PATHOGENIC APPROACH
to cardiac ischemia therapy

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RELEVANCE OF THE PROBLEM

Coronary artery disease (CAD) has steadily occupied one of the leading places in the structure of appealability, disability and mortality in cardiovascular diseases (CVD) in recent decades. In developed European countries, 30-40 thousand patients with this pathology per 1 million people were recorded [1]. According to S. Sutcliffe et al. (2003), effort angina is the first manifestation of CAD in 46% of cases, myocardial infarction (MI) – in 27%, sudden death – in 14%, and unstable angina – in 13% [2].

There are more than 15 million new cases of myocardial infarction recorded worldwide yearly: 35% of patients die on Days 1-7; and another 15-20% – during the first year. According to the American Heart Association, 18% of men and 35% of women experience a second infarction within the next six years after MI, 22% of men and 46% of women develop severe heart failure [3].

At the same time, according to the results of medical and statistical studies submitted by WHO, there is still no global trend for decreasing mortality from cardiovascular diseases, including CAD. This determines the urgency of developing and introducing new approaches to the diagnosis and treatment of this disease into clinical practice.

In recent years, the paradigm for treating patients with CAD has undergone changes associated with the evolution of understanding the pathogenesis of this disease. Therefore, not only atherosclerotic changes in coronary vessels, but also the degree of endothelial dysfunction, the lack of energy substrates in the mitochondria of cardiomyocytes, the state of the microcirculatory bed, that lead to ischemic changes occurring at the cellular level are currently in the focus for researchers and physicians. Accordingly, the emphasis in the strategy of drug treatment of patients with CAD shifted to innovative approaches that ensure not only full coronary blood flow, but also effective microcirculation, correction of endothelial dysfunction, and maintenance of energy processes at the level of cardiomyocytes.

The results of the studies in this field and related changes in official recommendations suggest that the introduction of such approaches into clinical practice in the near future will be crucial for improving the efficacy of therapy in patients with CAD [27].

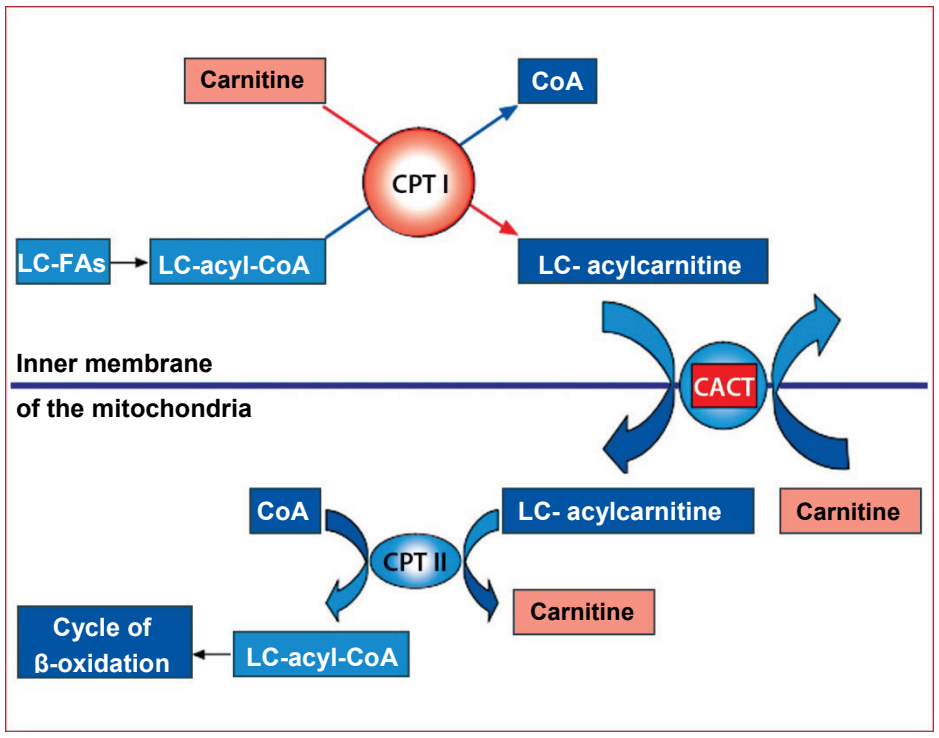

Figure 1. Transport of long-chain fatty acids from the cytoplasm in the mitochondria with the help of the carnitine shuttle

ENERGY METABOLISM IN CARDIOMYOCYTES IN PATHOLOGICAL CONDITIONS

In physiological conditions, at rest, the molecules of adenosine triphosphate (ATP) in the heart are formed as a result of oxidation of energy substrates, mainly long-chain fatty acids (DC-FAs), whose contribution to ATP formation is about 70-80%, as well as glucose and lactate [28], pyruvate, amino acids, and ketone bodies. The basic pathway of energy metabolism is associated with β-oxidation of fatty acids (FAs) in the mitochondria, and the auxiliary pathway is represented by glycolysis with subsequent oxidation in the mitochondria of pyruvate. Bioenergetic processes in the myocardium are normally provided almost exclusively by ATP produced in the mitochondria as a result of oxidative phosphorylation in the presence of oxygen.

To convert the energy contained in fatty acids into ATP binding energy, there is a metabolic pathway for the oxidation of fatty acids to CO_2 and water, which is closely related to the cycle of tricarboxylic acids and the respiratory chain. This pathway is called β-oxidation, since the oxidation of the third carbon atom of the fatty acid (β-position) to the carboxyl group occurs; simultaneously, the acetyl group comprising C_1 and C_2 of the initial fatty acid is cleaved from the acid. β-oxidation reactions occur in the mitochondria of most body cells (except for the nerve cells). For oxidation, fatty acids that enter the cytosol from the blood (from food) or appear in the lipolysis of intracellular triacylglycerols are used.

The main energy-producing compounds in the myocardium are free fatty acids (FFAs), for the oxidation of which about 70% of the oxygen consumed by the heart is used. The remaining 30% is spent mainly on the aerobic oxidation of carbohydrates.

The oxidation of FFA starts from the preparatory stage – the preliminary activation of the fatty acid, which is expressed in the formation of esters of fatty acids with coenzyme A (CoA) and their subsequent interaction with carnitine, resulting in the formation of carnitine esters with fatty acids. These events occur in the cytoplasm in the extra-mitochondrial space of the cell. Binding of carnitine to FA contributes to the transfer of FFA through the membranes into the mitochondria by enzymatic complexes – carnitine palmitoyltransferase I and II (CPT I and CPT II). Thereat, L-carnitine plays an important role in the energy metabolism of the heart, as it participates in the transport of the acyl residues of FA by means of the "carnitine shuttle" system. It consists of carnitine, CPT I located on the outer membrane of mitochondria, carnitine-acylcarnitine translocase (CACT) localized in the internal membrane, which exchanges mitochondrial carnitine and acylcarnitines of the cytoplasm, and CPT II located in the matrix of the mitochondria. Since activated FFAs in the form of acyl-CoA are soluble in water, rather than in lipids, they are unable to penetrate the inner membrane of the mitochondria. Consequently, CPT I catalyzes the transfer of FA acyl residue from CoA to L-carnitine, resulting in the formation of an ether, acylcarnitine, which is transported by carrier molecules, CACT, through the inner membrane of the mitochondria in exchange for free matrix carnitine which is transferred from the mitochondria to the cytoplasm [5].

In the matrix of the mitochondria, long-chain acylcarnitine undergoes CPT II exposure and is converted to acetyl-CoA. The latter enters the cycle of β-oxidation of FAs, during which its acyl residue is shortened to the length of medium-chain acyl-CoA and then of short-chain, with formation of one acyl-CoA in each turn of oxidation. When the intake of acyl-CoA exceeds its consumption in the cycle of β-oxidation, acyl-CoA is re-converted into acylcarnitines which are removed from the mitochondria into the cytoplasm, and then from the cells to the blood. This process prevents accumulation of acyl-CoA in the cytoplasm and inhibits the onset of lipotoxic effect [6].

From acetyl-CoA, formed in the mitochondria and entered into the cytoplasm, malonyl-CoA is synthesized. It reduces the activity of the key, the slowest enzyme in the oxidation of lipids of CPT I, thereby inhibiting the transport of long-chain FAs in the mitochondria and their oxidation, reducing the contribution of FAs to the synthesis of ATP.

Thus, along with CPT I, CACT and CPT II, L-carnitine appears to control the rate of oxidation of long-chain FAs, acting as a specific cofactor facilitating their transfer through the inner membrane of the mitochondria.

Impaired functional activity of the mitochondria plays a key role in a number of pathological states, including damage caused by ischemia/reperfusion, observed in angina pectoris, myocardial infarction, heart failure, and acute coronary syndrome (ACS). At the same time, it is known that even in severe ischemia, FFAs remain the predominant substrate of oxidation, despite the increased utilization of glucose [7, 8]. Even with a 50% restriction of cardiac blood flow, the myocardium satisfies its energy requirements by 50-70% due to the oxidation of FFAs, despite the concurrent sharp increase in lactate production [9]. In order to understand the nature of this distribution, one should pay attention to the efficiency (expressed in the number of ATP molecules) of obtaining energy for a cardiomyocyte from aerobic cleavage of FFA in β-oxidation or anaerobic cleavage of glucose. For example, the oxidation of one molecule of palmitic acid leads to formation of 129 ATP molecules, and the oxidation of glucose to pyruvic acid leads to formation only 2 ATP molecules during glycolysis.

Some authors believe that this circumstance – the imbalance in the processes of FFAs oxidation and glycolysis – can initiate a cascade of molecular-

cellular events that exacerbate the course of myocardial ischemia and cause such pathophysiologic manifestations as reperfusion injury and myocardial dysfunction, as well as severe heart rhythm disorder [10, 11].

In ischemia, the lack of oxygen leads to inhibition of ATP formation, decrease in contractile activity of the heart, occurrence of sympathoadrenal stress which results in an additional release of a large number of long-chain FAs, coming from the blood into cardiomyocytes to cover energy deficiency, from adipocytes. Excessive level of acetyl-CoA, formed in the cycle of β-oxidation of FAs, inhibits the activity of pyruvate dehydrogenase, preventing the oxidation of pyruvate in the mitochondria and promoting its conversion into lactic acid. As a result, the intracellular acidosis is developed, the ion homeostasis is affected. Removal of the excess of acetyl-CoA from the mitochondria by means of formation of acetylcarnitine and activation of carnitine acetyltransferase leads to the synthesis of a large amount of CPT I inhibitor, malonyl-CoA, which inhibits the oxidation of long-chain FFas in the mitochondria. In addition, L-carnitine participates in the removal of excess long-chain FFAs from the mitochondria, and then from the cytoplasm, preventing their cytotoxic effect. With this regard, the important protective effect of L-carnitine in ischemia becomes clear. Thus, it is obvious that a sufficiently high content and exchange of L-carnitine in the body plays an important role in supporting the normal functioning of cells [12, 13].

L-CARNITINE IN CLINICAL STUDIES IN PATIENTS WITH CARDIOVASCULAR DISEASES

The effect of L-carnitine in patients with angina has been studied and confirmed in several randomized, placebo-controlled studies that differ in design, duration of drug administration, and dosage used. All studies demonstrated a significant increase in exercise tolerance (an average of 14%), time to onset of ST depression during stress testing (15-25%) and load before the onset of an angina attack, as well as a decrease in the severity of ST segment depression [14].

The evidence that L-carnitine limits the volume of the affected myocardium in acute MI can be retrieved from the data obtained in several clinical studies. Thus, in a placebo-controlled randomized study, the administration of L-carnitine (2 g/day) produced significantly lower levels of creatine phosphokinase (CPK) compared to placebo at Day 28 of the onset of the disease (101 patients). The smaller was the QRS index on ECG, defined as the sum of the Q- and R-waves in the V_1-V_6 leads [15]. Similarly, in another multicenter, placebo-controlled randomized study (351 patients with MI), after administration of L-carnitine for 48 hours, started within 8 hours of the onset of the disease, a decrease in the voltage of the R-wave was 15% less compared to placebo [16]. Furthermore, in the group of patients treated with L-carnitine, the incidence of ischemia was lower (17.6 versus 36%), as well as the number of patients with NYHA class III/IV heart failure, combined with an increase in the left ventricle – 23.4 versus 36% of patients [16].

A double-blind, placebo-controlled study CEDIM involving 472 patients with primary anterior MI showed a significant reduction in heart volume in L-carnitine group compared to the placebo group. L-carnitine was administered intravenously at a daily dose of 9 g for 5 days, with subsequent switch to oral intake at 6 g daily for 12 months [17]. 2,330 patients with acute anterior MI participated in a randomized, double-blind, placebo-controlled study CEDIM-2. One of the most important outcomes of this study was a significant decrease in early mortality after MI, which was accounted for 39% on Day 5 of the acute period [18].

Another parallel, double-blind, placebo-controlled study involved 56 patients with myocardial infarction participated, who were injected with L-carnitine at a dose of 100 mg/kg every 12 hours for a period of 5 to 12 hours of the onset of the disease within 36 hours. It was shown that in L-carnitine group, the incidence of heart rhythm disorder reduced by 80% and the recording time for multiform or paired extrasystoles significantly decreased [19].

ENDOTHELIUM DYSFUNCTION: L-ARGININE AS A DONATOR OF NO

To date, it has been established that the vascular endothelium plays an important role in the signal transduction from various neurohumoral systems to subendothelial structures, providing a dynamic equilibrium between vasodilator and vasoconstrictor factors, and regulates the growth and proliferation of the subendothelial cell clusters of various origins, mediates platelet and coagulation hemostasis, vascular permeability and mechanical properties of the vascular wall [20].

Noteworthy that in physiologic conditions, the release of vasorelaxing factors (a special role belongs to NO) maintaining vasodilation predominates [20].

In addition to vasodilatation, NO performs a number of other important functions: modulates the release of vasoactive mediators, inhibits the adhesion of leukocytes, participates in the regulation of the vascular wall re-modeling, inhibits the expression of pro-inflammatory genes, adhesion and aggregation of thrombocytes, and inhibits migration and proliferation of SMC [21].

Endothelial dysfunction is one of the earliest stages in the pathogenesis of many cardiovascular diseases, including arterial hypertension, coronary artery disease and systemic atherosclerosis. Furthermore, the ability of endothelial cells to synthesize vasodilators decreases, and the synthesis of vasoconstrictor factors preserves or increases. Impaired endothelium-dependent vasodilation of coronary vessels, increase in plasma endothelin-1 and angiotensin-2 can play a significant role in the implementation of the mechanism of circulatory arrest in ischemia/reperfusion. In this case, the central role in the occurrence of dysfunction belongs to the impaired bioavailability of NO due to its inadequate production from L-arginine [20].

The availability of L-arginine for endothelial NO synthase is considered as one of the predisposing factors for the production of endogenous NO. In the study of J.S. Pollock et al. (1991), L-arginine has been shown to contribute to the improvement of endothelial function regardless of the causes that led to a decrease in the bioavailability of NO [22]. This phenomenon was named “L-arginine paradox” and it initiated the studies that allowed to explain its occurrence [23]. The latter appeared to be caused by low concentrations of the methylated analogue of L-arginine – asymmetric dimethylarginine (ADMA) whose main biological role is restricted to a competitive inhibition of endothelial NO synthase [24]. Moreover, the circulating endogenous ADMA promotes a shift in the reaction of the formation of L-arginine towards the latter. And even minor changes in the plasma concentration of ADMA were quite sufficient for the manifestation of clinically significant violation of the regulation of vascular tone [20]. Then, ADMA is metabolized by dimethylarginine dimethylaminohydrolase (DDAH) to L-citrulline and dimethylamine.

It was found that suppression of DDAH isoforms is also accompanied by endothelial dysfunction, increased resistance of pulmonary and peripheral arteries, a decrease in the volume rate of cerebral and renal blood flow, as well as an increase in arterial pressure, development of insulin resistance and thickening of the intima-medial segment of the arteries [20, 21].

Besides, L-arginine activates guanylate cyclase and increases the level of cyclic guanidine monophosphate in the vascular endothelium, reduces activation and adhesion of leukocytes and thrombocytes, inhibits the synthesis of adhesion molecules VCAM-1 and MCP-1, inhibits the synthesis of endothelin-1 which is a potent vasoconstrictor and a stimulator of the proliferation and migration of smooth vascular wall myocytes. It also suppresses the synthesis of asymmetric dimethylarginine, a potent endogenous stimulant of oxidative stress [25].

CLINICAL EVIDENCE OF THE EFFEC OF L-ARGININ ON THE ENDOTHELIAL DYSFUNCTION IN CARDIOVASCULAR PATHOLOGY

There is evidence of the beneficial effect of prolonged use of L-arginine on the endothelial function of the arteries in elderly patients [20], which was accompanied by a significant decrease in the circulating level of pro-inflammatory cytokines, such as C-reactive protein and interleukin-6 (Ellis A.C. et al., 2015).

In 2009 V. Bai et al., presented the results of a meta-analysis of 13 randomized trials conducted to study the effect of L-arginine on the functional state of the endothelium. In these trials, the effect of L-arginine in hypercholesterolemia, stable angina, peripheral artery disease and chronic heart failure (duration of treatment varied from 3 days to 6 months) was observed. Meta-analysis has shown that even short-term administration of L-arginine significantly increases the severity of endothelium-dependent vasodilation of the brachial artery compared to the placebo, which suggests an improvement in endothelial function [22]. L-arginine has a positive effect on the platelet function secondary to hypercholesterolemia, namely, reduces the aggregation of platelets and the adhesion of monocytes [29].

In an analysis of 11 randomized, double-blind, placebo-controlled trials involving 387 patients, low doses of L-arginine demonstrated a protective effect in ischemia/reperfusion, provided a lower rate of perioperative myocardial infarction and a shorter stay in the intensive care unit and hospital [26].

Moreover, positive data were obtained with short-term use of L-arginine in patients with chronic CAD: after infusion of L-arginine at 150 μmol/L in patients with CAD, an increase in the diameter of the lumen of the vessel in the stenotic segment by 3-24% was seen [25].

TIVOR-L® - DONATOR OF NITRIC OXIDE WITH ENHANCED EFFECT ON PATHOGENESIS OF CAD

Taking into account all the above, in order to correct energy metabolism in cardiomyocytes and endothelial dysfunction, the pharmaceutical company Yuria-Pharm created a combined drug TIVOR-L® containing 20 mg of levocarnitine and 42 mg of L-arginine hydrochloride in 1 ml of solution for infusion (100 ml) intended for use in the complex treatment of coronary artery disease, angina, and acute coronary syndrome.

Levocarnitine plays an important role in the oxidation of fatty acids. At the same time, acting as a specific cofactor, it controls the oxidation rate of long-chain FAs and facilitates their transfer through the inner membrane of the mitochondria, and then from the cytoplasm. Thus, it prevents the development of a cytotoxic effect. In ischemia, removing excess of acyl groups from the mitochondria, levocarnitine promotes the formation of malonyl-coenzyme A. Therefore, a sufficient level of carnitine ensures the efficient performance of the carnitine shuttle, increasing the rate of β-oxidation of the FAs to restore the energy balance of the cell.

Arginine, being a substrate for the formation of the enzyme MO synthase, catalyzes the synthesis of nitric oxide in endotheliocytes, contributes to the improvement of endothelial function. It also reduces the activation and adhesion of leukocytes and platelets, inhibits the synthesis of adhesion molecules CAV-1 and MCP-1, inhibits the synthesis of endothelin-1, a potent vasoconstrictor and a stimulator of proliferation and migration of smooth vascular wall myocytes.

Thus, the nitric oxide donator complex – L-arginine enhanced by the action of L-carnitine, presented as a ready-to-use TIVOR-L® solution for infusion – satisfies the need in correcting endothelial dysfunction and providing energy supply of the cardiomyocyte, according to the current concept of the pathogenesis of CAD.

The efficacy of TIVOR-L® administration in non-ST elevation acute coronary syndrome was studied by Vakalyuk I.P. (2016) in an open randomized comparative parallel study involving 100 patients with this condition [25].

In addition to background therapy, patients of the treatment group (n = 50) received TIVOR-L® 100 ml intravenously at a rate of 10 drops per minute for the first 10-15 minutes (then the rate of infusion could be increased to 30 drops per minute) once a day within 10 days. Patients of the control group (n = 50) were administered only with background therapy.

The drug Tivor-L®, prescribed as a part of combination therapy, has been found to improve the electrophysiological properties of the myocardium and prevents transient ECG abnormalities. Within the first hours after onset of AMI, late ventricular potentials (LVP) were less frequently recorded in patients of the treatment group (9.6%) compared to 19.8% of the controls. During follow-up, LVP in control group resolved, and in Tivor-L® group – did not occur. This suggests the presence of a pronounced anti-ischemic effect of the drug, which is confirmed by clinical data.

In addition, the lower incidence of ventricular arrhythmias, such as group ventricular extrasystole and ventricular tachycardia, was observed in the treatment group receiving TIVOR-L®; the atrioventricular block occurred 3 times less than in the control group.

Moreover, the author has noted the positive clinical dynamics of ACS: reduced frequency and severity of angina attacks, decreased blood pressure, and increased exercise tolerance. In the group of patients receiving TIVOR-L®, the attacks of anginal pain were less common (20.8% of cases in the treatment group vs. 32.0% in the control group); the demand for the use of narcotic analgesics decreased – 22.1 and 36.2% of cases, respectively.

In the course of the study, it was established that administration of TIVOR-L® within the first hours against conventional therapy of non-ST elevation ACS reduced the time required to reach the peak of CPK activity in patients of the treatment group: 13.5 ± 0.6 μkat/L vs. 17.1 ± 0.8 μkat/L in patients of the control group. The level of MB-CPK was 9.9 ± 0.4 μkat/L and 13.9 ± 0.6 μkat/L, respectively.

A necrotic lesion area was 26.4% less in treatment group compared to the control group. This was due to the reduced period of normalization of CPK and CPK-MB activity in serum by an average of 8.1 and 9.4 hours, respectively. Relatively early onset of the peak of cardiac enzyme activity (CPK, CPK-MB) indicates a more rapid restriction of the ischemic area, and a shorter period of their washing out – a prevention of further damage to the cardiomyocytes and the extension of necrotic lesion in patients of the treatment group.

Thus, urgent therapy with TIVOR-L® is a pathogenetically grounded method that allows to reduce the severity of myocardial metabolic disturbances in acute coronary and reperfusion syndrome. In hypoxic conditions, levocarnitine inhibits β-oxidation of fatty acids. Reducing the rate of their oxidation has a positive effect on the metabolism in the ischemic myocardium due to the enhancement of the production of alternative energy by oxidation of glucose. This way allows much more efficient use of a limited amount of oxygen. Removing toxic metabolites of acetyl-CoA from the mitochondria, levocarnitine provides a cytoprotective effect.

L-arginine, reducing the negative effect of oxidative stress on the endothelium of the vascular wall and inducing the formation of nitric oxide, restores vascular endothelial function and improves coronary blood flow.

In the study of Vakalyuk I.P., the use Tivor-L® in addition to background therapy helped to improve the management of patients with non-ST elevation ACS: a more rapid regression of ACS clinical manifestations, stabilized condition of patients and lower incidence of complications were observed. Tivor-L® was well-tolerated by the patients and caused no serious adverse reactions. The overall efficacy of treatment with Tivor-L® was superior to conventional therapy for patients with non-ST elevation ACS.

The list of references is located in the editorial office