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Inhibition of postinfarction ventricular remodeling by high molecular weight polyethylene glycol

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ABSTRACT

Background: Myocardial infarction (MI) is a major etiology for the development of heart failure. We have previously shown that high molecular weight polyethylene glycol (PEG) can protect cardiac myocytes from hypoxia-reoxygenation injury *in vitro*. In this study, we investigated the potential protective effects of 15-20 kD PEG postinfarction without reperfusion.

Methods: One milliliter of PEG 15-20 was delivered intravenously following permanent left anterior descending ligation in adult male rats with phosphate buffer saline (PBS) as control ($n = 9$ in each group). Echocardiography was performed at baseline and at 8 wk post-MI. Left ventricles (LVs) were harvested to quantify fibrosis, apoptosis, cell survival signaling, regulation of β-adrenergic signaling, and caveolin (Cav) expression.

Results: The PEG group had significant recovery of LV function at 8 wk compared with the PBS group. There was less LV fibrosis in both the infarct and remote territory. Cell survival signaling was upregulated in the PEG group with increased Akt and ERK phosphorylation. PEG inhibited apoptosis as measured by terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick-end labeling positive nuclei and caspase-3 activity. There was maintenance of Cav-1, Cav-2, and Cav-3 expression following PEG treatment *versus* a decline in the PBS group. Negative regulators of β-adrenergic signaling, G protein-coupled receptor kinase-2, and β-arrestin 1 and 2 were all upregulated in PBS-treated samples compared to normal control; however, PEG treatment led to decreased expression.

Conclusions: These data suggest that PEG 15-20 may have significant protective effects post-MI even in the setting of no acute reperfusion. Upregulation of Cav expression appears to be a key mechanism for the beneficial effects of PEG on ventricular remodeling and function.

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Introduction

Ischemic heart disease continues to be a primary etiology for the development of heart failure (HF). It has been reported that within 6 y of the clinical event of an acute myocardial infarction (MI), 22% of male and 46% of female patients develop HF.[1] This occurs despite optimal medical management. Loss of cardiac myocytes in the setting of ischemic injury occurs via several processes including necrosis and apoptosis.[2] Although both of these mechanisms of cell death occur acutely, apoptotic cell death of cardiac myocytes has been shown in the myocardium years after the ischemic injury.[3] There are currently no therapies to prevent or minimize cell death in the setting of acute MI, and this promotes the development of adverse ventricular remodeling and dysfunction in the longer term. This process is characterized by drop out of cardiac myocytes in the infarct zone and replacement fibrosis, which are well described through inflammatory and profibrotic responses to the injury.[4] In addition, remote (noninfarct) territory fibrosis can occur, which has been demonstrated to play a highly significant role in the maladaptive remodeling and progression to HF.[5]

Our group has been investigating the potential therapeutic role of the synthetic polymer, polyethylene glycol (PEG), in the setting of ischemia-reperfusion (I-R) injury. We have used the 15-20 kD molecular weight PEG (PEG 15-20) to demonstrate a protective role from cardiac myocyte apoptosis *in vitro* following hypoxia-reoxygenation injury[6] and more recently in an *in vivo* rat model of I-R injury.[7] The *in vivo* studies proved that PEG 15-20 could be taken up in the myocardium following intravenous delivery just before reperfusion, and that there was a robust protective effect as measured by decreased apoptotic signaling and apoptosis, upregulation of cell survival signaling, inhibition of myocardial fibrosis and adverse remodeling, and decreased mortality.

The objective of the present study is to determine whether PEG 15-20 may have beneficial effects on ventricular remodeling and function when administered intravenously following an acute MI without reperfusion. This was done using a rat model of left anterior descending (LAD) coronary artery ligation. Our hypothesis is that PEG will provide significant myocardial protection following MI and that the mechanism would include increased caveolin (Cav) signaling and inhibition of deleterious G protein-coupled receptor kinase-2 (GRK2) and β-arrestin signaling, which have been shown to be major drivers for the development of postinjury HF.[8,9]

Materials and methods

Rat MI model and delivery of PEG

All animal procedures and experiments were performed in accordance with the guidelines of the Institutional Animal Care and Use Committee of the University of Wisconsin–Madison. Adult male Sprague–Dawley rats were used for all procedures. MI was induced as described previously using a LAD coronary artery ligation that produces a highly reproducible infarction.[10] Briefly, anesthetized adult male rats were intubated and mechanically ventilated. Anesthesia was maintained using a 2% isoflurane (v/v) oxygen mixture. Rats were placed in the left lateral decubitus position and the chest cavity was opened through a left thoracotomy at the third intercostal space to achieve visualization of the heart. The pericardium was opened. MI was induced through permanent ligation of the LAD coronary artery with an 8-0 silk suture proximal to its bifurcation from the main stem. Left ventricles (LVs) blanching indicated successful occlusion of the LAD. The incision was subsequently closed with 5-0 silk suture, and the rats were allowed to recover. One milliliter of 10% PEG solution or saline (phosphate buffer saline [PBS]) control ($n = 9$ per group) was administered intravenously immediately after ligation. Animals were sacrificed 8 wk post-MI surgery. Control rats did not undergo surgery ($n = 9$). The mortality of animals operated on in this manner was 5% within 48 h. High molecular weight PEG (15,000-20,000 Da), referred to as PEG 15-20, was purchased from Sigma (St. Louis, MO).

Echocardiography

Echocardiography was performed at baseline and at 8 wk post-MI to assess global cardiac function as previously described.[11] The VisualSONICS Vevo 2100 (Visual Sonics Inc. Toronto, Ontario, Canada) imaging system with a 250 scan head was used in anesthetized animals (2% isoflurane, v/v). The internal diameter of the LV was measured in the short-axis view from M-mode recordings in end diastole and end systole. Vevo 2100 Imaging System analysis software was used to calculate ejection fraction (EF) and fractional shortening (FS).

Histology

The LV of control and post-MI rats were fixed in 4% paraformaldehyde, embedded with paraffin, and cut cross-sectionally into 5-μm thick sections along the center of the fibrotic scar. Masson trichrome staining was used to evaluate cardiac fibrosis and collagen deposition. Sections were imaged at 20× magnification by bright-field microscopy. The quantity of cardiac fibrosis was assessed by calculating the percent fibrotic area by modification of the previously described method of Pan *et al.*[12] Percent fibrotic area was determined as the ratio of collagen surface area stained blue by trichrome staining to the myocardial surface area. Five areas were selected at random from the remote (noninfarct) territories of each LV and values were averaged for each rat heart over all five sections. Infarct territory fibrosis was assessed by examining images obtained at low magnification and calculated as the ratio of scar area to total LV cross sectional area. All quantitative evaluations were carried out by NIH ImageJ software.

TUNEL staining

Using the terminal deoxynucleotidyl transferase [TdT]-mediated dUTP nick-end labeling (TUNEL) method, cardiac sections were stained for the detection of fragmented DNA, which is indicative of apoptotic cells, according to the manufacturer's protocol (Roche). The number of TUNEL positive cells was counted in five different fields.