BIOCHEMICAL PHARMACOLOGY 71 (2006) 441–452

available at www.sciencedirect.com

SCIENCE DIRECT®

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

Critical role of PI3-kinase/Akt activation in the PARP inhibitor induced heart function recovery during ischemia–reperfusion

Krisztina Kovacs [a], *Ambrus Toth* [a], *Peter Deres* [a], *Tamas Kalai* [b], *Kalman Hideg* [b], *Ferenc Gallyas Jr.* [a], *Balazs Sumegi* [a,*]

[a] *Department of Biochemistry and Medical Chemistry/Hungarian Academy of Sciences, Research Group for Mitochondrial Function and Mitochondrial Diseases, Hungary*

[b] *Institute of Organic and Medicinal Chemistry, University of Pecs Medical School, Pecs, Hungary*

ARTICLE INFO

Article history:
Received 11 March 2005
Accepted 5 May 2005

Keywords:
Ischemia–reperfusion
PARP inhibitors
ROS
Myocardial energy metabolism
Signal transduction
PI3-kinase/Akt

Abbreviations:
GSK-3β, glycogen synthase kinase-3β
IR, ischemia–reperfusion
NAD^+, nicotinamide adenine dinucleotide
PARP, poly(ADP-ribose) polymerase
PI3-kinase, phosphatidylinositol-3-kinase
ROS, reactive oxygen species
TBARS, thiobarbituric acid reactive substances

ABSTRACT

Poly(ADP-ribose) polymerase (PARP) inhibitors protect hearts from ischemia–reperfusion (IR)-induced damages by limiting nicotinamide adenine dinucleotide (NAD^+) and ATP depletion, and by other, not yet elucidated mechanisms. Our preliminary data suggested that PARP catalyzed ADP-ribosylations may affect signaling pathways in cardiomyocytes. To clarify this possibility, we studied the effect of a well-characterized (4-hydroxyquinazoline) and a novel (carboxaminobenzimidazol-derivative) PARP inhibitor on the activation of phosphatidylinositol-3-kinase (PI3-kinase)/Akt pathway in Langendorff-perfused hearts. PARP inhibitors promoted the restoration of myocardial energy metabolism (assessed by ^{31}P nuclear magnetic resonance spectroscopy) and cardiac function compared to untreated hearts. PARP inhibitors also attenuated the infarct size and reduced the IR-induced lipid peroxidation, protein oxidation and total peroxide concentration. Moreover, PARP inhibitors facilitated Akt phosphorylation and activation, as well as the phosphorylation of its downstream target glycogen synthase kinase-3β (GSK-3β) in normoxia and, more robustly, during IR. Blocking PI3-kinase by wortmannin or LY294002 reduced the PARP inhibitor-elicited robust Akt and GSK-3β phosphorylation upon ischemia–reperfusion, and significantly diminished the recovery of ATP and creatine phosphate showing the importance of Akt activation in the recovery of energy metabolism. In addition, inhibition of PI3-kinase/Akt pathway decreased the protective effect of PARP inhibitors on infarct size and the recovery of heart functions. All these data suggest that contrary to the original view, which considered preservation of NAD^+ and consequently ATP pools as the exclusive underlying mechanism for the cytoprotective effect of PARP inhibitors, the activation of PI3-kinase/Akt pathway and related processes are at least equally important in the cardioprotective effects of PARP inhibitors during ischemia–reperfusion.

* *Corresponding author*. Present address: Department of Biochemistry and Medical Chemistry, University of Pecs Medical School, Szigeti u. 12, H-7624 Pecs, Hungary. Tel.: +36 72 536 277; fax: +36 72 536 277.
E-mail address: balazs.sumegi@aok.pte.hu (B. Sumegi).

0006-2952/$ – see front matter
doi:10.1016/j.bcp.2005.05.036

1. Introduction

Acute myocardial ischemia accounts for the highest percentage of morbidity and mortality in the Western world [1]. Persistent ischemia can result in cardiomyocyte death and lead to congestive heart failure. Coronary reperfusion utilizing thrombolytics and coronary angioplasty can partially rescue the ischemic myocardium and limit the development of an infarct. However, reperfusion, though prerequisite for tissue salvage, might also lead to increased cell mortality, possibly as a result of the inflammatory response, a burst of oxygen-free radical production and calcium overload [2,3].

Several studies have suggested that both neutrophils and reactive oxygen species (ROS) play important roles in ischemia–reperfusion (IR)-induced cardiac dysfunction [4–6]. High levels of ROS are generated from a variety of sources, such as the xantine oxidase system [4], the leakage of electrons from the mitochondrial respiratory chain [5,7], the cyclooxygenase pathway of arachidonic acid metabolism [6,8] and the respiratory burst of phagocytic cells [9,10]. In the heart, ROS can evoke cytotoxicity [11], cardiac stunning [12], arrhythmia [13], reduction of the calcium transient and contractility, elevated diastolic calcium levels [14] and intracellular ATP depletion [15].

During ischemia–reperfusion cycle ROS and peroxynitrite formation causes lipid peroxidation, protein oxidation as well as DNA breaks [16]. Poly(ADP-ribose) polymerase (PARP), a protein-modifying and nucleotide-polymerizing enzyme, is present abundantly in the nucleus. In response to DNA damage, PARP becomes activated and produces homopolimers of adenosine diphosphate-ribose units using nicotinamide adenine dinucleotide (NAD^+) as a substrate. This process rapidly depletes the intracellular NAD^+ and ATP pools, which slows the rate of glycolysis and mitochondrial respiration leading to cellular dysfunction and death [17,18]. Accordingly, inhibition of PARP can improve the recovery of different cells from oxidative injury [19]. Our previous data showed that PARP inhibitors were able to reduce the oxidative damage of cellular components without having an obvious scavenger activity [16].

External stress-related tissue injury, such as ischemia–reperfusion can initiate protein kinase cascades and inflammatory reactions. Previous results indicate that the growth factor-associated kinase Akt (also known as protein kinase B) is phosphorylated following ischemia–reperfusion in cardiomyocytes in a phosphatidylinositol-3-kinase (PI3-kinase)-dependent manner [20]. However, some data suggest that Akt can be activated by a PI3-kinase-independent way, as well [21,22]. Akt kinase pathway is one of several signal transduction pathways implicated in cell survival [23,24]. Akt can phosphorylate a number of downstream targets leading to the inactivation of glycogen synthase kinase-3β (GSK-3β), the pro-apoptotic Bcl-2 family member Bad [25], caspase-9 [26] and Forkhead transcription factor (FKHR) [24], as well as to the activation of nuclear factor-κB (NF-κB) [27], p70 ribosomal S6 kinase and endothelial nitric oxide synthase (eNOS) [28,29]. PARP inhibitors have been shown to improve the survival of mice with lipopolysaccharide-induced septic shock in a PI3-kinase/Akt-dependent manner [30]. However, it needs to be elucidated whether the proven cardioprotective properties of PARP inhibitors in ischemia–reperfusion models are, at least in part, mediated via Akt signaling.

In the present study, we investigated the molecular mechanism by which PARP inhibitors promote the recovery of energy metabolism and heart function during ischemia–reperfusion, and provided evidence that PARP inhibitors activated PI3-kinase/Akt pathway in postischemic hearts. Furthermore, data presented here provide the first evidence that the activation of PI3-kinase/Akt pathway in postischemmic heart is responsible in a significant extent for the recovery of energy metabolism and heart function, as well as preservation of viable myocardium in ischemia–reperfusion, indicating a novel molecular mechanism in the cardioprotective effect of PARP inhibitors.

2. Materials and methods

2.1. PARP inhibition

The IC_{50} of 4-hydroxyquinazoline and HO-3089 was studied in an in vitro assay as described before [31].

2.2. Cell culture and MTT assay

H9c2(2-1) cardiomyoblasts (American Type Culture Collection number CRL-1446), a clonal line derived from embryonic rat heart, were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum and 2 mM pyruvate in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C. Before reaching confluence, the cells were split, plated at low density in culture dishes (approximately 2×10^4 cells/well) and cultured for 24 h. Cardiomyocytes were then incubated without (negative control) and with 1 mM hydrogen peroxide for 3 h either untreated (positive control) or treated with 4-hydroxyquinazoline (in 5, 10, 50, 100 and 200 μM) or HO-3089 (in 0.02, 0.05, 0.1, 10 and 50 μM). At the end of the incubation period the survival of cells was determined by the MTT assay as described before [32]. Briefly, the cells were incubated for 3 h in fresh medium containing 0.5% of the water-soluble yellow mitochondrial dye, 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT^+). The MTT^+ reaction was terminated by adding HCl to the medium at a final concentration of 10mM. The amount of water-insoluble blue formasan dye formed from MTT^+ was proportional to the number of live cells, and was determined with an Anthos Labtech 2010 ELISA reader at 550 nm wavelength after dissolving the blue formasan precipitate in 10% sodium dodecyl sulphate. All experiments were run in at least four parallels and repeated three times.

2.3. Heart perfusion

Male Wistar rats weighing 300–350 g were heparinized with sodium heparin (100 IU, i.p.) and anesthetized with ketamine (200 mg/kg, i.p.). The investigation conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health, and was approved by the Animal Research Review Committee of the University of Pecs. Hearts were perfused via the aorta according to the Langendorff