Lauren Wendt, Jelena Vider, Louise E. See Hoe, Eugene Du Toit, Jason N. Peart, John P. Headrick

Complex Effects of Putative DRP-1 Inhibitors on Stress Responses in Mouse Heart and Rat Cardiomyoblasts

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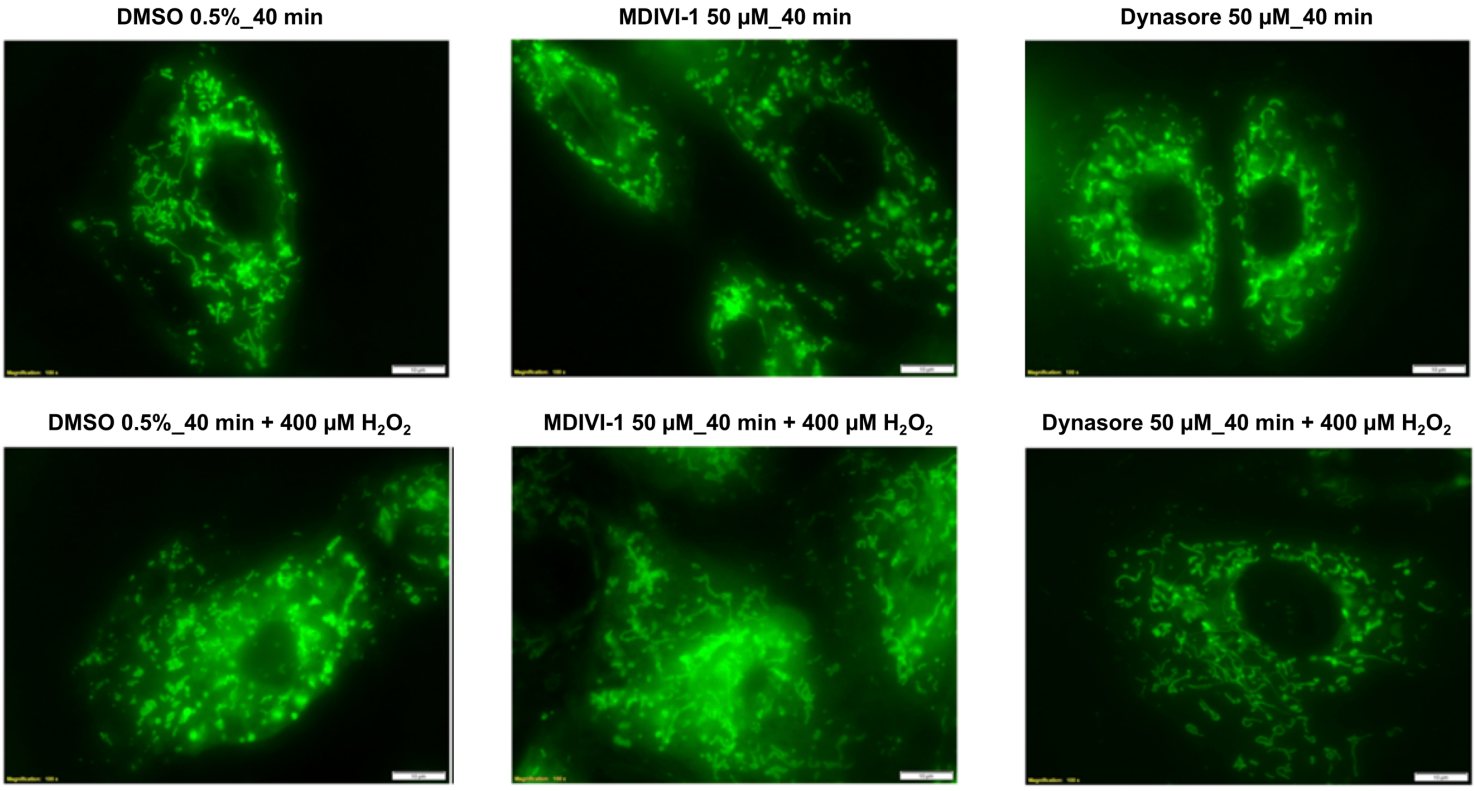
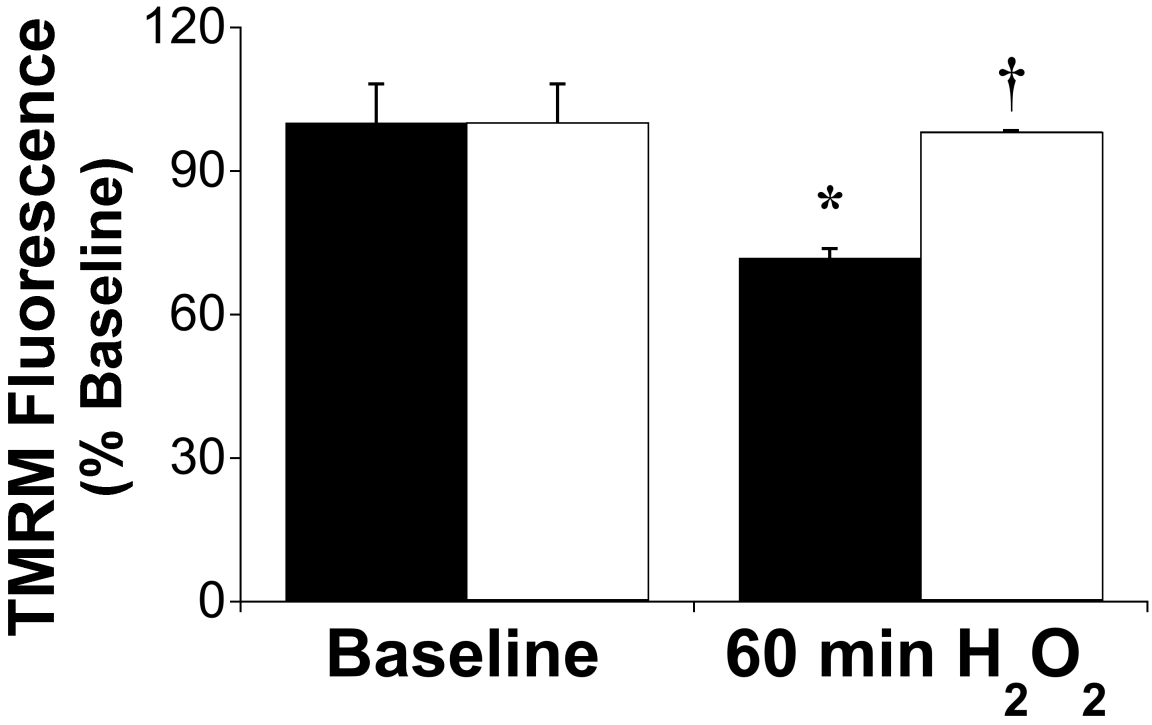
Supplemental Figures

Fig. 1



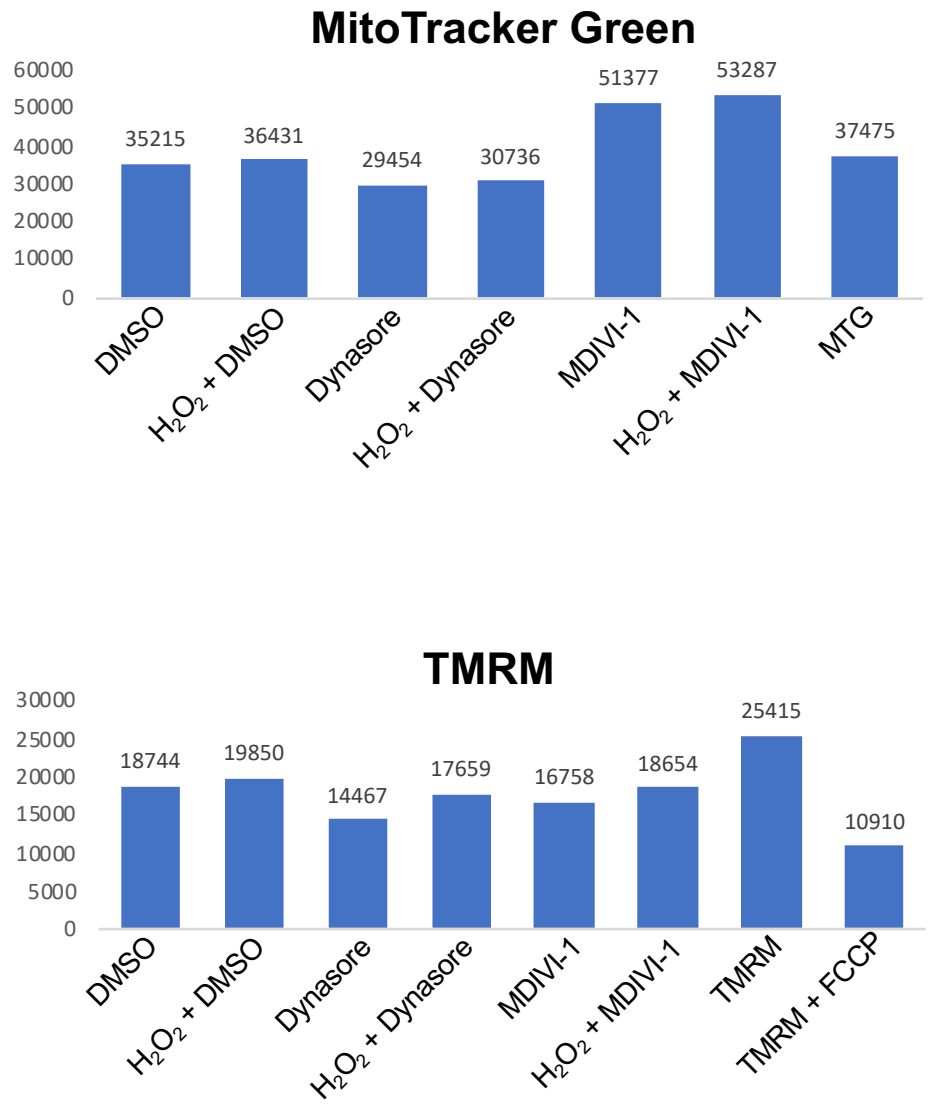
Mitochondrial morphology in H9c2 cardiomyoblasts exposed to H_2O_2 ± DRP-1 inhibitors. H9c2 cells were stained with 100 nM MitoTracker green (Thermo Fisher Scientific, Waltham, Massachusetts) in HBSS for 20 min at 37°C. After staining, HBSS was replaced for full media with 50 µM MDIVI/Dynasore or DMSO (0.5% final concentration) and incubated for 40 min. H_2O_2 was added to corresponding wells at 400 µM final concentration prior to imaging using fluorescence microscopy (CellM; Olympus, Japan). Note mitochondrial fragmentation (punctate staining) in H_2O_2 exposed cells, an effect limited by DRP-1 inhibitors.

Fig. 2



Preliminary analysis of mitochondrial membrane potential in H9c2 cardiomyoblasts exposed to H_2O_2 ± MDIVI-1. For assessment of mitochondrial membrane potential (Δψm) the fluorescent probe tetramethylrhodamine methyl ester perchlorate (TMRM) was employed, accumulating within mitochondria in proportion to Δψm. After experimentation cells were incubated with 100 μM TMRM for 20 min at 37°C (protected from light), harvested and re-suspended in PBS for analysis in a FACS-Calibur flow cytometer ((BD Bioscience, San Jose, CA, USA), with excitation at 549 nm and emission at 575 nm. Data were analyzed using FlowJo software, with median fluorescent intensity values calculated. Results suggest a ~20% fall in membrane potential with H_2O_2, an effect reversed by MDIVI-1, however it must be noted data were acquired with a potentially high quenching concentration (100 μM) of TMRM.

Fig. 3.



Supplementary flow cytometry data for MitoTracker green (MTG) and TMRM in H9c2 cardiomyoblasts exposed to H_2O_2 ± DRP-1 inhibitors. H9c2 cells were stained with 100 nM MitoTracker green (Thermo Fisher Scientific, Waltham, Massachusetts) in HBSS for 20 min at 37°C. After staining HBSS was replaced for full media with 50 µM MDIVI/Dynasore or DMSO (0.5% final concentration) and incubated for 40 min. After treatment cells were harvested and stained with 20 nM TMRM at 37°C (protected from light) in HBSS+2% FBS. H_2O_2 was added to corresponding tubes at 400 µM final concentration prior to analysis on a BD LSRII Fortessa flow cytometer (BD Bioscience, San Jose, CA, USA) with excitation at 561 nm, 88/12 BP. Data were analyzed using FlowJo software, with median fluorescent intensity values calculated. Note an apparent drop of mitochondrial potential with MDIVI/Dynasore treatment and increase in MitoTracker green MFI with MDIVI treatment.