Cardioprotective Effects of Selective Mitochondrial-Targeted Antioxidants in Myocardial Ischemia/Reperfusion (I/R) Injury

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Introduction

During myocardial ischemia, coronary blood flow interruption deprives cardiomyocytes of oxygen, glucose and fatty acids. Ischemic damage is exacerbated by a burst of reactive oxygen species (ROS) generated at reperfusion when oxygen interacts with damaged mitochondrial electron transport chains (ETC), especially uncoupled complexes I and III (Fig. 1,2). Nicotinamide adenine dinucleotide phosphate oxidase (Nox) activity can also release ROS, inducing additional tissue/organ damage[1,2,3].

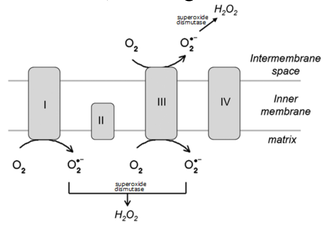

Figure 1. Electrons leaking from complexes I and III are accepted by oxygen (O_2) to generate superoxide (O_2^-). Adapted from Szeto 2006.

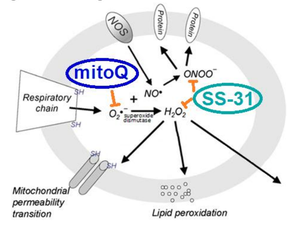

Figure 2. Decreasing mitochondrial ROS production prevents destructive effects, including loss of membrane integrity. Modified from Szeto 2006.

Surgical intervention or thrombolytic treatments can restore coronary blood flow. However, as blood flow reestablishes, oxidative stress leads to I/R injury. Clinical treatment remains a challenge as no pharmaceutical agents effectively limit I/R-induced damage. Mitochondria are implicated in I/R as a major source of ROS[3,4,5]. Excess ROS leads to mitochondrial and cardiac contractile dysfunction[6]. Conventional antioxidants have limited efficacy in myocardial I/R because they are not targeted selectively to where most I/R damage occurs, in mitochondria (Fig. 3)[3,4,5]. Mitoquinone (mitoQ, MW=600 g/mol), a coenzyme Q analog, easily crosses phospholipid bilayers and is driven by the large electrochemical membrane potential to concentrate mitoQ several hundred-fold within mitochondria. The respiratory chain reduces mitoQ to its active ubiquinol antioxidant form to limit myocardial I/R injury[5]. The SS-31 (Szeto-Schiller) peptide ((D-Arg)-Dmt-Lys-Phe-Amide, MW=640 g/mol, Genemed Synthesis, Inc., San Antonio, TX) is also of interest since it is cell-permeable, specifically targeted to inner mitochondrial membranes based on its alternating cationic aromatic residue sequence, with an antioxidant dimethyltyrosine moeity. SS peptides scavenge ROS in I/R models[4].

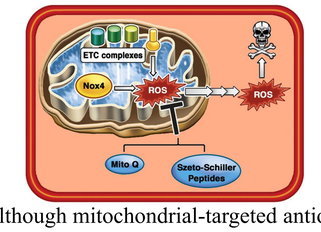

Figure 3. Mitochondrial ROS stimulate ROS production outside mitochondria, increasing oxidative stress and apoptosis signaling cascade activation. ETC complexes and Nox4 are major ROS sources in dysfunctional mitochondria. Specific mitochondrial-targeted antioxidants exert cardioprotective effects. Adapted from Bayeva *et al*. 2013 and modified.

Although mitochondrial-targeted antioxidant pretreatment can effectively limit I/R injury, pretreatment is not always possible in cases of myocardial infarction. Therefore, evaluating cardioprotective efficacy of mitochondrial-targeted antioxidants when given at reperfusion is of high significance.

Hypothesis

We hypothesized that antioxidants specifically targeted to the mitochondria will attenuate myocardial I/R injury by limiting cardiac contractile dysfunction, cardiac tissue damage and SO release in isolated perfused rat hearts subjected to I/R compared to untreated I/R hearts.

Methods

Isolated Rat Heart Preparation

Male Sprague Dawley rats (275-325g, Charles River, Springfield, MA) were anesthetized with a pentobarbital sodium (60 mg/kg) and sodium heparin (1,000 U) injection intraperitoneally (i.p.). Each heart was rapidly excised and subjected to retrograde perfusion via the aorta, while immersed in a 160 mL water-jacketed reservoir, with a modified Krebs' buffer (in mmol/L: 17.0 dextrose, 120.0 NaCl, 25.0 $NaHCO_3$, 2.5 $CaCl_2$, 0.5 EDTA, 5.9 KCl and 1.2 $MgCl_2$). The perfusate was maintained at 37°C, kept at 80 mmHg constant pressure, aerated with 95% O_2-5% CO_2 and equilibrated at a pH of 7.35-7.45. A side arm in the perfusion line was used for the infusion of 5 mL of autologous plasma with or without mitoQ (4, 40, 80 µM) or SS-31 (25, 50, 100 µM). A flow meter (T106, Transonic Systems, Inc., Ithaca, NY) monitored coronary flow. Left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), heart rate and the peak rates of rise and fall in the first derivative of left ventricular pressure (dP/dt_{max} and dP/dt_{min}, respectively) were monitored using a pressure transducer (SPR-524, Millar Instruments, Inc., Houston, TX) positioned in the left ventricular cavity and recorded using a Powerlab Station acquisition system (AD Instruments, Grand Junction, CO). Left ventricular developed pressure (LVDP) was calculated by subtracting LVEDP from LVESP. Cardiac function parameters were measured every 5 min for 15 min to obtain stable baseline measurements. Ischemia was induced for 30 min by stopping Krebs' buffer flow. After ischemia, Krebs' buffer flow was restored while infusing 5 mL of plasma with or without mitoQ or SS-31 at a rate of 1 mL/min for 5 min. Cardiac function parameters were recorded every 5 min for 45 min. Three left ventricle sections from apex to middle were used in 1% 2,3,5- triphenyltetrazolium chloride (TTC) staining for 20 min at 37°C to detect infarct size. Frozen sections (8 µm) of the left ventricle base were subjected to dihydroethidium (DHE) staining for 2 min at 22°C to fluoroscopically detect SO release. Fluorescence intensity is expressed in arbitrary units quantified by Image J.

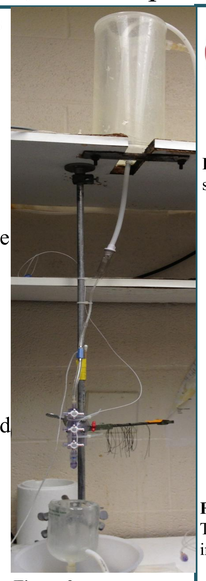

Figure 2. Myocardial I/R apparatus.

Statistical Analysis

All data in the text and figures are presented as means ± SEM. The cardiac function and TTC staining data were analyzed by ANOVA using post hoc analysis with the Student-Newman-Keuls test. Probability values of <0.05 were considered statistically significant.

Results

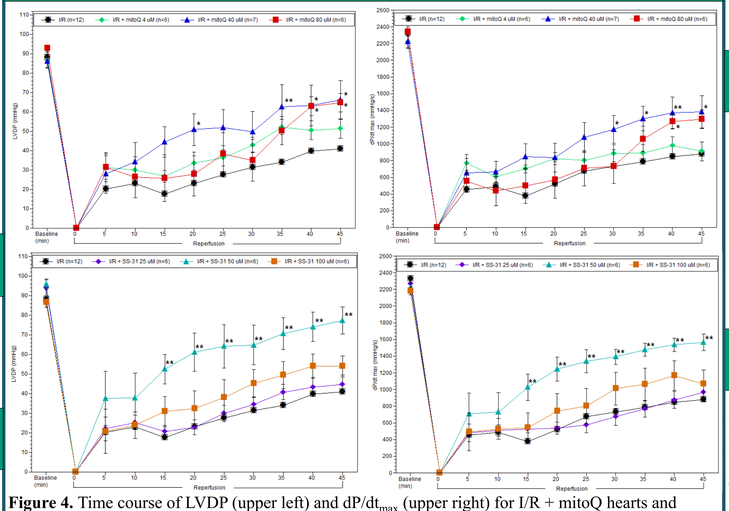

Figure 4. Time course of LVDP (upper left) and dP/dt_{max} (upper right) for I/R + mitoQ hearts and LVDP (lower left) and dP/dt_{max} (lower right) for I/R + SS-31 hearts. MitoQ and SS-31 restored post-reperfused cardiac function significantly compared to untreated I/R hearts (*p<0.05, **p<0.01).

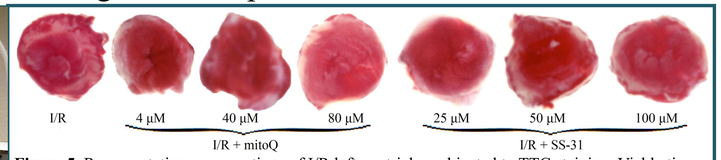

Figure 5. Representative cross sections of I/R left ventricles subjected to TTC staining. Viable tissue stained red while the infarcted tissue remained unstained (white).

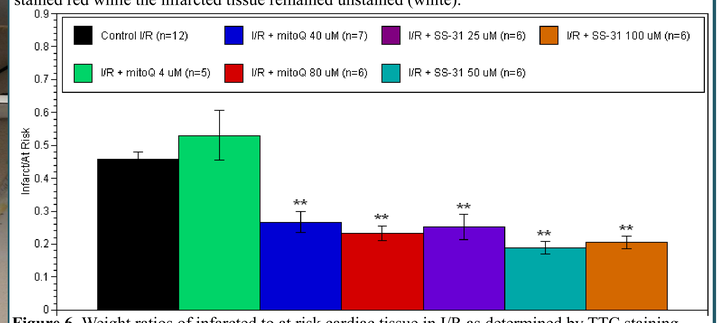

Figure 6. Weight ratios of infarcted to at risk cardiac tissue in I/R as determined by TTC staining. Treatment with mitoQ (40, 80 µM) or SS-31 (25, 50, 100 µM) at reperfusion significantly reduced infarct size compared to untreated I/R hearts (**P<0.01).

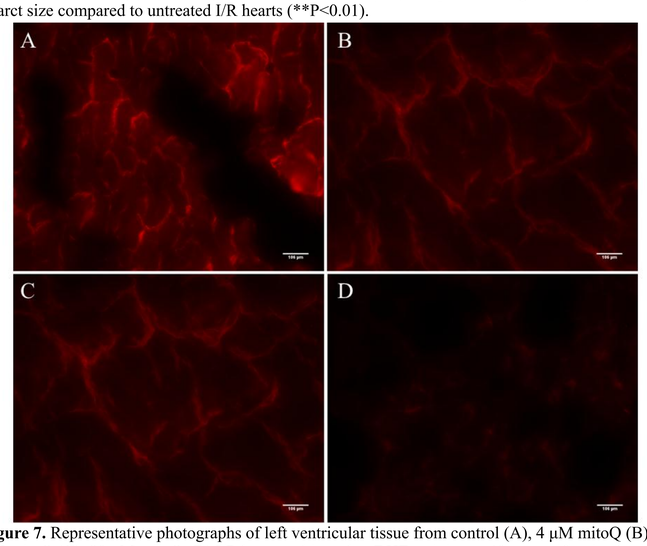

Figure 7. Representative photographs of left ventricular tissue from control (A), 4 µM mitoQ (B), 40 µM mitoQ (C), 80 µM mitoQ (D) I/R hearts subjected to DHE staining at 40x magnification by fluorescence microscopy. I/R hearts treated with mitoQ (40, 80 µM) expressed less SO release than untreated I/R hearts, suggesting mitoQ attenuates SO release in myocardial I/R.

Conclusions

The data suggest that inhibiting mitochondrial derived ROS with selective mitochondrial-targeted antioxidants, such as mitoQ and SS-31, can attenuate myocardial I/R injury by reducing ROS formation to limit cardiac contractile dysfunction and infarct size. Additionally, the data suggest that agents such as mitoQ and SS-31 can work expeditiously and effectively when administered at the time of coronary blood flow restoration. Therefore, treatment with mitoQ or SS-31 may be useful in the clinical setting in cases of myocardial infarction, where pretreatment is not always a practical option.

References

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