Investigating the Role of Mitochondrial Fission in Cardiac Myocyte Hypoxia/Reoxygenation

Amina Pratt, Erin Heine, Qian Chen, Robert Barsotti and Lindon Young

Department of Bio-Medical Sciences, Philadelphia College of Osteopathic Medicine

4170 City Avenue, Philadelphia, PA 19131

Introduction
Myocardial ischemia reperfusion (MiR) injury results in increased cell death which is initiated in part by uncoupling of the electron transport chain in mitochondria leading to the reduction of reactive oxygen species (ROS) and opening of the mitochondrial permeability transition pore (MPTP) (Fig. 1)\(^2\). These events lead to loss of the mitochondrial membrane potential and augments mitochondrial fission in MiR. Mitochondrial fission is associated with shortening of mitochondria, decreased ATP production, and is thought to promote post-reperfused cardiomyocyte cell death (Fig. 2)\(^3\,4\).

Methods

**HL-1 Cell Preparation**

An immortalized line of cardiac murine myocytes derived from the atria (HL-1 cells) were grown in 100 mm plates using supplemented Claycomb media (Sigma) (5). Plates were incubated at 37°C, 5% CO\(_2\). Once the plates reached 90-100% confluence, they were split into 12 fibronectin-coated 12 well plates (9 wells used/ plate, 1 ml re-suspended cells/well) at a concentration of 2.7 x10\(^5\) cells/ml and incubated overnight at 37°C, 5% CO\(_2\).

**Hypoxic and Normoxic Buffers**

The next day the media from each plate was replaced with 1 ml per well of hypoxic buffer (1.0 mM KH\(_2\)PO\(_4\), 10 mM NaHCO\(_3\), 1.2 mM MgCl\(_2\) 6 H\(_2\)O, 25 mM Na HEPES, 74 mM NaCl, 16 mM KCl, 1.2 mM CaCl\(_2\), and 20 mM Na Lactate, at pH 6.2) or normoxic buffer (1.0 mM KH\(_2\)PO\(_4\), 10 mM NaHCO\(_3\), 1.2 mM MgCl\(_2\), 6 H\(_2\)O, 25 Na HEPES, 98 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl\(_2\), 10 mM d-glucose, and 2.0 mM Na Pyruvate at pH 7.4). Both buffers were prepared with or without Mdivi-1 (MW = 353 g/mol, S#M or 25 µM, Sigma).

**Normoxic and SIR procedures**

Figure 1. Acute myocardial ischemia results in a decrease in pH due to the build up of lactic acid from anaerobic conditions. The acidic conditions during ischemia prevent the opening of the mitochondrial permeability transition pore (MPTP) and cardiomyocyte hypoxia-induced apoptosis at this time. Reperfusion results in restoration of physiological pH, opening of the MPTP, Ca\(_2+\) overload, and cardiomyocyte hypertrophy and cell death. MPTP opening induces uncoupling of oxidative phosphorylation which generates mitochondrial ROS and ATP depletion. Neutrophils accumulate in the infarcted myocardial tissue, generate ROS and accelerate reperfusion injury. Adapted from Hausenloy & Yellon 2013.

Therefore, inhibiting mitochondrial fission may be an effective strategy to attenuate cell death in MiR. Mdivi-1, a mitochondrial fission inhibitor, which acts by selectively inhibiting dynamin related protein 1 (Drp1), a GTPase that causes mitochondrial fragmentation. Drp1 translocates to the outer mitochondrial membrane, where it interacts with Fis-1 to promote mitochondrial fragmentation. Mdivi-1 inhibits Drp-1 GTPase activity and would attenuate mitochondrial fission (steps c thru e) in SIR. Adapted from Chan 2006.

**Statistical Analysis**

All data in the text and figures are presented as means ± SEM. A student’s-t test was used to assess statistical difference between Mdivi-1 treated and non-treated HL-1 cells. Probability values of <0.05 were considered statistically significant.

Results

**Cell Viability After 19 Hrs**

Figure 5. Cell viability of HL-1 cells subjected to 18 hours of hypoxic/ 1 hour reoxygenation or time-matched normoxic conditions. Mdivi-1 (5 and 25 µM) given at the beginning of experiment significantly attenuated cell death in cells subjected to SIR compared to untreated SIR cells (*p<0.05). Untreated normoxic HL-1 cells exhibited significantly enhanced cell viability compared to untreated SIR cells (δp<0.05). No differences were observed between untreated and Mdivi-1 groups under normoxic conditions (n=3 for all groups).

**Conclusions**

These data suggest mitochondrial fission contributes to MiR injury. Mdivi-1 treatment (5 and 25µM) significantly improved cell viability during 12 and 18 hours or hypoxia / 1hr reoxygenation. These preliminary results suggest that inhibition of mitochondrial fission may be an effective strategy to mitigate heart injury in MI patients. Future studies will determine levels of Drp-1 translocation to the outer mitochondrial membrane in the presence/absence of additional Mdivi-1 concentrations under extended SIR and normoxic conditions.

References