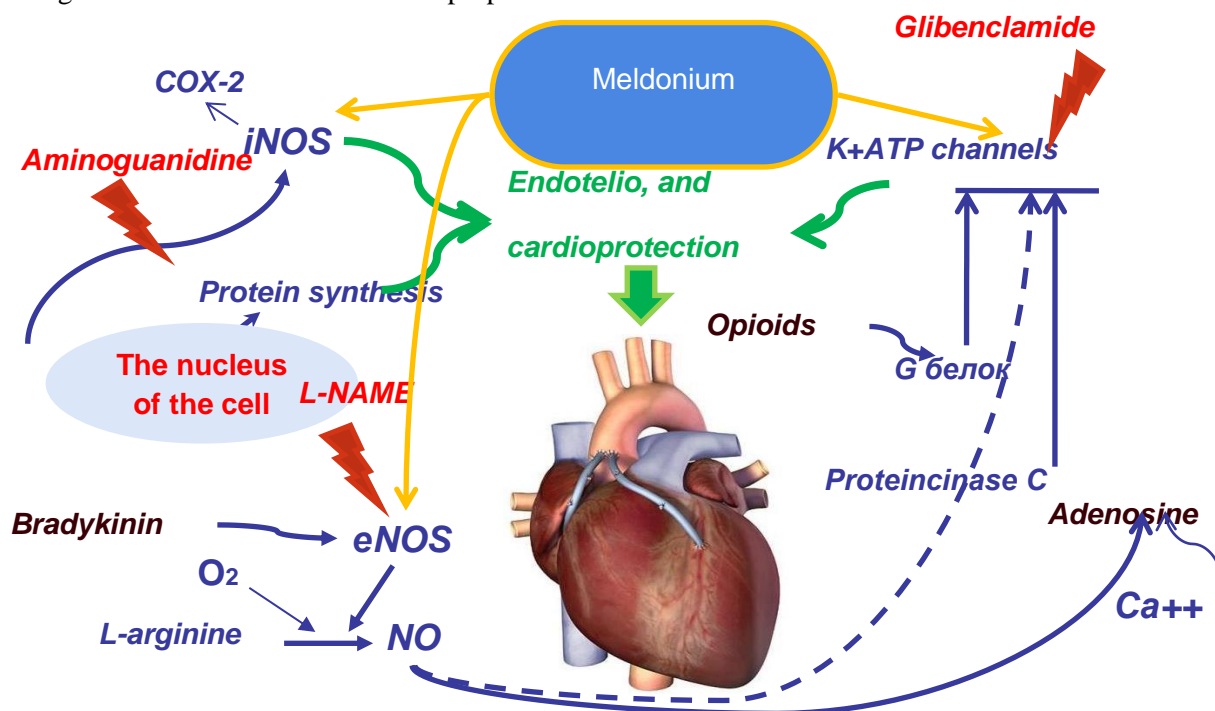


Figure 1. The hypothetical role meldonium in the mechanism of ischemic preconditioning.

Note. K<sup>+</sup> АТФканалы – ATP-dependent potassium channel; eNOS – endothelial NO-synthase; iNOS – inducible NO-synthase; L-NAME – N- nitro-L-arginine-methyl ester; NO – Nitric oxide; ПФК – reactive oxygen species; ЦОГ-2 – cyclooxygenase-2

ATP-dependent potassium channels are the effector mechanism in the implementation of anti-ischemic effect, acts as a distant ischemic preconditioning and meldonium/ Nitric oxide acts as a trigger of ischemic preconditioning [56, 57, 58].

In either case, its synthesis is carried out by activation of the inducible and endothelial NO-synthase. Meldonium competing for the receptors, gamma-butyrobetaine, it reduces the concentration of carnitine. Due to the geometric similarity gamma-butyrobetaine to acetylcholine it activates endothelial acetylcholine receptors which lead to the induction of NO synthesis. It follows that the meldonium can be recommended as the drug for pharmacological preconditioning.

Thus, the therapeutic mechanism and protective action of meldonium is its effect on carnitine levels, trimethylamine oxide and metabolic energy pathways, which provides a more economical and efficient functioning of cells under conditions of oxygen shortage. Antiischemic effects of meldonium are achieved by reducing the intensity of fatty acid oxidation in ischemia (oxygen savings), activation mechanisms to capture and oxidation of glucose for energy production pharmacological preconditioning, which includes compensatory mechanisms – training through the suppression of the synthesis of carnitine, and induction

of NO biosynthesis, vasoactive effects by reducing peripheral vascular resistance and endothelial protective effect via induction of NO biosynthesis.

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