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Journal of Molecular and Cellular Cardiology 38 (2005) 367–374
Journal of Molecular and Cellular Cardiology
www.elsevier.com/locate/yjmcc

Original article

Specific inhibition of the mitochondrial permeability transition prevents lethal reperfusion injury

Laurent Argaud [a,b], Odile Gateau-Roesch [a], Danina Muntean [a], Lara Chalabreysse [a], Joseph Loufouat [a], Dominique Robert [b], Michel Ovize [a,*]

[a] *Inserm E 0226, Laboratoire de Physiologie Lyon-Nord, Faculté de Médecine Lyon-Nord, Université Claude Bernard Lyon I, 8, avenue Rockefeller, 69373 Lyon cedex 8, France*

[b] *Département d'Urgence et de Réanimation Médicale, Hôpital Edouard Herriot, Hospices Civils de Lyon, 5, place d'Arsonval, 69437 Lyon cedex 3, France*

Received 13 October 2004; received in revised form 25 November 2004; accepted 3 December 2004

Available online 26 January 2005

Abstract

The aim of the present study was to determine whether specific inhibition of mitochondrial permeability transition (MPT) by NIM811 at the time of reperfusion following acute myocardial infarction may protect the heart. MPT pore opening appears to be a pivotal event in cell death following acute myocardial infarction. Recently, MPT pore opening has been involved in ischemic preconditioning. In protocol 1, NZW rabbits underwent either no intervention (sham) or 10 min of ischemia followed by 5 min of reperfusion, preceded (preconditioned, PC) or not (control, C) by 5 min of ischemia and 5 min of reperfusion. Additional rabbits were treated by cyclosporin A (CsA) or its non-immunosuppressive and more specific derivative (NIM811) (10 mg kg^{-1}, IV bolus), either 10 min before ischemia or 1 min before reperfusion. Hearts were excised and mitochondria isolated for further assessment of Ca^{2+}-induced MPT. In protocol 2, animals were randomly assigned into similar experimental groups and underwent 30 min of ischemia and 4 h of reperfusion. Infarct size was assessed by TTC staining, and apoptosis by TUNEL assay. The Ca^{2+} overload required to induce MPT pore opening was significantly higher in NIM811, CsA and PC groups than in controls. Both necrotic and apoptotic cardiomyocyte death were significantly reduced by NIM811, CsA and PC. In both protocols, administration of NIM811 at reperfusion provided full protection. These data indicate that specific inhibition of MPT pore opening at reperfusion following acute myocardial infarction provides a powerful antinecrotic and antiapoptotic protection.

Keywords: Preconditioning; Cyclosporin A; NIM811; Ischemia; Reperfusion

1. Introduction

There is no doubt that reperfusion limits irreversible injury following a prolonged ischemic insult, and reperfusion strategies have been extensively studied and developed in clinical practice over the last two decades [1,2]. Reperfusion has also been named a "double-edged sword", since it is associated with reversible functional alterations including myocardial stunning, arrhythmias or no-reflow [3]. The existence of another form of reperfusion, namely lethal reperfusion injury, has been widely investigated and remains a matter of debate [4,5]. Demonstration of the existence of lethal reperfusion injury would imply reduction of infarct size following administration of a pharmacological agent at the time of reperfusion. Recently, the demonstration that several peptide growth factors as well as caspase inhibitors can attenuate myocardial cell death when administered at the time of reflow, reactivated the issue of lethal reperfusion injury and the hope for the development of adjunctive treatments to thrombolysis or coronary angioplasty during acute myocardial infarction [6–9]. It has been recently demonstrated that brief episodes of ischemia–reperfusion performed at the time of reflow (named "postconditioning") may similarly attenuate lethal reperfusion injury, likewise the previously described controlled reperfusion [10–12].

In the past 3 years, increasing research has been focused on the role of mitochondrial permeability transition (MPT)

* Corresponding author. Tel.: +33-4-78-77-70-74; fax: +33-4-78-77-71-75.
E-mail address: ovize@rockefeller.univ-lyon1.fr (M. Ovize).

0022-2828/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.yjmcc.2004.12.001

in lethal ischemia–reperfusion injury, with specific attention to the mechanism of preconditioning [13–16]. MPT pore opening is recognized as a pivotal event in necrotic and apoptotic cell death [17,18]. Following ischemia–reperfusion injury, opening of this nonspecific pore in the inner mitochondrial membrane is favored by Ca^{2+} overload, adenine nucleotide depletion, accumulation of inorganic phosphate, production of reactive oxygen species [19]. It results in inner membrane potential ($\Delta\Psi_m$) collapse, uncoupling of the respiratory chain, and efflux of small molecules such as cytochrome *c* and other proapoptotic factors [20]. According to the energy status of the cell, this event may further lead to either apoptosis or necrosis. Griffiths and Halestrap [21] demonstrated in the isolated rat heart that the MPT pore remains closed throughout ischemia but opens at the time of reperfusion. It is therefore logical to investigate whether inhibiting MPT opening at the time of reperfusion might limit cardiomyocyte death following a prolonged ischemic insult. Cyclosporin A (CsA) is a powerful inhibitor of the MPT pore, and several reports indicate that it protects the isolated heart from ischemia–reperfusion injury [13,15,22–25]. Unfortunately, besides its inhibitory effect on the MPT through it is binding to cyclophylin D, CsA also interacts (via its binding to cyclophilin A) with several molecular targets in the cytosol and the mitochondria, which may impact on the cardiomyocyte prosurvival mechanisms [26,27].

NIM811 is a non-immunosuppressive derivative of CsA that does not bind to cytosolic cyclophilin A, but specifically inhibits MPT pore opening. NIM811 allowed us to directly investigate whether a more specific inhibition of the MPT pore at the onset of reperfusion is cardioprotective. In the present study, we demonstrated that administration of the specific MPT pore inhibitor NIM811 at the time of reperfusion attenuates myocardial apoptosis and necrosis to a similar extent as ischemic preconditioning.

2. Materials and methods

All animals were treated in compliance with the "Position of the American Heart Association on Research Animal Use", adopted by the AHA on November 11, 1984.

2.1. Surgical preparation

Male New Zealand White rabbits, weighing 2.2–2.5 kg were anesthetized by intramuscular injections of xylazine (5 mg kg^{-1}) and ketamine (50 mg kg^{-1}), as previously described [28]. An intravenous infusion of a mixture of xylazine (20–50 μg kg^{-1} min^{-1}) and ketamine (40–100 μg kg^{-1} min^{-1}) was then maintained throughout the experiment. After a midline cervical incision, a tracheotomy was performed and animals were ventilated with room air. A cannula was inserted into the right internal jugular vein for administration of drugs and fluids and into the left carotid artery for measurement of blood pressure. After an intravenous bolus administration of fentanyl (10 mg kg^{-1}), a left thoracotomy was performed in the fourth left intercostal space. The pericardium was opened and the heart exposed. A 3.0 silk suture attached to a small curved needle was passed around a marginal branch of the left circumflex coronary artery. Both ends of the thread were passed through a small vinyl tube to form a snare that could be tightened to occlude and loosened to reperfuse the artery. Body temperature was monitored via an intraperitoneal thermometer and kept constant by means of a heating pad. Heart rate (HR) and arterial pressure were monitored continuously throughout the experiment on a Gould® recorder (Gould Inc., Cleveland, OH). After the surgical procedure, a 15 min stabilization period was observed.

2.2. Protocol 1: Specific inhibition of Ca^{2+}-induced MPT pore opening by NIM811

Protocol 1 investigated whether and how administration of NIM811 at the time of reperfusion altered Ca^{2+}-induced MPT pore opening.

2.2.1. Experimental design

Fifty-six rabbits were randomly assigned to one of seven groups (Fig. 1). All animals (n = 8 per group) underwent a test ischemic insult consisting of 10 min of coronary artery occlusion followed by 5 min of reperfusion, as previously described [16]. Prior to this, control rabbits underwent no intervention for 15 min (control groups, C), while preconditioned (PC) received 5 min of ischemia followed by 5 min of reperfusion (PC groups). Treated rabbits received an intravenous bolus of CsA or NIM811 (10 mg kg^{-1}), either 10 min before coronary occlusion (CsA-I and NIM811-I groups), or 1 min before the reperfusion period (CsA-R and NIM811-R groups). An additional group of rabbits (sham) underwent no ischemia/reperfusion for the 30 min period of the protocol. At the end of the experiment, hearts were harvested while still beating, and mitochondria isolated from the myocardium at risk for further assessment of Ca^{2+}-induced MPT pore opening.

2.2.2. Preparation of isolated mitochondria

Preparation of mitochondria was adapted from a previously described procedure [16,29,30]. All operations were carried out in a cold room at 4 °C. Heart pieces (0.5–1.0 g) were placed in isolation buffer A containing 70 mM sucrose, 210 mM mannitol, 1 mM EDTA in 50 mM Tris–HCl pH 7.4. The tissue was finely minced with scissors and then homogenized in the same buffer (10 ml buffer per g tissue), using successively a Kontes tissue grinder and a Potter Elvejem. The homogenate was centrifuged at 1300 g for 3 min. The supernatant was poured through cheesecloth and centrifuged at 10,000 × *g* for 10 min. The mitochondrial pellet was suspended in isolation buffer B containing 70 mM sucrose, 210 mM mannitol, 0.1 mM EDTA in 50 mM Tris–HCl pH 7.4. Protein content was routinely assayed according to Gornall et al. [31] procedure using bovine serum albumin as a