Cardioprotective Effects of Cell Permeable NADPH oxidase inhibitors in Myocardial Ischemia/Reperfusion (I/R) Injury

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Introduction

In myocardial infarction, heart tissue becomes ischemic and completely deprived of oxygenated blood and its essential nutrients. Blood must quickly be restored to ischemic heart tissue to avoid irreversible cell death. The reperfusion of blood to previously ischemic areas can cause additional heart damage, which is referred to as myocardial I/R injury. Reperfusion injury is closely related to the accumulation of reactive oxygen species (ROS) once oxygen is available during reperfusion. ROS can reduce vascular endothelial-derived nitric oxide (NO) bioavailability resulting in reduced flow to ischemic area because NO is a potent vasodilator. In myocardial infarction, heart tissue becomes ischemic and completely deprived of oxygenated blood. Reduced flow to ischemic area because NO is a potent vasodilator. Reduced flow to ischemic area because NO is a potent vasodilator. In myocardial infarction, heart tissue becomes ischemic and completely deprived of oxygenated blood. Reduced flow to ischemic area because NO is a potent vasodilator.

Results

We hypothesize that reducing ROS formation through inhibition of NADPH oxidase will attenuate myocardial I/R injury by limiting cardiac contractile and diastolic dysfunction associated with reduced infarct size and attenuated SO production in myocytes.

Methods

Isolated Rat Heart Preparation

Male Sprague-Dawley (SD) rats (275-325 g) were anesthetized intraperitoneally (i.p.) (pentobarbital sodium 60 mg/kg and 1.08% of sodium lauryl sulfate). Hearts were rapidly excised and perfused with modified Krebs' buffer (in mmol): 117.0 NaCl, 25.0 NaHCO3, 2.5 CaCl2, 5.0 EDTA, 5.9 KC1, and 1.2 MgCl2 maintained at 37°C, 80 mm Hg constant pressure, aerated with 95% O2-5% CO2, pH kept at 7.3-7.4 by langendorff preparation. Hearts were subjected to 15 minutes of baseline perfusion, 30 minutes of ischemia, and a 45 minute reperfusion period. Shelf of plasma (control), or plasma containing apocynin (400 µM) were injected during the first five minutes of reperfusion by a side arm line proximal to the heart inflow at a rate of 1 ml/min. Coronary blood flow and left ventricular developed pressure (LVPD), which is the left ventricular end-systolic pressure (LVESP) minus left ventricular end-diastolic pressure (LVEDP), the maximal and minimal rate of LVDP (+dP/dt max and −dP/dt min), and heart rate were taken every 5 minutes during baseline and reperfusion using a flow meter (T106, Transonic Systems, Inc., Ithaca, NY) and pressure transducer (SPR-524, Millar Instruments, Inc., Houston, TX) respectively. Data was recording using a PowerLab acquisition system (AD Instruments, Grand Junction, CO). Sham hearts received no drug and experienced no ischemia. After 45 minutes of reperfusion, the left ventricle was isolated and cross-sectioned into three pieces from apex to base. Two pieces were subjected to 1% triphenyltetrazolium chloride (TTC) staining for 15 min at 37°C to detect infarct size (visible stained red, intact left unstained (white)). The third piece was frozen sectioned (5 µm) and subjected to dihydroethidium (DHE) staining for 2.5 min at room temp to fluoroscopically detect SO release for control and apocynin treated groups. Fluorescence intensity was expressed as arbitrary units and was quantitated by ImageJ. Statistical Analysis

All data in the text and figures are presented as means ± S.E.M, and analyzed by analysis of variance (ANOVA) followed by Tukey’s least significant difference (LSD) test for the heart function, infarct size and SO data. Probability values of <0.05 are statistically significant.

Conclusions

This study showed that both NADPH oxidase inhibitors, gp91 ds-tat and apocynin significantly improved post-reperfusion cardiac function associated with reduction of infarct size. When given at reperfusion, apocynin exerted the cardioprotective effects dose-dependently associated with decreased myocyte SO release. The lag time during reperfusion may be due to apocynin corrosion to deacetylation in tissue where vascular NADPH oxidase assembly. By contrast, all 100 µM gp91 ds-tat concentrations significantly restored post-reperfusion cardiac function and reduced infarct size, suggesting that these effects are independent of the concentration range (i.e. 1000 µM). This study indicates that NADPH oxidase, especially in vascular endothelium and myocytes, is a significant source of ROS in myocardial I/R. Therefore, both NADPH oxidase inhibitors may be potential agents to reduce SO production and mitigate reperfusion induced heart damage.

References