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 myocardial I/R injury. Reperfusion injury is closely related to an overproduction of reactive oxygen species (ROS) since oxygen is available during reperfusion. ROS can reduce vascular endothelial-derived nitric oxide (NO) bioavailability resulting in a reduced flow to ischemic area because NO is a potent vasodilator. ROS can also damage the mitochondria by opening mitochondrial permeability transition pore, leading to a reduction in ATP production and myocyte necrosis/apoptosis. ROS can also damage proteins, lipids, and DNA and disrupt cell integrity. Furthermore, clinical trials suggest that nontoxic antioxidants are not effective at attenuating reperfusion injury possibly because they do not specifically target the source of ROS. It has been proposed that NADPH oxidase, xanthine oxidase, uncoupled endothelial NO synthase (eNOS), and mitochondrial dysfunction can serve as ROS sources under I/R conditions. Moreover, it has been shown that an overproduction of superoxide (SO) by NADPH oxidase can cause dysfunctional modifications that can add other ROS sources, such as mitochondrial dysfunction and eNOS uncoupling. So far, there are limited effective treatment strategies targeted at limiting reperfusion injury through inhibition of NADPH oxidase. In this study, selective peptide NADPH oxidase inhibitors, gp91 ds-tat, and a well-known NADPH oxidase inhibitor, apocynin, will be used to determine how such inhibition will effect myocardial I/R injury (see figure 1).

Methods

Isolated Rat Heart Preparation

Male Sprague-Dawley (SD) rats (275-325g) were anesthetized intraperitoneally (i.p.) (pentobarbital sodium 60 mg/kg and 1.080C of sodium lepromin). Hearts were rapidly excised and perfused with modified Krebs’ buffer (in mmol): 117.6 NaCl, 120.0 NaHCO3, 2.5 KCl, 5.0 EDTA, 5.9 KHC, and 1.2 MgCl2 maintained at 37°C, 80 mm Hg constant pressure, aerated with 95% O2-5% CO2, pH kept at 3.7-3.7 by langendorff preparation. Hearts were subjected to 15 minutes of baseline perfusion, 30 minutes of ischemia, and a 45 minute reperfusion period. 5ml of plasma (control), or plasma containing apocynin (40 µM) was 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 flow and left ventricular developed pressure (LVDP), 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 System acquisition system (ADInstruments, 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, infarct 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 fluoscopically detect SO release for control and apocynin treated groups. Fluorescence intensity is expressed in arbitrary units and was quantitated by Image J.

Hypothesis

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 myocardium.

Results

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 myocardial I/R injury. Reperfusion injury is closely related to an overproduction of reactive oxygen species (ROS) since oxygen is available during reperfusion. ROS can reduce vascular endothelial-derived nitric oxide (NO) bioavailability resulting in a reduced flow to ischemic area because NO is a potent vasodilator. ROS can also damage the mitochondria by opening mitochondrial permeability transition pore, leading to a reduction in ATP production and myocyte necrosis/apoptosis. ROS can also damage proteins, lipids, and DNA and disrupt cell integrity. Furthermore, clinical trials suggest that nontoxic antioxidants are not effective at attenuating reperfusion injury possibly because they do not specifically target the source of ROS. It has been proposed that NADPH oxidase, xanthine oxidase, uncoupled endothelial NO synthase (eNOS), and mitochondrial dysfunction can serve as ROS sources under I/R conditions. Moreover, it has been shown that an overproduction of superoxide (SO) by NADPH oxidase can cause dysfunctional modifications that can add other ROS sources, such as mitochondrial dysfunction and eNOS uncoupling. So far, there are limited effective treatment strategies targeted at limiting reperfusion injury through inhibition of NADPH oxidase. In this study, selective peptide NADPH oxidase inhibitors, gp91 ds-tat, and a well-known NADPH oxidase inhibitor, apocynin, will be used to determine how such inhibition will effect myocardial I/R injury (see figure 1).

Figure 1. Apocynin and gp91 ds-tat inhibit NADPH oxidase subunit assembly and attenuate SO release under I/R conditions (upper panel). Structures of apocynin and gp91 ds-tat are shown in the bottom. Apocynin inhibits NADPH oxidase by binding p47(phox) and p22(phox) assembly after forming complexes by peptide-phox docking. In contrast, gp91 ds-tat contains a docking sequence (ds) which prevents NADPH oxidase p47(phox) and gp91 assembly. The red portons indicate the peptide diffusion into the cell.

Figure 2. gp91 ds-tat and apocynin significantly reduced infarct size, reduced SO release and increased LVDP, 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 (HR) 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 System acquisition system (ADInstruments, 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, infarct 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 fluoscopically detect SO release for control and apocynin treated groups. Fluorescence intensity is expressed in arbitrary units and was quantitated by Image J.

Statistical Analysis

All data in the text and figures are presented as means ± S.E.M. and analyzed by analysis of variance and post hoc analysis with the Student-Newman-Keuls 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 disaccharin by tissue peroxidase in order to inhibit NADPH oxidase assembly. By contrast, all 10 g/ml ds-tat concentrations significantly reduced post-reperfusion cardiac function and reduced infarct size, suggesting that these effects are dose-dependent in this concentration range (i.e. all 10 g/ml ds-tat concentrations significantly reduced post-reperfusion cardiac function). 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