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METABOLIC THERAPY: CARDIOPROTECTIVE EFFECTS OF OROTIC ACID AND ITS DERIVATIVES

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Metabolic therapy involves the administration of a substance normally found in the human body to enhance cellular reactions involved in the pathogenesis of disease. Myocardial ischaemia/reperfusion injury represents a leading cause of morbidity and mortality, also in cardiovascular disease. Therapeutic strategies aimed at limiting cardiomyocyte death during the postischaemic reperfusion and in the perioperative settings are nowadays extensively studied. Conceived originally as a dietary constituent (known as vitamin B13) only, it is now apparent that most orotic acid is synthesized in the human body where it arises as an intermediate in the biosynthetic pathway of pyrimidine nucleotides. Previous investigations in the heart suggest that orotate and its derivatives could be of significant clinical benefit in the treatment of heart disease. The present brief review is concerned with the current knowledge of the major effects of these compounds in both experimental and clinical cardiology. The potential mechanisms and biochemical pathways responsible for cardioprotection are highlighted. ***Biomed Rev*** **2010; 21: 47-55.**

Key words: cardioprotection, ischaemia-reperfusion injury, orotate

INTRODUCTION

Myocardial ischaemia/reperfusion injury represents a major cause of morbidity and mortality worldwide being associated with acute coronary syndromes, particularly acute myocardial infarction that has been predicted to become by the year 2020 the first cause of death in the world (1). Timely coronary reperfusion by either thrombolysis or primary coronary artery angioplasty has become the established routine therapy that effectively decreases infarct size and reduces mortality. Despite the unequivocal beneficial effects in stopping the progression of irreversible damage, reperfusion *per se* is considered a double-edged sword (2) as it can induce severe myocardial lesions, known as lethal reperfusion injury that paradoxically may alleviate the beneficial effects of revascularization (3). Moreover, there is no clinically available therapeutic intervention able to reduce infarct size in association with the revascularization procedures. Accordingly, cardioprotection, defined as the totality of pharmacological

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and mechanical interventions aimed at reducing cell death during/after myocardial ischaemia/reperfusion, continues to be the focus of considerable research effort for both understanding the underlying mechanisms (4) and translating the experimental findings into clinical therapy (5,6).

This article attempts to summarize the present state of knowledge of cardioprotective potentials of orotate-based metabolic therapy in both experimental and clinical settings.

BIOCHEMISTRY OF OROTIC ACID

Orotic acid is a natural substance present in high concentration in dairy products (*oros* means "whey" in Greek) classified more than 40 years ago as vitamin B13 and mainly used in the past for the treatment of pernicious anaemia. Its major metabolic role within the human body consists of that it is the first fully formed intermediate in the manufacture of the pyrimidine bases (7) required for the RNA/DNA synthesis.
In the *de novo* pathway of pyrimidines biosynthesis, the enzyme orotate phosphoribosyltransferase (OPRTase), found in organisms ranging from yeast to humans, catalyzes the formation of orotidine 5'-monophosphate (OMP) from orotic acid and 5-phosphoribosyl-1-pyrophosphate (PRPP) which in turn is further used as a substrate for uridine nucleotides: uridine monophosphate (UMP) and uridine triphosphate (UTP) synthesis (Fig. 1). Because the enzyme OPRTase requires magnesium ions for its activity, early studies suggested that the true substrate for OPRTase is not orotate itself, but rather a magnesium-orotate complex (8). These researchers hypothesized that the magnesium complex helps position orotate within the enzyme in the proper orientation for conversion to OMP. In contrast, some of the mineral orotates such as copper and nickel either inhibit OPRTase or, in the case of calcium orotate, neither activates nor inhibits the enzyme activity. However, subsequent work of Bhatia and Grubmeyer (9) infirmed this hypothesis by showing that, at least in yeast, the role of divalent metal must be to bind PRPP, because orotate-metal complex is not necessary for OPRTase catalysis and only weak metal-enzyme complexes may form in bacterial OPRTase. UTP thus synthesized can be further utilized for the synthesis of cytidine triphosphate (CTP): the amino group of CTP is donated from glutamine in an ATP-dependent reaction catalyzed by CTP synthase (Fig. 1).

Uridine nucleotides are also the precursors for *de novo* synthesis of the thymine nucleotides: this pathway requires the use of dUMP which is converted to dTMP by the action of thymidylate synthase. The methyl group (thymine is 5-methyl uracil) is donated by tetrahydrofolate (similarly to the donation of methyl groups during the biosynthesis of the purines).

Besides this energetically expensive, multistep *de novo* pathway, the pyrimidine nucleotide pools are sustained by the recycling of the basic components derived from their catabolism during the normal process of cell turnover, the so-called "salvage", PRPP independent pathway.

Uracil can be "salvaged" to form UMP through the concerted action of uridine phosphorylase and uridine kinase, as indicated below:

uracil + ribose-1-phosphate $\rightleftharpoons$ uridine + P_i
uridine + ATP $\longrightarrow$ UMP + ADP

Deoxyuridine is also a substrate for uridine phosphorylase. Formation of dTMP, by salvage of dTMP requires thymine phosphorylase and thymidine kinase:

thymine + deoxyribose-1-phosphate $\rightleftharpoons$ thymidine + P_i
thymidine + ATP $\longrightarrow$ dTMP + ADP

Thymidine kinase can also use deoxyuridine as substrate:

deoxyuridine + ATP $\rightleftharpoons$ dUMP + ADP

The activity of thymidine kinase (one of the various deoxyribonucleotide kinases) is unique in that it fluctuates with the cell cycle, rising to peak activity during the phase of DNA synthesis; it is inhibited by dTTP.
The salvage of deoxycytidine is catalyzed by *deoxycytidine kinase*:

deoxycytidine + ATP $\rightleftharpoons$ dCMP + ADP

The major function of the pyrimidine nucleoside kinases is to maintain a cellular balance between the level of pyrimidine nucleosides and pyrimidine nucleoside monophosphates. However, since the overall cellular and plasma concentrations of the pyrimidine nucleosides (as well as those of ribose-1-phosphate) are low, the "salvage" of pyrimidines by these kinases is relatively inefficient.

EXPERIMENTAL EVIDENCE

Experimental evidence with OA can be traced back to the late 60s when a Russian physiologist, E.Z. Meerson, demonstrated for the first time the cardioprotective effect of OA administration during his pioneering studies on experimental hypertrophy induced by aortic ligation in rabbits. In this respect, he described the first stage of acute hypertrophy, known as the

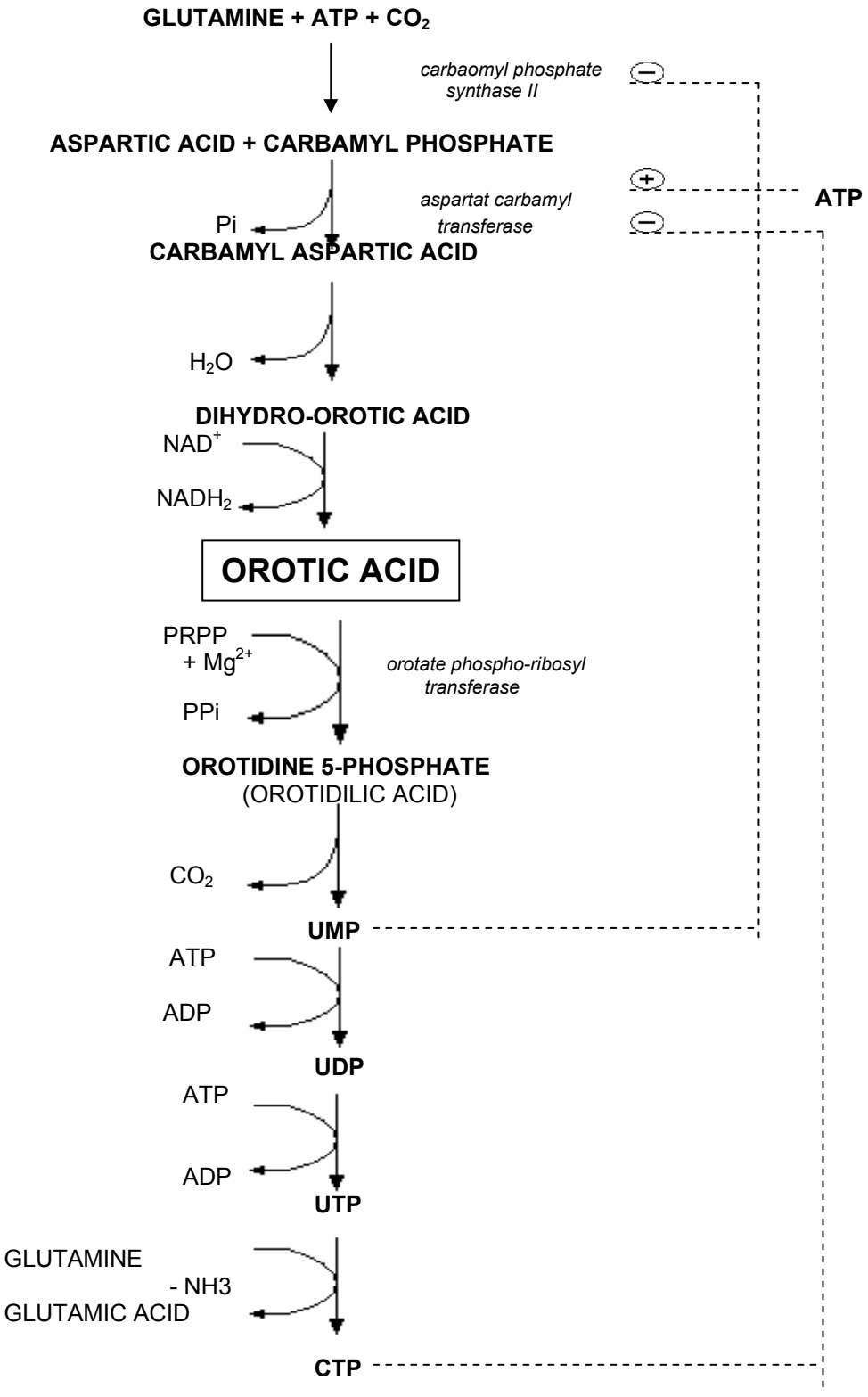

Figure 1. **De novo** ***Biosynthesis of Pyrimidine Nucleotides.***

'emergency' or 'damage' stage, as being characterized by the impairment of contractility together with an acute increase in protein synthetic rates and considered the latter as the major responsible for the structural adaptive changes of myofibrils enabling the heart to undergo transition to the second stage of 'stable compensation' which was allowing a better contraction (10,11). In this same vein, Meerson postulated the 'relative deficiency theory', i.e., the deficiency of cofactors such as OA as being responsible for the limitation of the extent of protein and nucleic acids synthesis activation during hypertrophy. Administration of potassium orotate, as a precursor of RNA synthesis, for the first 4 days after the aortic ligation, enhanced the rate of protein synthesis, improved contractility and almost completely prevented the appearance of acute heart failure (12). These investigators further confirmed the beneficial effects of chronic orotate administration post-experimental myocardial infarction in rats (13) and they postulated as putative mechanism, the prevention of drop in cathecolamines concentration in the non-ischaemic zone of the myocardium (14).

The first Western author who confirmed the protective role of OA in the experimental setting was an Australian biochemist, J.F. Williams. His seminal studies (reviewed in 15) on the biochemistry and functional role of OA in the acute hypertrophying rat heart provided extensive evidence concerning the major metabolic pathways activated by chronic treatment with OA (10 mg/kg/day started immediately after the induction of hypertrophy). The proposed inter-organ connections of these metabolic activations thought to be responsible for the enhancement of contractility and functional performance improvement during the first four days of developing cardiac hypertrophy in rat are summarized in Figure 2. The orotate-related cardioprotection can be attributed to the following mechanisms: (*i*) stimulation of the pyrimidine synthesis in liver via the PRPP independent salvage pathway followed by the transport of the bases (mostly as uridine) to the heart where their conversion to nucleotides will be performed (with a subsequent increase in the RNA synthesis rate within the heart); (*ii*) enhancement of the myocardial glycogen stores due to the high levels of uridine and UDP-glucose in the heart coupled with a higher flux in cardiac glycolysis with two consequences: (*a*) the expansion of the glycolytic intermediates concentration, and (*b*) the preservation of the cytoplasmic NAD^+/NADH ratio in the physiological range, (*iii*) the elevated levels of glucose-6-P and fructose-6-P can force glycolytic intermediates to entry into the non-oxidative reaction segment of the pentose pathway. As a consequence, the activation of ribose-5-P formation via reversal of the L-type pentose pathway in the heart will increase the PRPP generation whose concentration regulates both *de novo* and the salvage pathways for adenine nucleotide synthesis; the subsequent augmentation of adenylates (AMP, ADP) concentration will further increase the ATP availability, and (*iv*) the predominant use of the pyrimidine *salvage* pathway in the heart together with the enhanced production of pyrimidine bases in organs distant from the heart spares myocardial PRPP for the more dominant *de novo* adenine nucleotide synthesis (16). These latter authors demonstrated the ability of OA treatment to "energize" cardiomyocytes evaluated by the phosphorylation state of the adenine nucleotides, a parameter that is considered the best predictive index of the cellular energy status in normal and hypertrophying hearts (17).

The beneficial effects of orotate on the cardiac function were further reproduced by Williams' group in infarcted working rat hearts exposed to a 60 minutes period of hypothermic cardioplegic arrest (18,19). These investigators demonstrated that: (*i*) regardless the protective effect of hypothermic cardioplegia, a rather small-sized left ventricular myocardial infarction induced a marked reduction of the ability of the heart to recover from global ischaemia, and (*ii*) pretreatment with OA produced a substantial improvement of infarcted hearts. The same research team reported the lack of a direct effect of OA on cardiac function in normal isolated working rat hearts in both acute and chronic administration. Protection elicited by orotate in this setting was ascribed to the same mechanisms described for the hypertrophying hearts, namely: (*i*) the high rate of protein synthesis in non-infarcted myocardium with the putative restoration of the metabolic enzymes in the surviving areas that might allow a better recovery from ischaemia, (*ii*) enhancement of the myocardial glycogen stores (via increasing the UDP-glucose levels) that will supply the substrate for anaerobic metabolism during ischaemia, (*iii*) augmentation of phospholipids biosynthesis (via increased cytidine nucleotide production) will allow the repair of the ischaemia induced membrane damage, and (*iv*) prevention of adenine nucleotides depletion in the surviving myocardium together with the subsequent increase in the ATP availability will allow a better recovery post-global ischaemia (17,20). The observation that orotic acid improves cardiac performance of the ischaemic/reperfused rat hearts via the elevation of myocardial glycogen content was further confirmed (21).

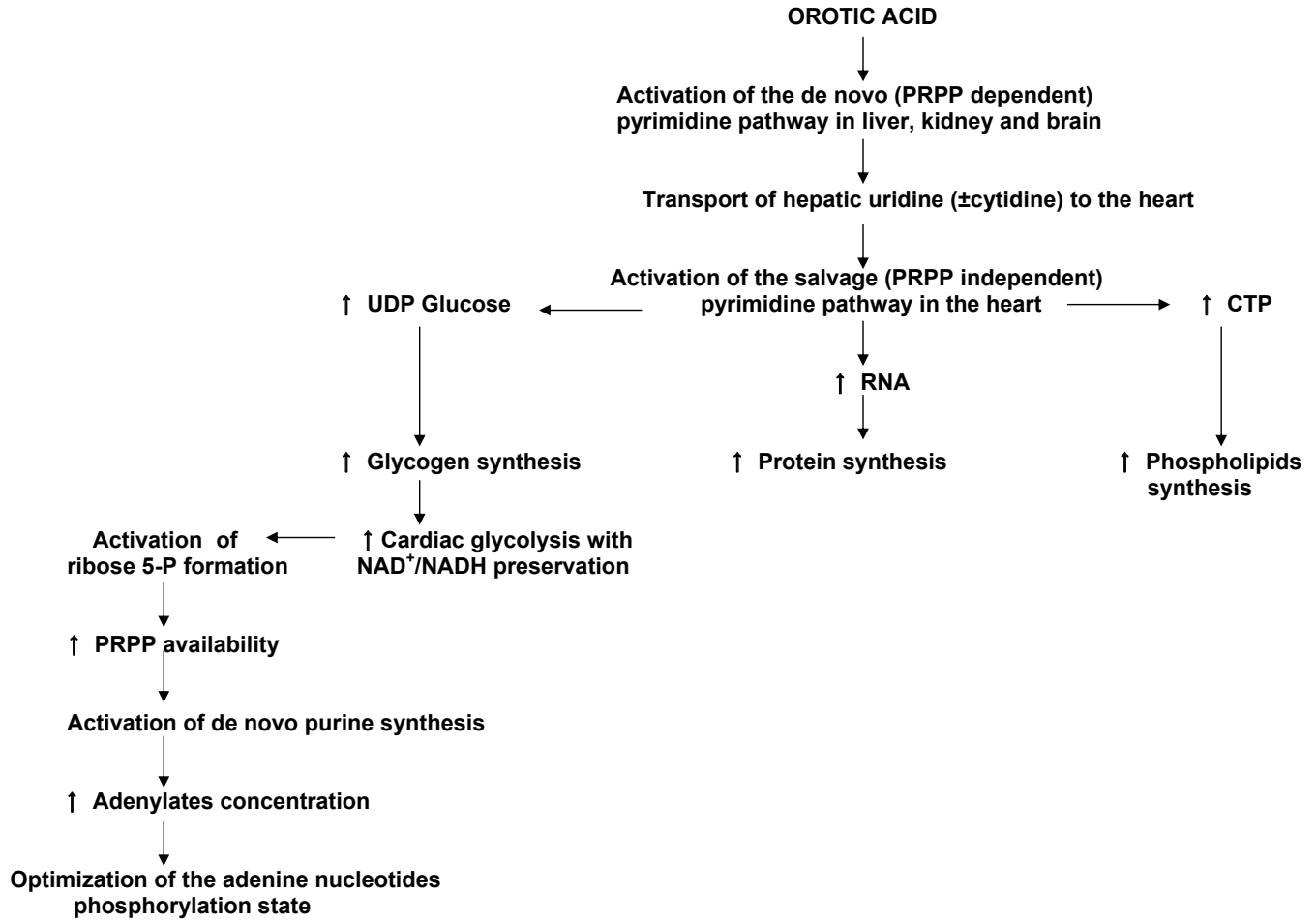

Figure. 2 Possible Metabolic Pathways Activated by Orotic Acid
(UTP = uridine triphosphate, CTP = cytidine triphosphate, PRPP = 5-phosphoribosyl-1-pyrophosphate)

The salts of OA have been also shown to exert protective effects on cardio-vascular system in both experimental and clinical studies, albeit the mechanisms of protection are not entirely elucidated. Thus, Bajusz *et al* (22) demonstrated the beneficial effect of potassium orotate in preventing the development of the dystrophic Syrian hamster cardiomyopathy, a chronic heart disease associated with skeletal muscle dystrophy. The authors attributed it initially to the effect of potassium (as these animals were extremely sensitive to hypokaliemia). However, it was shown that sodium orotate was equally effective in improving myocardial contractility, suggesting that the beneficial effect should be ascribed to OA (23). These latter authors revealed that OA, in chronic administration, was able to restore the sialyltransferase activity to the normal levels; this sarcolemmal enzyme is responsible for the sialic acid incorporation into the interstitial glycoproteins involved in the excitation-contraction coupling process. Sialic acid is the terminal monosaccharide within the membrane glycoproteins with at least two major roles in mammalian myocardium: as binding sites for the calcium involved in excitation-contraction coupling, also regulator of membrane permeability to calcium ions.

More recently, Jasmin *et al* (24) confirmed the superiority of chronic treatments (30 and 50 days, respectively) with magnesium orotate as compared to orotic acid in reducing the myocardial degeneration (especially the severity of calcifications) and the development of congestive heart failure in cardiomyopathic hamsters with a similar efficiency for both compounds in prolonging their survival. Similarly, Jellinek and Takacs (25) reported a beneficial effect of magnesium

orotate that performed better than OA which in turn outperformed magnesium chloride in preventing the development of atherosclerotic lesions in rabbits chronically fed with an enriched cholesterol diet. These authors also speculated on possible mechanism underlying this effect: they suggested that OA in the magnesium orotate complex might become coupled with ribose (ribosylated) in the walls of blood vessels and the subsequent release of magnesium during this process will render the ions locally available for activating cholesterol-metabolizing enzymes.

At variance from its beneficial effects in chronic administration, we have investigated the cardioprotective effect of magnesium orotate in acute administration during the reperfusion post-global ischemia in isolated perfused rat hearts (26). In this study, the postischaemic recovery of contractile function was assessed and the effects of MO (1 mM) were compared to the ones elicited by cyclosporine A (0.2 μM), a recognized cardioprotective agent at mitochondrial level (27).

We reported a comparable degree of protection on contractile function for both magnesium orotate and cyclosporine A in isolated heart subjected to 30 min of global ischemia and 2 hours of reperfusion (Fig. 3).

In order to identify the major agent responsible for cardioprotection at reperfusion we have compared the effects of magnesium orotate on postischemic recovery of contractile function and infarct size with the ones elicited by an equivalent dose of OA and magnesium chloride, respectively (unpublished results). Our preliminary data show that, when given at reperfusion, magnesium orotate was associated with a better cardioprotective effect when compared to OA (suggesting that a synergy might exist between magnesium and OA) whereas magnesium chloride administration had no beneficial effects. Current investigations are ongoing in order to identify the mechanisms underlying acute cardioprotection related to magnesium orotate administration at reperfusion with a possible contribution of mitochondria.

CLINICAL EVIDENCE

Orotate (in form of potassium salt, 500 mg t.i.d) was initially used during in the early 1970s in cardiovascular patients in the former Soviet Union and in Bulgaria, but comprehensive information available in English concerning this early experience was rather limited (28). In 1991, Rosenfeldt (29) briefly reviewed during an international symposium the clinical trials reported in the Russian literature, showing that potassium orotate was effective when added to standard therapy, mainly in 2 major settings: acute myocardial infarction (30-32) and severe heart failure (33). In the case of the former indication, some authors underlined the importance of early administration, reporting that giving orotate in the first 1-3 days after infarction was followed by a reduction in the incidence of cardiac complications as compared with patients starting therapy after 4-7 days. All these studies, even if including a small number of patients, showed a quicker restoration of myocardial contractile function, a lower incidence of early arrhythmic complications and a decreased mortality rate after myocardial infarction in patients receiving orotate together

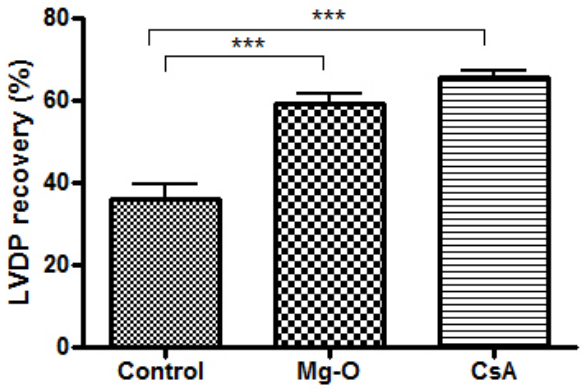

Figure 3. Postischemic recovery of contractile function in isolated rat hearts (percentage of their pre-ischemic value).
LVDP = left ventricular developed function. Mg-O = magnesium orotate, CsA = cyclosporine A ($p < 0.001$ Mg-O and CsA vs. Control)

with the conventional therapy. The same beneficial effects together with a clinical improvement and less digoxin toxicity were reported in the case of chronic treatment with orotate in patients with heart failure (33). Moreover, the treatment with OA and its salts was further extended to other heart diseases such as severe angina (34), rheumatic myocarditis (35) and mitral valve disease (36). Surprisingly - or, as often happened in science - for the next almost two decades, these studies with few exceptions (37,38) have been neglected.

Recently, Stepura and Martynow (39) reported the beneficial effects of adjuvant magnesium orotate on clinical symptomatology, survival rate and quality of life in patients with severe heart failure under optimal cardiovascular medication. In line with the "upregulation" of scientific interest to orotate and its derivatives, recent studies provided experimental evidence for their cardioprotective significance, including antinecrotic and anti-arrhythmic effects (40-42, cf. 43,44). However, the precise mechanism by which orotate combinations are more effective than OA alone remains still elusive in the metabolic therapy of cardiovascular diseases (45).

CONCLUSION

We have highlighted the cardioprotective effects caused by OA and/or its derivatives. A better understanding of the complex mechanisms responsible for these effects may further widen the therapeutic potential of such a simple and safe metabolic therapy. In future, possible implications of OA and/or its derivatives in the pathogenesis and therapy of certain cardiometabolic diseases such as atherosclerosis (25), type 2 diabetes and the metabolic syndrome might represent a novel research field to be pursued.

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