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Original article

PARP inhibition prevents postinfarction myocardial remodeling and heart failure via the protein kinase C/glycogen synthase kinase-3β pathway ☆

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Abstract

The inhibition of glycogen synthase kinase-3β (GSK-3β) via phosphorylation by Akt or protein kinase C (PKC), or the activation of mitogen-activated protein kinase (MAPK) cascades can play a pivotal role in left ventricular remodeling following myocardial infarction. Our previous data showed that MAPK and phosphatidylinositol-3-kinase/Akt pathways could be modulated by poly(ADP-ribose)polymerase (PARP) inhibition raising the possibility that cardiac hypertrophic signaling responses may be favorably influenced by PARP inhibitors.

A novel PARP inhibitor (L-2286) was tested in a rat model of chronic heart failure following isoproterenol-induced myocardial infarction. Subsequently, cardiac hypertrophy and interstitial collagen deposition were assessed; additionally, mitochondrial enzyme activity and the phosphorylation state of GSK-3β, Akt, PKC and MAPK cascades were monitored.

PARP inhibitor (L-2286) treatment significantly reduced the progression of postinfarction heart failure attenuating cardiac hypertrophy and interstitial fibrosis, and preserving the integrity of respiratory complexes. More importantly, L-2286 repressed the hypertrophy-associated increased phosphorylation of panPKC, PKC α/βII, PKC δ and PKC ε, which could be responsible for the activation of the antihypertrophic GSK-3β.

This work provides the first evidence that PARP inhibition beneficially modulates the PKC/GSK-3β intracellular signaling pathway in a rat model of chronic heart failure identifying a novel drug target to treat heart failure.

Keywords: PARP inhibition; Intracellular signaling; Glycogen synthase kinase-3β; Heart failure; Protein kinase C; Ventricular remodeling

1. Introduction

Increased activation of poly(ADP-ribose) polymerase (PARP) enzyme is a crucial step in the development of oxidative stress-induced cell dysfunction and tissue injury [1,2]. Oxidative stress also plays a pathogenic role in chronic heart failure that can be characterized by altered intracellular signaling and gene expression [3–7]. Several studies demonstrated that inhibition of PARP enzyme can efficiently reduce oxidative myocardial damage; nevertheless, little is known about the mechanism of cardioprotection by PARP inhibitors in chronic heart failure [1,2,8]. This molecular mechanism is worth elucidating because

Abbreviations: BNP, B-type natriuretic peptide; BW, body weight; ERK1/2, extracellular signal-regulated kinase; GSK-3β, glycogen synthase kinase-3β; HE, hematoxylin and eosin; ISO, isoproterenol hydrochloride; JNK, c-*jun* N-terminal kinase; MAPK, mitogen activated protein kinase; NAD^+, nicotinamide adenine dinucleotide; NF-κB, nuclear factor-κB; NIH, National Institutes of Health; PARP, poly(ADP-ribose) polymerase; PDC-1α, pyruvate dehydrogenase complex-1α; PI3K, phosphatidylinositol-3-kinase; PKC, protein kinase C; ROS, reactive oxygen species; SEM, standard error of mean; TBS, Tris-buffered saline; TEF-1, transcriptional enhancer factor-1; TL, length of right tibia; WV, weight of ventricles

☆ List of special characters: β, μ, ±, <.

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PARP inhibition already represents a potential clinical approach to fight against myocardial remodeling: as we have previously reported, carvedilol, an antiadrenergic drug used, among others, to treat the hypertrophic and failing heart, exhibited antioxidant and PARP-inhibitory properties [9,10]. It has been widely known that the beneficial effects of PARP enzyme blockade in oxidative injuries partially rely on the preservation of the cellular NAD^+ and ATP pools [1,2]. Recent studies, however, suggested that PARP inhibition could beneficially influence various intracellular signaling routes further contributing to the cardioprotective effect of these agents [2,8,11,12]. Although PARP enzyme-mediated direct protein interaction and poly(ADP-ribosyl)ation were shown to influence the function of a variety of transcription factors and to interfere with the expression of several proinflammatory genes, to date, limited information is available on how PARP inhibition might modulate intracellular signaling during the progression of postinfarction heart failure [11].

Remodeling of the left ventricle following myocardial infarction ultimately progresses into heart failure, which is a leading cause of mortality worldwide [5]. Cardiac remodeling is associated with alterations in intracellular signaling including the inhibition of glycogen synthase kinase-3β (GSK-3β) via phosphorylation by Akt, protein kinase C (PKC), p70-S6 kinase, p90-RSK and protein kinase A [13]. These pathways and the mitogen-activated protein kinases (MAPK) have all been demonstrated to alter their activation state in response to hypertrophic stimuli and may therefore contribute to myocardial hypertrophy [5–7]. We have recently found that inhibition of PARP protected cells from oxidative stress by modulating the phosphorylation and activation of Akt and/or MAP kinases in a cell culture system, ex vivo and in vivo animal models of myocardial injury and also in a rodent septic shock model. These findings also suggest that PARP inhibition could modulate these signaling pathways which are also participants in the development of cardiac hypertrophy [17,18]. Notably, ventricular remodeling is also accompanied by changes in energy metabolism, mitochondrial dysfunction and by increased expression of fetal genes and extracellular matrix components [14–16].

To elucidate the role of protein kinase signaling in the mechanism of cardioprotection afforded by PARP inhibition, L-2286 (Fig. 1), a novel quinazoline-type PARP inhibitor was tested in a rat model of chronic heart failure after isoproterenol-induced myocardial infarction [8]. L-2286 attenuated the myocardial hypertrophy and interstitial deposition of type III collagen, and beneficially influenced the mitochondrial enzyme activity in the failing heart. In addition, our study first demonstrated that PARP inhibition could reduce the progression of chronic myocardial remodeling possibly via its ability to interfere with PKC-mediated GSK-3β inhibition.

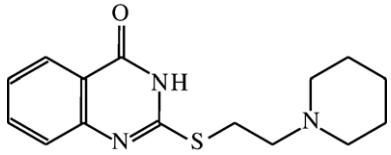

Fig. 1. Chemical structure of L-2286 (2-[(2-Piperidin-1-yletil)thio]quinazolin-4(3*H*)-one).

2. Materials and methods

2.1. Chronic heart failure model

Male CFY-strain Sprague–Dawley rats (350–380 g) received two subcutaneous injections (separated by a 24-h interval) of 80 mg/kg isoproterenol (ISO, Sigma-Aldrich Co, Budapest, Hungary) [19]. Twenty-four hours after the second injection, the surviving animals were randomly assigned to receive either 5 mg/kg/die L-2286 (n = 15), an effective and selective water-soluble PARP inhibitor, or water (n = 12). PARP inhibitor treatment was delayed 24 h to avoid suppression of infarct size by the PARP inhibition [20,21]. L-2286 was given for 8 weeks. The drug-only control group (C + L-2286) received 5 mg/kg/die L-2286 for 8 weeks. At the end of the 8-week period, body weights were measured and standard electrocardiogram (ECG) was recorded to determine R wave amplitude and J point deviation (lead II) (Schiller AG Electrocardiograph, Switzerland). Animals were subsequently sacrificed, their hearts were removed, the atria and great vessels were trimmed from the ventricles and the weight of the ventricles was measured. It was then normalized to the body mass and the length of right tibia. Hearts were freeze-clamped or fixed in 10% formalin. The investigation conformed to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85–23, revised 1996), and was approved by the Animal Research Review Committee of the University of Pecs Medical School.

2.2. Determination of plasma B-type natriuretic peptide

Blood samples were collected into the Lavender Vacutaner tubes containing EDTA and aprotinin (0.6 TIU/ml of blood), centrifuged at 1600×g for 15 min at 4 °C. Supernatants (plasma) were collected and kept at −70 °C. Plasma B-type natriuretic peptide-45 (BNP-45) levels were determined by enzyme immunoassay method (BNP-45, Rat EIA Kit, Phoenix Pharmaceuticals Inc., CA, USA).

2.3. Measurement of mitochondrial enzyme activity

NADH:cytochrome c oxidoreductase was measured as described previously [22]. Enzyme activity was determined by measuring the rate of cytochrome c reduction at 550 nm in a medium containing 50 mmol/l sodium-phosphate, 1 mmol/l sodium-azide, 1.5 mM NADH and 50–75 μg mitochondrial protein/ml, pH 7.5. The reaction was started by addition of 40 μl cytochrome c.

2.4. Histology

Ventricles fixed in formalin were sliced and embedded in paraffin. Sections (5-μm thick) were cut serially from base to apex. Ten to twelve slices at 1-mm intervals were stained with