

Introduction

Myocardial ischemia reperfusion (MI/R) injury results in increased cell death which is initiated in part by uncoupling of the electron transport chain in mitochondria leading to the generation of reactive oxygen species (ROS) and opening of the mitochondrial permeability transition pore (MPTP) (Fig. 1)^{1,2}. These events lead to collapse of the mitochondrial membrane potential and augment mitochondrial fission in MI/R. 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}.

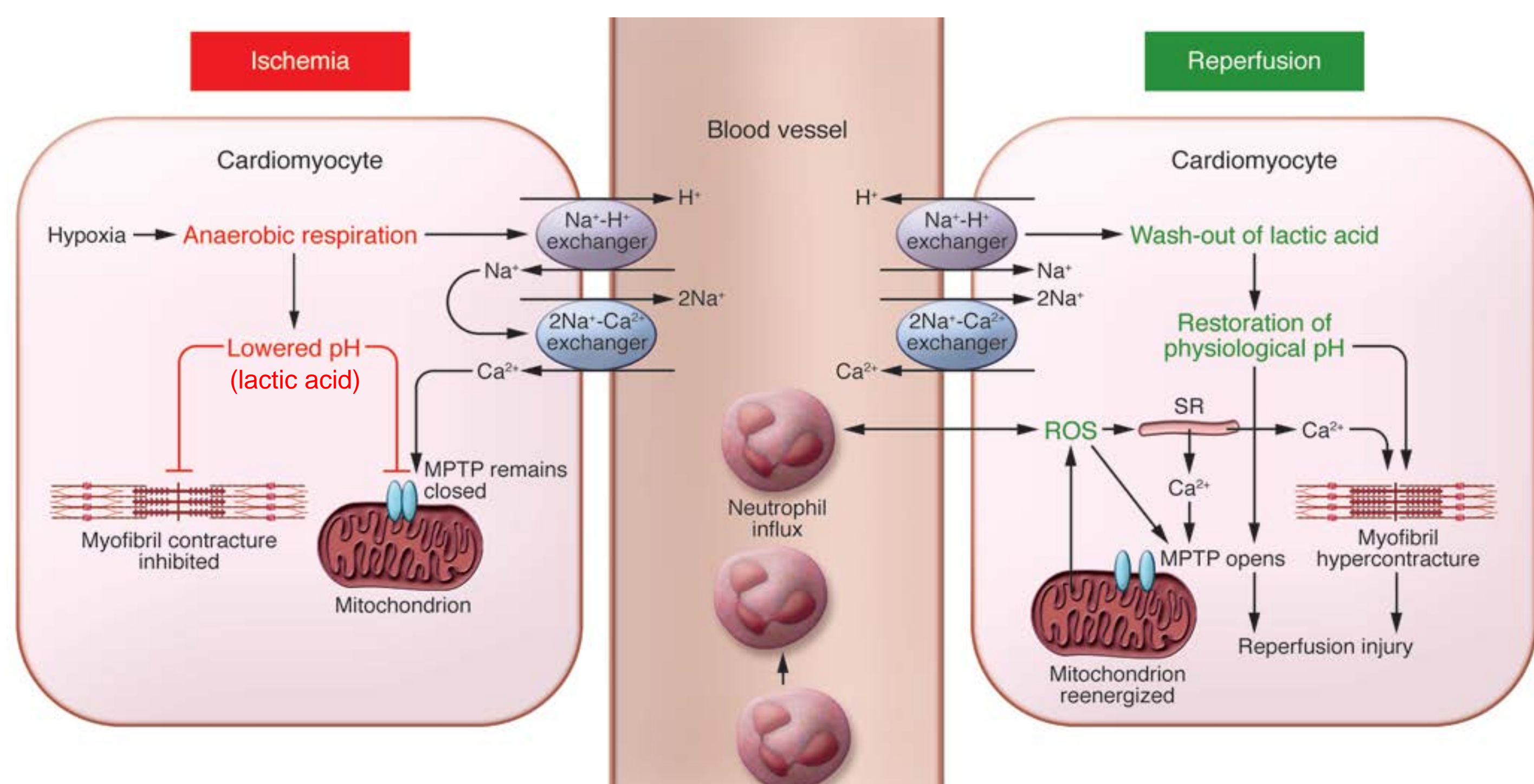


Figure 1. Acute myocardial ischemia results in a decrease in pH due to the build up of lactic acid from anaerobic conditions. The acidic conditions during ischemia prevent the opening of the mitochondrial permeability transition pore (MPTP) and cardiomyocyte hypercontracture at this time. Reperfusion results in restoration of physiological pH, opening of the MPTP, Ca²⁺ overload, and cardiomyocyte hypercontracture 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 exacerbate reperfusion injury. Adapted from Hausenloy & Yellon 2013.

Therefore, inhibiting mitochondrial fission may be an effective strategy to attenuate cell death in MI/R. Mdivi-1, a mitochondrial fission inhibitor, which acts by selectively inhibiting dynamin related protein 1 (Drp1), a GTPase that promotes mitochondrial fission via interaction with outer mitochondrial membrane proteins (e.g. human fission protein 1 (Fis1) was used to attenuate fission in cardiac myocytes subjected to simulated IR (SIR) (Figs. 2 & 3)^{3,4,5}.

Mitochondria Fission Schematic

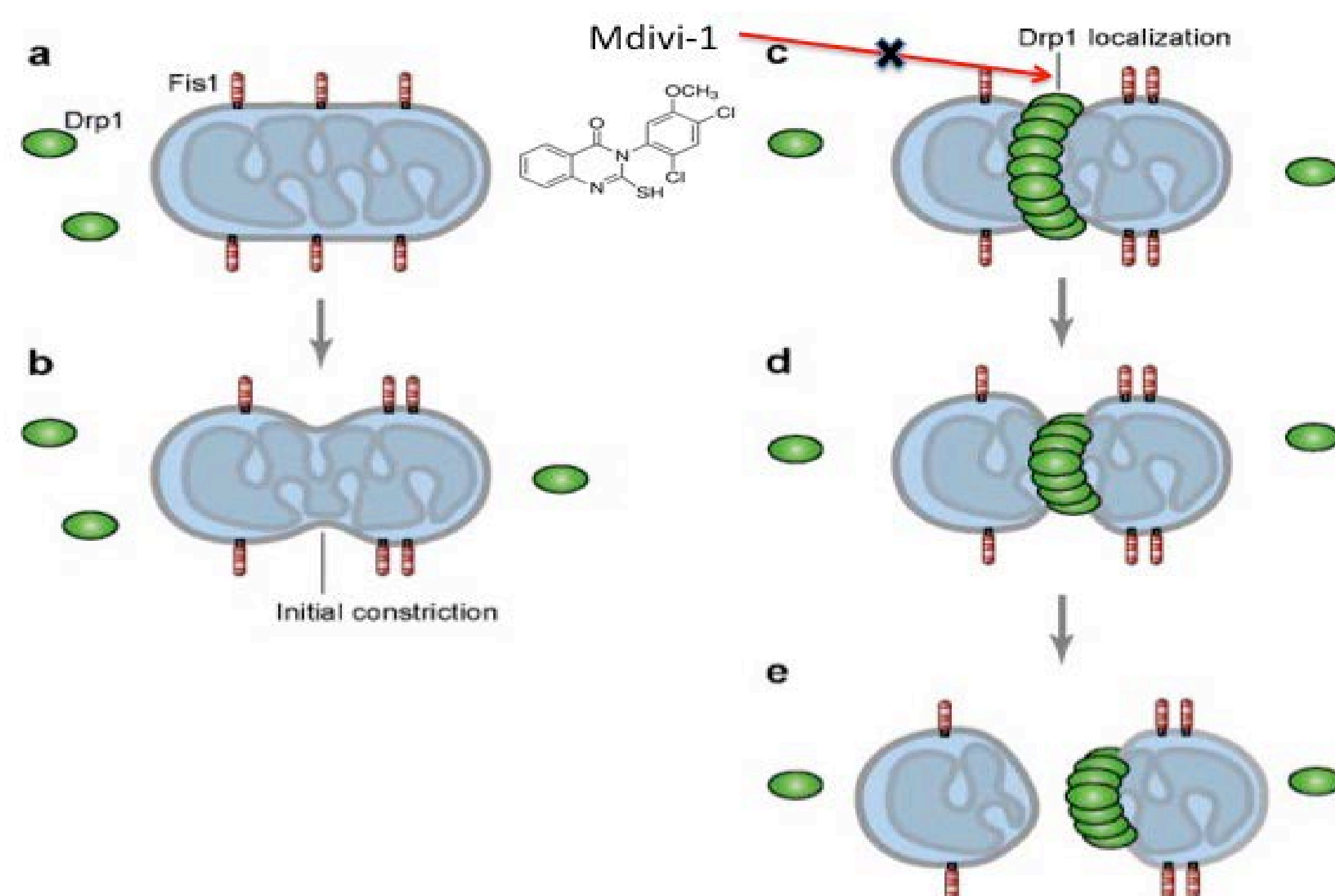


Figure 2. Drp-1, a GTPase that causes mitochondrial fragmentation. Drp1 translocates to the outer mitochondrial membrane, where it interacts with Fis-1 to promote mitochondrial fragmentation. Mdivi-1 inhibits Drp-1 GTPase activity and would attenuate mitochondrial fission (steps c thru e) in SIR. Adapted from Chan 2006.

Hypothesis

We hypothesize that HL-1 cells subjected to SIR would exhibit increased cell death compared to their normoxic control counterparts, and that Mdivi-1 treated cells would have a smaller percentage of SIR-induced cell death.

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% CO₂. Once the plates reached 90-100% confluence, they were split into 2 fibronectin-coated 12 well plates (9 wells used/ plate, 1 ml re-suspended cells/well) at a concentration of 2.7 x10⁵ cells/ml and incubated overnight at 37° C, 5% CO₂.

Normoxic and Hypoxic Buffers

The next day the media from each plate was replaced with 1 ml per well of hypoxic buffer (1.0 mM KH₂PO₄, 10 mM NaHCO₃, 1.2 mM MgCl₂ 6 H₂O, 25 mM Na HEPES, 74 mM NaCl, 16 mM KCl, 1.2 mM CaCl₂, and 20 mM Na Lactate, at pH 6.2 bubbled with 100% nitrogen for 30 min) or normoxic buffer (1.0 mM KH₂PO₄, 10 mM NaHCO₃, 1.2 mM MgCl₂ 6 H₂O, 25 mM Na HEPES, 98 mM NaCl, 3.0 mM KCl, 1.2 mM CaCl₂, 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; 5µM or 25µM; Sigma).

Normoxic and SIR procedures

First, the hypoxic plate was placed in a hypoxic chamber, and then both plates (normoxic and hypoxic) were put into an incubator at 37° C, 5% CO₂ for 12 or 18 hours. After 12 or 18 hours, the hypoxic plate was removed from the chamber and the hypoxic buffer was replaced with normoxic buffer (reoxygenation) and incubated at 37° C, 5% CO₂, for 1 hour. Thereafter, cells from both plates were assessed for cell viability using trypan blue (0.4%). The protocol is shown in Fig.3.

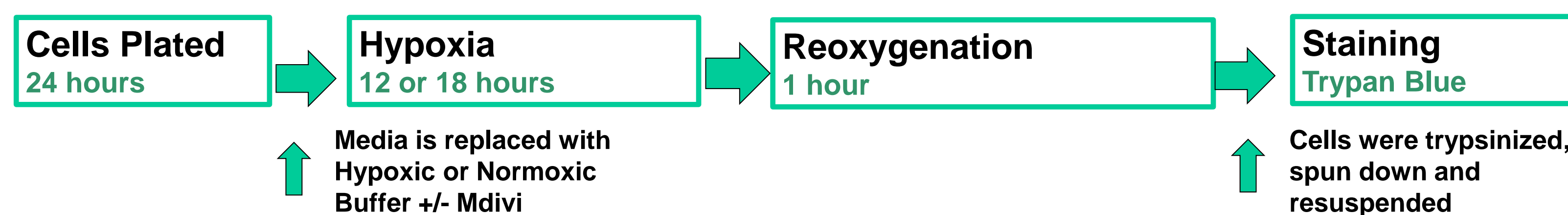


Figure 3. Cells were plated and allowed to attach overnight. Hypoxia was induced using hypoxic buffer and vacuum sealed in a chamber and then placed in the incubator.

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 13 Hrs

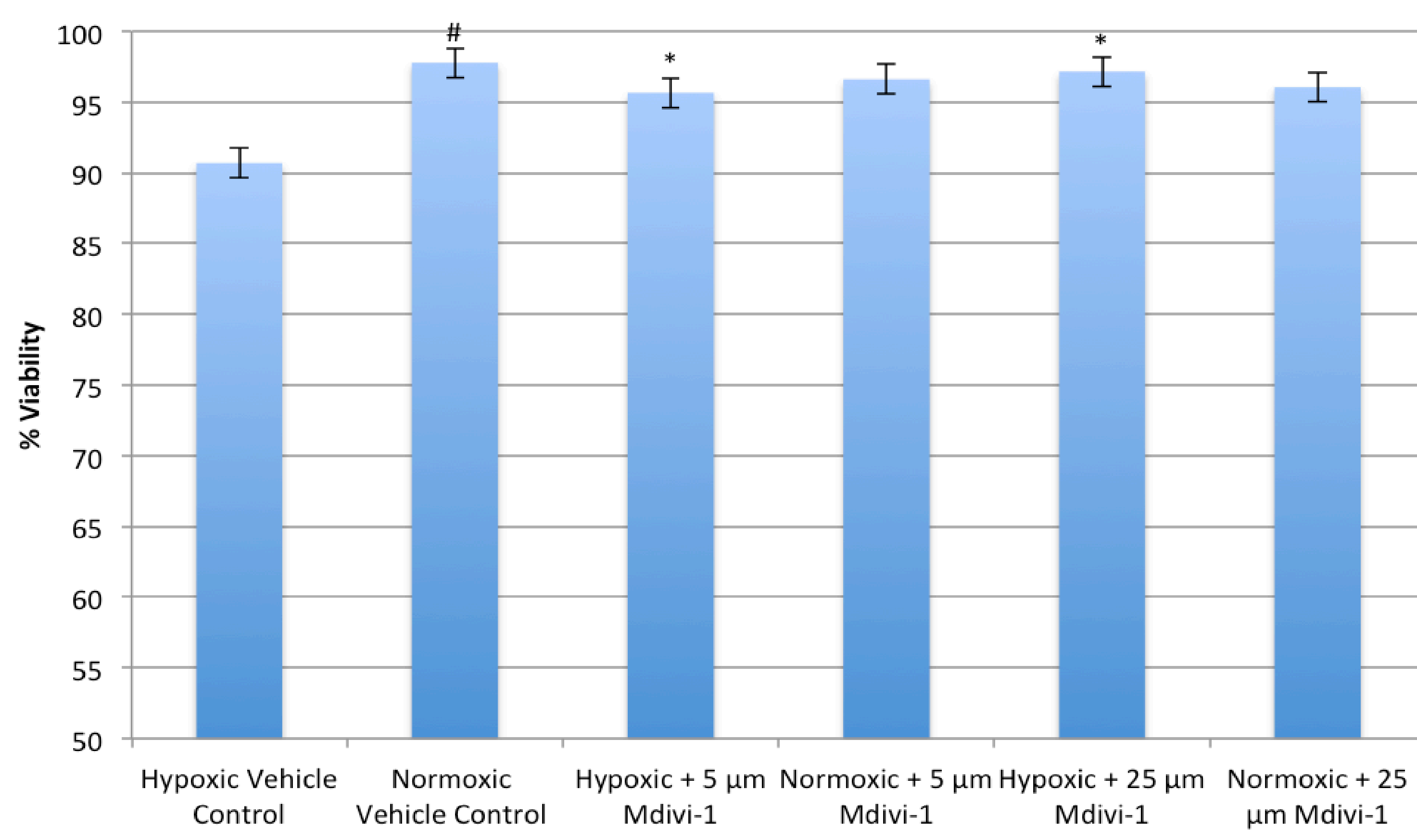


Figure 4. Cell Viability of HL-1 cells subjected to 12 hrs of hypoxia/ 1hr reoxygenation or time-matched normoxic conditions. Mdivi-1 (5 and 25µM) given at the beginning of experiment significantly attenuated cell death in cells subjected to SIR compared to untreated SIR cells (*p<0.05). Untreated normoxic HL-1 cells exhibited significantly enhanced cell viability compared to untreated SIR cells (#p<0.05). No differences were observed between untreated and Mdivi-1 groups under normoxic conditions (n=4 for all groups).

Cell Viability After 19 Hrs

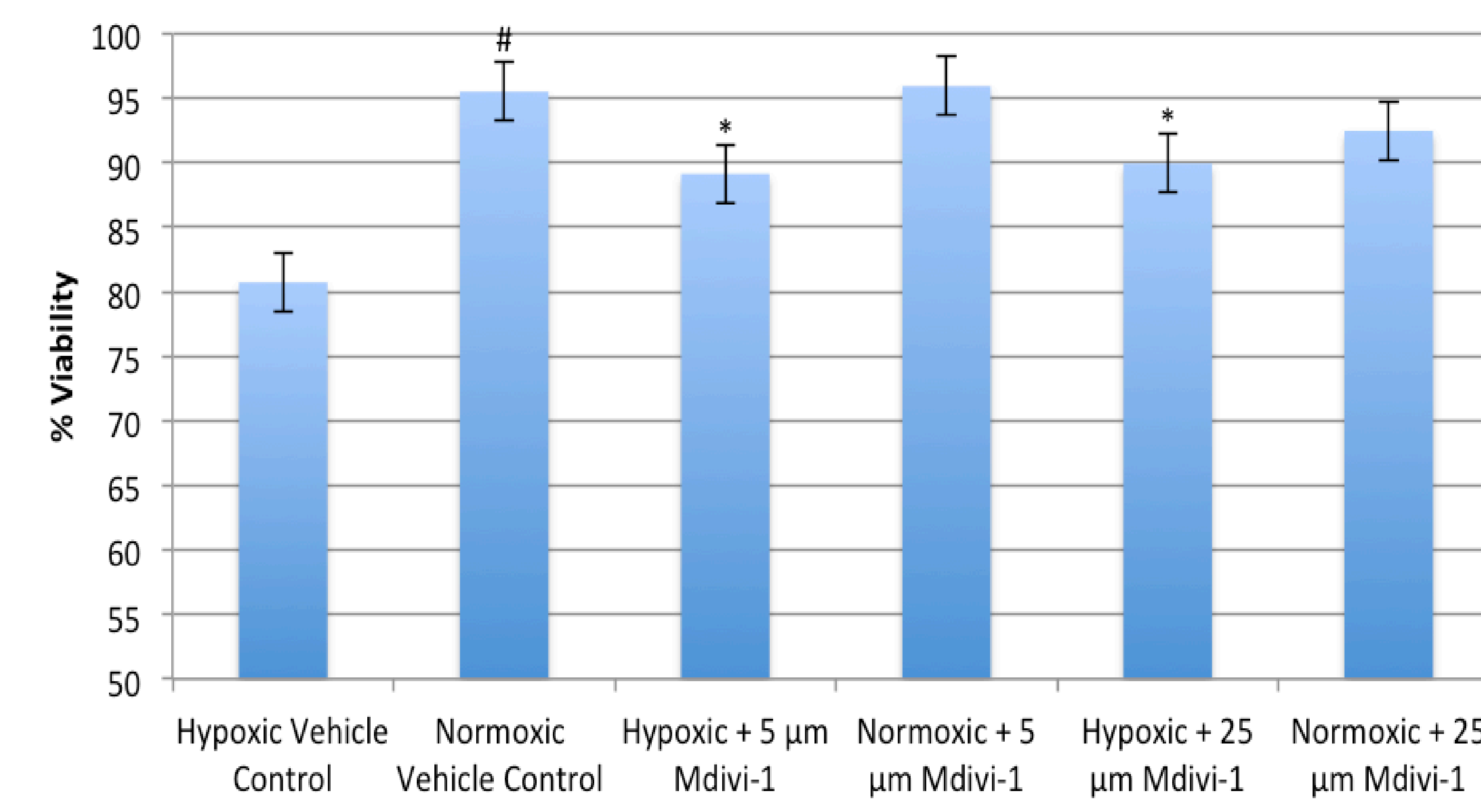


Figure 5. Cell viability of HL-1 cells subjected to 18 hours of hypoxia/ 1hour reoxygenation or time matched normoxic conditions. Mdivi-1 (5 and 25µM) given at the beginning of experiment significantly attenuated cell death in cells subjected to SIR compared to untreated SIR cells (*p<0.05). Untreated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

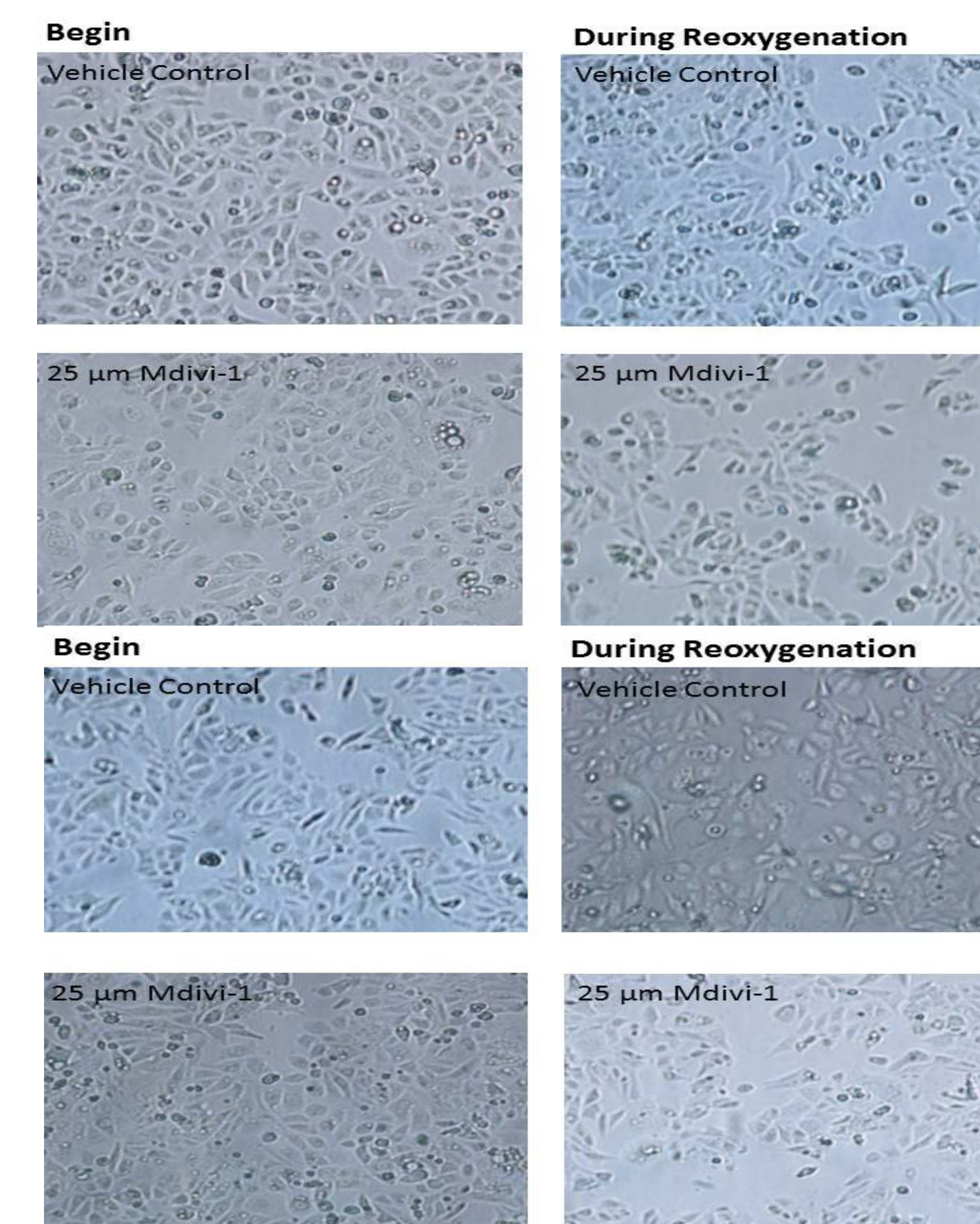


Figure 6. Representation of HL-1 cells subjected to Normoxic conditions before the experiment began and during reoxygenation (Fig. 4)

Figure 7. Representation of HL-1 cells subjected to Hypoxic conditions before the experiment began and during reoxygenation (Fig. 4).

Conclusions

These data suggest mitochondrial fission contributes to MI/R 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 mitochondria membrane in the presence/absence of additional Mdivi-1 concentrations under extended SIR and normoxic conditions.

References

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