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Comparable Cardioprotection at Reperfusion by Magnesium Orotate and Cyclosporin A: A Study in Isolated Rat Hearts

Silvia N. MIRICA[1], Oana M. DUICU[1], Andreea M. RĂDUCAN[1], Adrian STURZA[1], Valentin L. ORDODI[2], Ovidiu FIRA-MLADINESCU[1], Danina M. MUNTEAN[1]

[1]Department of Pathophysiology,
[2]Department of Cell and Molecular Biology,
University of Medicine and Pharmacy "Victor Babes" Timisoara,
14 Tudor Vladimirescu street. Timişoara, Romania; mirica_nicoleta@umft.ro

Abstract. Orotic acid and its salts has been previously shown to improve the energy status of cardiomyocytes and to protect myocardium following transplantation and cardioplegic arrest. The present study carried out in Langendorff perfused rat hearts was purported to compare cardioprotection elicited by CsA with the one afforded by magnesium orotate (Mg-O) when administrated at reperfusion. To this aim isolated rat hearts (n = 6-8/group) subjected to 30 min of global ischemia and 30 min of reperfusion were randomized to receive: (1) no intervention (Controls), (2) Mg-O (1 mM) and (3) CsA (0.2 μM) in the perfusion buffer throughout the reperfusion period. Recovery of post-ischemic ventricular function was assessed by the left ventricular developed pressure (LVDP), rate pressure product (RPP), and maximal and minimal first derivatives of left ventricular pressure (dLVP/dtmax and dLVP/dtmin) as indices of contractility and relaxation, respectively. All contractile parameters were expressed as percentage of their pre-ischemic values. At the end of the reperfusion period, both Mg-O and CsA induced a substantial recovery of contractile function parameters. Accordingly, LVDP was 59.4 ± 2.24% and 65.8 ± 1.56% in Mg-O and CsA groups, respectively *vs.* 36 ± 3.68% in Controls ($p < 0.001$). Similarly, both Mg-O and CsA administration improved contractility (59,4 ± 2.24% and 65.97 ± 1,6%, p=NS) and relaxation indices (67,6 ± 0,8% and 65,8 ± 1,5%, p=NS) when compared to Controls ($p < 0.001$). In isolated rat hearts, acute administration of Mg-O and CsA at reperfusion was associated with significant improvement of the postischemic contractile function.

Keywords: ischemia – reperfusion injury, rat heart, cardioprotection, Mg orotate, cyclosporine A

INTRODUCTION

Ischemia/reperfusion injury of the heart represents a major health burden mainly associated with acute coronary syndromes. Each year, acute myocardial infarction (AMI) is responsible for the death of millions of persons worldwide and represents the first cause of chronic heart failure (Keely *et al*, 2003). 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, it is a well known nowadays that: (i) reperfusion *per se* is considered a double-edged sword (Braunwald & Kloner, 1985) as it can itself induce severe myocardial lesions, known as *lethal reperfusion injury* which paradoxically alleviate the beneficial effects of revascularization (Yellon and Hausenloy, 2007; Garcia-Dorado et al, 2009), and (ii) there is currently no clinically available therapeutic

intervention able to further reduce infarct size in association with the revascularization procedures. Accordingly, cardioprotection, defined as the totality of mechanical and pharmacological interventions aimed at reducing cell death at reperfusion, continues to be the focus of considerable research effort for both understanding the underlying mechanisms (Murphy & Steenbergen, 2008) and translating the experimental findings into clinical therapy (Ruiz-Meana & Garcia-Dorado, 2009). In the present study performed in Langendorff-perfused rat hearts we have compared cardioprotection elicited by cyclosporin A, that has been unequivocally recognized to protect against mitochondria dysfunction during the acute ischemia/reperfusion injury in both animal models (Griffith et al, 1995; Halestrap et al, 1997; Massoudy et al, 1997; Weinbrenner et al, 1998) and humans (Piot et al, 2008; Mewton et al, 2010) to the one afforded by magnesium orotate, a compound recognized for its protective effect mainly in cronic administration (Donohoe et al, 1993; Ferdinandy et al, 1998; Stepura et al, 2009), yet less less investigated in acute settings (Munsch et al, 1991).

MATERIALS AND METHODS

All experimental procedures and protocols used in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (published by the National Institute of Health - NIH Publication no. 85-23, revised 1996) and with the consent of the Ethics and Deontology Committee of the "Victor Babeş" University for Medicine and Pharmacy Timişoara. Adult Sprague-Dawley rats were fed ad libitum and housed at a 12 h light/dark cycle. Twenty-four hours prior to the experiment solid food was withdrawn from the animals with no limitation in water supply. Most reagents were from Sigma Aldrich. Magnesium orotate was a kind gift from PharmaZell.

Heart Preparation: Hearts excised from Sprague-Dawley rats (250-350 g) anesthetized with Ketamine (30 mg/kg and 10 mg/kg Xylazine) and anticoagulated with heparin (1000 U/kg IV) were quickly removed and retrogradely perfused through the aorta in a noncirculating Langendorff apparatus (AD Instruments Ltd.) with Krebs-Henseleit (K-H) buffer having the following composition (in mmol/L): NaCl 118.0; KCl 3.2; MgSO4 1.2; $NaHCO_3$ 25.0; NaH_2PO_4 1.18; $CaCl_2$ 2.5; glucose 11.1. The buffer was saturated with 95% O2-5% CO2 (pH 7.4, 37°C) for 50 minutes. Hearts were perfused at a constant pressure of 80 mm Hg. A water-filled latex balloon-tipped catheter was inserted into the left ventricle through the left atrium and was adjusted to a left ventricular end-diastolic pressure (LVEDP) of 8 to 10 mmHg during the initial equilibration. Thereafter, the balloon volume was not changed (Fig. 1). The distal end of the catheter was connected to a ML870 PowerLab 4/30 Analyzer (AD Instruments) via a pressure transducer (MLT 0380/A, AD Instruments). Hearts were not paced. Once instrumentation of the heart has been completed, a temperature regulated heart chamber is placed around the heart to avoid heat loss due to external cooling. Coronary flow was measured by timed effluent collection. Temperature was maintained at 37°C. Protocols began after 25 minutes of stabilization.

Experimental Protocol

After stabilization, hearts (n = 6-8/group) subjected to 30 min of global ischemia and 30 min of reperfusion were randomly divided to receive: (1) no additional intervention (Control group), (2) 1 mM magnesium orotate (Mg-O group) and, (3) 0.2 microM cyclosporine A (CsA group) in the perfusion buffer throughout the reperfusion period (Fig. 2). The choice of the dosage was based upon the results of previous experimental studies in

the case of CsA, whereas for Mg-O it represented the equivalent per body weight of the dose chronically administered as mineral supplement in patients with cardiovascular disease.

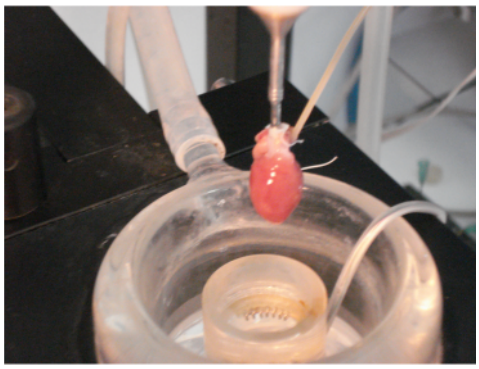


Fig. 1. The isolated rat retrogradely perfused according to the Langendorff technique.

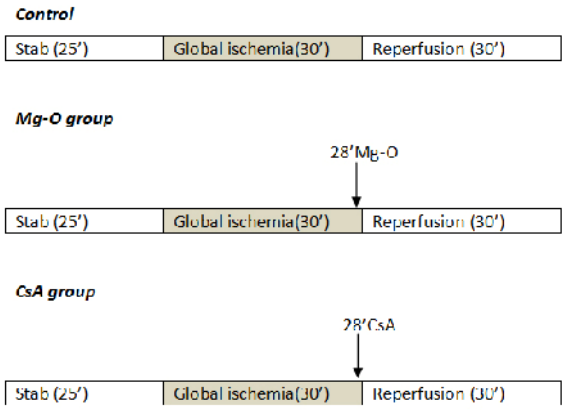


Fig. 2. Timeline of experimental protocol.

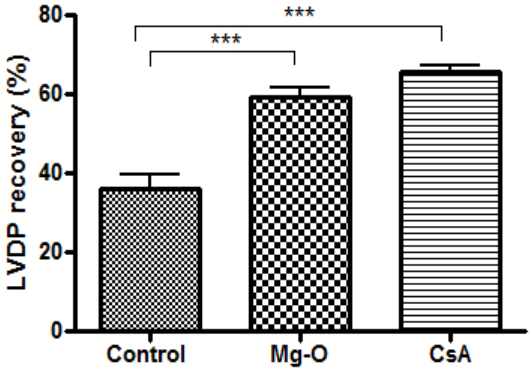
Recovery of post-ischemic ventricular function was assessed by the left ventricular developed pressure (LVDP), rate-pressure product (RPP =LVDP x HR) and the maximal and minimal first derivatives of left ventricular pressure (dLVP/dtmax and dLVP/dtmin) as indices of contractility and relaxation, respectively.

Statistical Analysis: Data were analyzed by GraphPad Prism 5 statistical software and are presented as means ± SEM. Group comparisons were performed by one way Analysis of Variance (ANOVA) and post-hoc Tukey`s multiple comparison test. A $p < 0.05$ was considered significant.

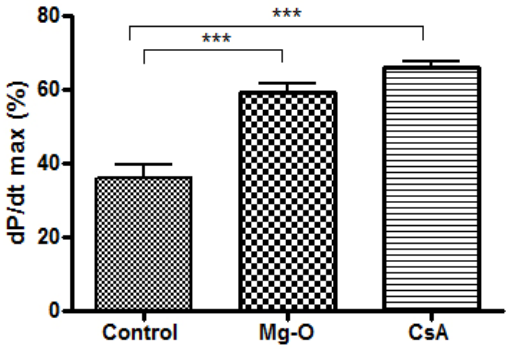
RESULTS AND DISCUSSION

All contractile parameters were expressed as percentage of their pre-ischemic values. At the end of the reperfusion period, both Mg-O and CsA induced a substantial recovery of contractile function parameters. Accordingly, LVDP was 59.4 ± 2.24% and 65.8 ± 1.56% in Mg-O and CsA groups, respectively *vs.* 36 ± 3.68% in Controls ($p < 0.001$).

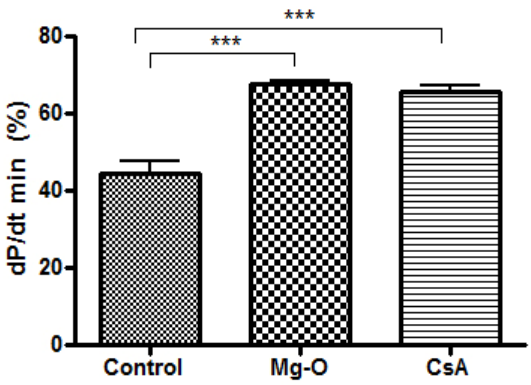
Similarly, both Mg-O and CsA administration improved contractility (59,4 ± 2.24% and 65.97 ± 1,6%, *p*=NS) and relaxation indices (67.6 ± 0,8% and 65.8 ± 1.5%, *p*=NS) when compared to Controls ($p < 0.001$) (Fig. 3).



A) $p < 0.001$ Mg-O and CsA vs. Control



B) $p < 0.001$ Mg-O and CsA vs. Control



C) $p < 0.001$ Mg-O and CsA vs. Control

Fig. 3. Postischemic recovery of functional parameters (percentage of their pre-ischemic value): A) LVDP; B) dP/dtmax (contractility index); C) dP/dt min (relaxation index).

Because heart rate and left ventricular developed pressure (LVDP) may recover to different degrees, rate pressure product (RPP) was calculated by multiplying the heart rate with LVDP and presented as reliable left ventricular functional parameter for the isolated heart. As presented in Fig. 4, in the Mg-O group and CsA-group RPP ultimately recovered to 65,6 ± 2.7% ($p < 0.001$) and 57.2 ± 2.8% ($p < 0.01$) basal value vs. 45 ± 2.4% in Control group at the end of 30 min of reperfusion.

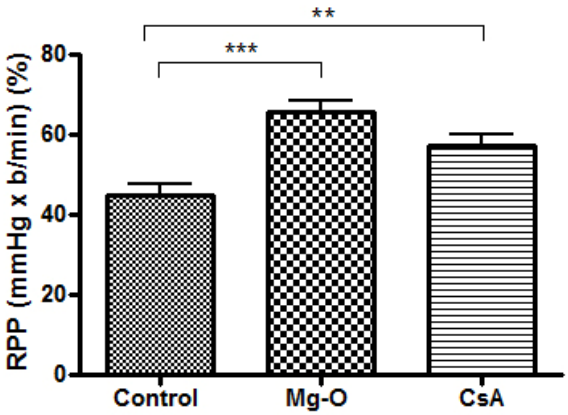


Fig. 4. Postischemic recovery of RPP ($p < 0.001$ Mg-O vs. Control; $p < 0.01$ CsA vs. Control).

DISCUSSION

Orotate, initially used (in the form of potassium salt, 500 mg t.i.d) during in the early 70s in cardiovascular patients within the former Soviet Union, was reported to be effective when added to standard therapy, in patients with acute myocardial infarction (Lukomsky et al, 1967; Kheinonen, 1970). These seminal studies showed a quicker restoration of myocardial contractile function, a lower incidence of early arrhythmic complications and a decreased mortality after myocardial infarction in patients receiving orotate when compared with patients receiving conventional treatment, even if the underlying mechanism(s) remained elusive. Surprisingly, for the next almost 2 decades, these studies have neither been reproduced in the Western world nor apparently continued by the Russian researchers. Recently, Stepura and Martynow (2009) reported 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 the 90s experimental work was carried out in order to elucidate the mechanisms of protection afforded by orotic acid and its salts. Hence, when given in chronic administration to animals that were further subjected to an ischemia/reperfusion protocol, Munsch et al (1991) and Donohoe et al (1993) attributed protection to the following mechanisms: (1) enhancement of the myocardial glycogen stores (via increasing the UDP-glucose levels) and thus supplying substrate for anaerobic metabolism during ischemia; the fact that orotic acid improves cardiac performance of ischemic/reperfused rat hearts via the elevation of myocardial glycogen content was also suggested by Ferdinandy et al (1998); (2) augmentation of phospholipids biosynthesis (via increased cytidine nucleotide production) and thus repairing the ischemia induced membrane damage and (3) improvement of myocardial tolerance to global ischemia in infarcted hearts by preventing the depletion of adenine nucleotides in the surviving myocardium, with the subsequent increase in the ATP availability.

The limit of the present study is that, despite the fact we have demonstrated that acute administration of the drug solely at reperfusion supported the postischemic recovery of contractile function, we did not provided insights into the protective mechanism(s).

With respect to CsA, a huge body of experimental and clinical evidence demonstrated that submicromolar doses of CsA protect against *in vivo* postischemic reperfusion-induced injury in small (Squadrito et al, 1999) and large animals (Skyschally et al, 2009) and, also, as recently demonstrated in a proof-of-concept study, the human heart (Piot et al, 2008). Cardioprotection afforded by CsA has been associated with the prevention of the phenomenon

of mitochondrial permeability transition, considered nowadays as being central to both necrotic cell death during the postischemic reperfusion (Halestrap, 2010) and to cardioprotection elicited by powerful strategies such as pre- and postconditioning (Di Lisa et al, 2010).

Our experimental data show a comparable degree of protection on contractile function for both Mg-O and CsA in isolated heart subjected to global ischemia. In particular, in the case of RPP, the recovery in Mg-O group was more significant than in CsA one; protection was considered to be mediated by a better recuperation of the heart rate values at reperfusion as compared to the preischemic values. Of note, in 3 additional animals we have applied a protocol of ischemic preconditioning consisting of 3 cycles of 5 min ischemia/5 min reperfusion prior to the 30 min of global ischemia and we have obtained a value of RPP equal to 75% (data not shown), i.e. close to the one reported in Mg-O group (p = NS).

CONCLUSIONS

In isolated rat hearts, acute administration of magnesium orotate and cyclosporine A during reperfusion after global ischaemia was associated with significant improvement of the postischaemic contractile function. A better understanding of the mechanisms responsible for cardioprotection in acute settings is mandatory in order to widen the therapeutic potential of old protective drug.

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