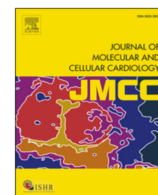
Journal of Molecular and Cellular Cardiology 84 (2015) 61–69

Contents lists available at ScienceDirect

Journal of Molecular and Cellular Cardiology

journal homepage: www.elsevier.com/locate/yjmcc



Original article

Inhibition of myocardial reperfusion injury by ischemic postconditioning requires sirtuin 3-mediated deacetylation of cyclophilin D

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ARTICLE INFO

Article history:
Received 5 November 2014
Received in revised form 26 March 2015
Accepted 27 March 2015
Available online 11 April 2015

Keywords:
Ischemia–reperfusion
Survival
Post-conditioning
Cyclophilin D
Sirtuin 3

ABSTRACT

Rationale. How ischemic postconditioning can inhibit opening of the mitochondrial permeability transition pore (PTP) and subsequent cardiac myocytes death at reperfusion remains unknown. Recent studies have suggested that de-acetylation of cyclophilin D (CyPD) by sirtuin 3 (SIRT3) can modulate its binding to the PTP.
Objective. The aim of the present study was to examine whether ischemic postconditioning (PostC) might activate SIRT3 and consequently prevent lethal myocardial reperfusion injury through a deacetylation of CyPD.
Methods and results. Using hypoxia–reoxygenation (H/R) in H9C2 cells, we showed that SIRT3 overexpression prevented CyPD acetylation, limited PTP opening and reduced cell death by 24%. In vitro modification of the CyPD acetylation status in MEFs by site-directed mutagenesis altered capacity of PTP opening by calcium. Calcium Retention Capacity (CRC) was significantly decreased with CyPD-KQ that mimics acetylated protein compared with CyPD WT (871 ± 266 vs 1193 ± 263 nmoles Ca^{2+}/mg protein respectively). Cells expressing non-acetylable CyPD mutant (CyPD-KR) displayed 20% decrease in cell death compared to cells expressing CyPD WT after H/R. Correspondingly, in mice we showed that cardiac ischemic postconditioning could not reduce infarct size and CyPD acetylation in SIRT3 KO mice, and was unable to restore CRC in mitochondria as it is observed in WT mice.
Conclusions. Our study suggests that the increased acetylation of CyPD following myocardial ischemia–reperfusion facilitates PTP opening and subsequent cell death. Therefore ischemic postconditioning might prevent lethal reperfusion injury through an increased SIRT3 activity and subsequent attenuation of CyPD acetylation at reperfusion.

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1. Introduction

Acute myocardial infarction (AMI) is one of the leading causes of death worldwide. Myocardial infarct size is strongly correlated with heart failure and mortality [1,2]. Early reperfusion is the most effective treatment to reduce myocardial damage due to a prolonged ischemic insult [3,4]. Unfortunately, the benefit of the restoration of blood flow to the jeopardized myocardium is partly attenuated by irreversible damage to cardiomyocytes that were still viable at the end of the ischemic period [5].

Evidence indicates that this lethal reperfusion injury is due to mitochondrial dysfunction, following the opening of a mega-channel in the inner membrane named the permeability transition pore (PTP) [6]. Therapeutic interventions like ischemic postconditioning (PostC) significantly blunt lethal reperfusion injury likely through the inhibition of PTP opening [7,8]. Although molecular structure of the PTP remains unclear, it is well accepted that PTP opening is facilitated by the translocation of cyclophilin D (CyPD), a mitochondrial matrix protein, to the inner mitochondrial membrane [9]. CyPD KO mice which display an increased resistance to PTP opening in vitro are protected from lethal reperfusion injury, and can no longer be postconditioned [10,11].

Previous studies have demonstrated that post-translational modifications of CyPD by phosphorylation and nitrosylation regulate PTP opening [12–14]. Other studies showed that CyPD can also be acetylated on lysine 166 [15,16]. Protein acetylation is regulated by sirtuins which belong to a family of Nicotinamide Adenine Dinucleotide (NAD^+)-dependent deacetylases. Sirtuins are considered as “metabolic sensors”

Abbreviations: AN, area of necrosis; AR, area at risk; CRC, Calcium Retention Capacity; CyPD, cyclophilin D; DMEM, Dulbecco's Modified Eagle Medium; FBS, Fetal Bovine Serum; FFC, Fédération Française de Cardiologie; H/R, hypoxia–reoxygenation; LCA, left coronary artery; MEFs, Mouse Embryonic Fibroblasts; NAD+, Nicotinamide Adenine Dinucleotide; PI, Propidium Iodide; PostC, postconditioning; PTP, permeability transition pore; SIRT3, sirtuin 3.

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http://dx.doi.org/10.1016/j.yjmcc.2015.03.017
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[17] and the human genome encodes seven sirtuins isoforms named SIRT1 to SIRT7 [18]. SIRT3, SIRT4 and SIRT5 [19] are located in the mitochondria, with SIRT3 being the most studied [20]. In 2010, Hafner et al. demonstrated that SIRT3 is able to deacetylate CyPD which in turn inhibits PTP induction [15].

We hypothesized that ischemic PostC might attenuate lethal reperfusion injury via the activation of SIRT3 with subsequent deacetylation of CyPD and inhibition of PTP opening.

2. Methods

2.1. Animals

Experiments were carried out according to the NIH Guide on the Use of Laboratory Animals and the Lyon 1 Claude Bernard University Committee on Animal Care approved all procedures. A total of 120 males 8 to 12-week-old 129S6/SvEvTac mice used in this study were divided into 2 groups; wild-type (WT) or knocked-out (KO) SIRT3 mice ($SIRT3^{-/-}$) and were randomly assigned to different experimental groups.

2.2. In vivo model of acute myocardial ischemia–reperfusion injury

WT and SIRT3KO mice were anesthetized by intraperitoneal injection of 73 mg/kg body weight of pentobarbital. Analgesia was provided by an intraperitoneal injection of buprenorphine. Animals were intubated and subjected to open-chest surgery to induce myocardial infarction by the transient ligation of the left coronary artery (LCA) as previously described [21,22].

Animals underwent 60 min of ischemia followed by either 24 h of reperfusion (for determination of infarct size) or 1 h of reperfusion (for western-blot analysis). WT and SIRT3 KO mice were randomly allocated to one of the following groups (n = 7 to 9 per group) (Fig. 1):

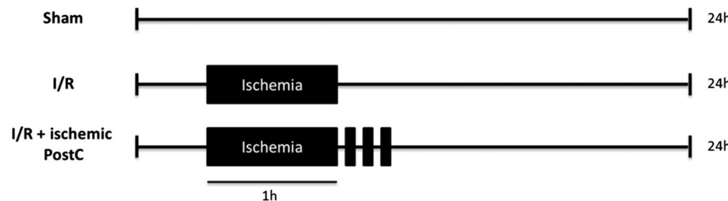
- Sham animals underwent no LCA ligation and no intervention.
- The control group: 60 min of ischemia followed by reperfusion but no other intervention.
- The ischemic PostC group: immediately (within one minute) after the 60-minute ischemia, mice underwent ischemic PostC consisting of 3 cycles of 1 min of ischemia/1 min of reperfusion followed by a prolonged reperfusion period.

2.3. Area at risk (AR) and area of necrosis (AN) determination

At the end of the final reperfusion, the coronary artery was briefly reoccluded and 0.5 mg/kg of Uniperse blue pigment was injected intravenously to delineate the in vivo area at risk (AR), as previously described [21,22]. Each heart slice was weighed and photographed for later measurement of the AR. After 15 min of incubation in a 1% solution of triphenyltetrazolium chloride at 37 °C, infarcted (pale) and viable (brick red) myocardial areas were photographed. Enlarged projections of these slices were traced for determination and quantification of the boundaries of the AR and area of necrosis (AN). Total weight of the AR and the AN was then calculated and expressed in grams and as percentage of LV weight and of the AR weight, respectively.

2.4. SIRT3 activity

Mitochondria isolated from WT and SIRT3 KO mice hearts from Sham, control or PostC groups were used to quantify SIRT3 activity using SIRT3 fluorescent assay kit (BPS Bioscience).



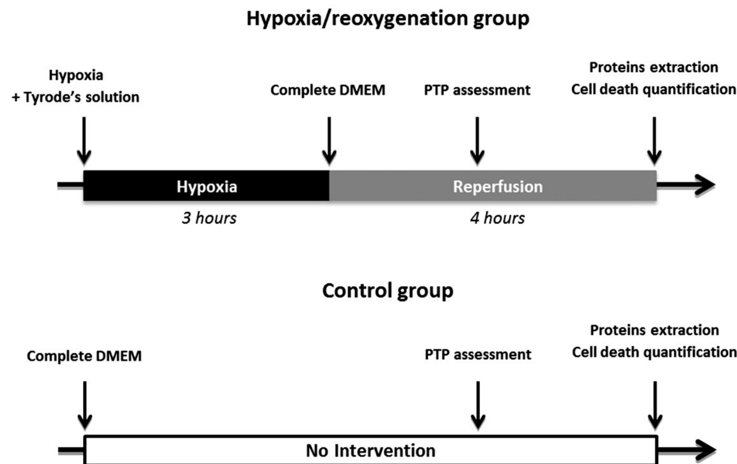


Fig. 1. Experimental design. In vivo: Sham animals underwent no LCA. The control group underwent 60 min of ischemia followed by reperfusion. The ischemic postconditioning (PostC) group underwent a procedure consisting of 3 cycles of 1 min of ischemia/1 min of reperfusion followed by the prolonged reperfusion period immediately after the 60 min ischemic period. In vitro: Cells from the hypoxia/reoxygenation (H/R) group were submitted to 3 h of hypoxia followed by 4 h of reperfusion. PTP assessment was performed after 2 h of reperfusion. Cell death quantification and protein extraction were carried out after 4 h of reperfusion. The control group underwent no intervention.

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