Comparison of the effects of Myristoylated and Transactivating peptide (TAT) conjugated Mitochondrial Fission Peptide Inhibitor (P110) in Myocardial Ischemia/Reperfusion (I/R) Injury

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Introduction

During ischemic events, coronary blood flow is restricted causing cardiomyocytes to enter a hypoxic state. This in turns leads to uncoupling of the mitochondrial electron transport chain. When bloodflow and oxygen delivery is restored during reperfusion, reactive oxygen species (ROS) are generated which leads to the loss of mitochondrial membrane potential and opening of the mitochondrial permeability transition pore (MPTP) which potentiates mitochondrial fission in MI/R. Mitochondrial fission is associated with mitochondrial fragmentation and decreased ATP production, leading to cardiac contractile dysfunction and increased infarct size in MI/R (Figure 1) (1,2,3).

Therefore, inhibiting mitochondrial fission which results from the vital act of reperfusion, may be a strategy to salvage damaged cardiomyocytes and protect them from MI/R injury. Myristoylated TAT conjugated mitochondrial fission peptide inhibitor functions by selectively inhibiting the interaction between human fission protein 1 (Fis1), which is located on the outer mitochondrial membrane and dynamin related protein 1 (Drp1), a GTPase (Figure 2).

Hypothesis

We hypothesize that Myr-P110 (1 µM) will significantly improve post-ischemic cardiac function and reduce infarct size compared to untreated control I/R hearts or native P110 (1 µM) given 10min, prior to ischemia and for 20min, at the beginning of reperfusion. We also predict that TAT-P110 (1 µM), will significantly improve post-ischemic cardiac function and reduce infarct size compared to untreated control I/R hearts or native P110 (1 µM) but to a lesser extent than Myr-P110.

Methods

Isolated Rat Heart Preparation

Male Sprague Dawley rats (275-325 g, Charles River, Springfield, MA) were anesthetized with a pentobarbital sodium (50 mg/kg) and sodium heparin (1,000 U) injection intraperitoneally. Each heart was rapidly excised, immersed in a 160 mL water-jacketed reservoir and subjected to retrograde perfusion via the aorta with a modified Krebs-Henseleit buffer. The perfuse was maintained at 37°C, kept at 80 mmHg constant pressure, aerated with 95% O2-5% CO2, and equilibrated at a pH of 7.35 ± 0.15. A flow meter (T106, Transonic Systems, Inc., Ithaca, NY) placed in the inflow line monitored coronary flow. Left ventricular end-systolic pressure (LVESP), left ventricular end-diastolic pressure (LVEDP), heart rate and peak rates of rise and fall in the first derivative (dP/dtmax and dP/dtmin, respectively) of left ventricular developed pressure (LVDP) 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 (ADInstruments, Grand Junction, CO). LVDPs were calculated by subtracting LVEDP from LVESP. At the end of reperfusion, five heart slices (2 mm/slice) were subjected to 1% triphenyltetrazolium chloride (TTC) staining to detect dead (unstained) and viable (red stained) area. Infarct size was expressed as the percentage of dead tissue to the total tissue weight. Experimental protocol is shown in Figure 4 (6).

Conclusions

These data suggest excessive mitochondrial fission contributes to MI/R injury. Myr-P110 (1µM) significantly improved post-reperfusion dP/dtmin and reduced infarct size as compared to untreated, control, Tat, and Native P110 hearts. Tat-P110 transiently improved postreperfusion cardiac function but did not reduce infarct size compared to Native P110. The data suggest that Myr-P110 is the more effective formulation and may be an attractive strategy to attenuate MI/R injury and salvage heart tissue in MI patients.

References