Mitochondrial Directed Therapy for Prophylaxis in Oxygen Toxicity Exposures Relevant to Diving Operations

Tanvir Hossain, PhD[Ψ], Ruwani Dewathanthridge, MS[Ψ], and David M. Eckmann, PhD, MD[Ψ, §,€]

[Ψ]Department of Anesthesiology, [§]Department of Biomedical Engineering, and [€]Center for Medical and Engineering Innovation, The Ohio State University, Columbus, Ohio

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INTRODUCTION

The diving community seeks mitigation strategies to prevent oxygen toxicity (OT) and decompression sickness (DCS). Mitochondrial dysfunction in both OT and DCS is a driver of adverse pathophysiology in multiple organs (lung, central nervous system, heart, kidney, other) through loss of cellular bioenergy availability, and can even lead to death.

Maintenance of diver performance ability and safety both require that cellular bioenergetic function be preserved and protected in the face of incipient or sustained environmental challenges. Exploration of a variety of pharmacological strategies to alter the genesis of injury and incapacity from hyperoxic exposures and DCS has yet to yield an operational therapeutic drug that restores or preserves mitochondrial bioenergetic function and subverts development of relevant pathophysiology.

Mitochondrial-directed therapeutics is a new and rapidly emerging class of subcellular organelle-specific drug therapies for preserving or restoring mitochondrial integrity. In these experiments we have studied the effects of Elamipretide (SS31) and DRP1i27, two compounds in this new drug class, to mitigate mitochondrial dysfunction induced by hyperbaric oxygen in a highly relevant pulmonary cell type. We hypothesized that Elamipretide (SS31) and DRP1i27 would mitigate hyperbaric/hyperoxic-induced derangements of mitochondrial dynamics and bioenergetics underpinning the cellular pathogenesis of OT. We expected these drugs to have distinctive signatures for cellular effects due to their unique mitochondrial targets. We have tested our hypotheses via experiments designed to determine effects of the two mitochondrial-targeted therapeutics to mitigate *in vitro* mitochondrial and cellular responses to induction of OT. Measurements include mitochondrial dynamics and bioenergetics as well as relevant cell biochemical markers of OT.

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Use Established Model of O_2Tox Induction in Pulmonary Cells

Our two previous models to induce O_2Tox in pulmonary cells:

Hossain T, et al., Chem Biol Interact 2024
Hossain T, et al., Oxid Med Cell Longev 2025

CURRENT STUDY:

Human Lung Microvascular Endothelial Cells (HLMVECs)

Cell Exposure Conditions:

*Normobaric / Normoxic [Control]
*Hyperbaric / Hyperoxic [HBO]
± Targeted mitochondrial therapeutics

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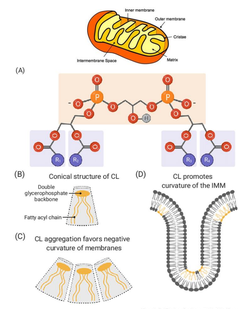
Mitochondrial Therapeutics I: Cardiolipin Targeting

- Cardiolipin is a unique phospholipid found almost exclusively in the inner mitochondrial membrane (IMM)
- It typically accounts for 18% of the IMM mass in mammalian cells
- Its distinctive conical shape facilitates
  - membrane curvature
  - assembly of respiratory-chain supercomplexes
  - mitochondrial bioenergetics
- ***Cardiolipin is a therapeutic target***



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Mitochondrial Therapeutics I: Elamipretide (SS31)

- Elamipretide (SS31) binds the IMM in proximity to cardiolipin via electrostatic/hydrophobic interactions
- Binding to cardiolipin
  - stabilizes cardiolipin–protein complexes
  - reduces cardiolipin oxidation
  - maintains cristae architecture
- **FDA approved as of September 19, 2025 as *Forzinity* for Barth Syndrome**

Anticipated Effects of Therapy

- ✓ Optimize Electron Transport Chain efficiency
- ✓ Maintain $\Delta\Psi_m$
- ✓ Maintain the structural integrity of mitochondria
- ✓ Preserve mitochondrial outer membrane integrity
- ✓ Maintain mitochondrial dynamics (fusion/fission balance)

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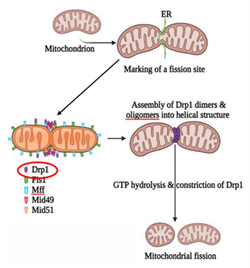
Mitochondrial Therapeutics II: DRP1 Targeting

- Dynamin-related protein 1 (DRP1) is a cytosolic GTPase that plays a central role in mitochondrial fission
- DRP1 is recruited from the cytosol to the outer mitochondrial membrane via adaptor proteins (Mff, MiD49/MiD51, and Fis1)
- Cardiolipin recruits, binds, and functionally activates Drp1 at the outer mitochondrial membrane
- DRP1 oligomerizes into a ring around the mitochondrial constriction site and hydrolyzes GTP, leading to fission
- Many pathologies involve increased mitochondrial fission with resultant dysfunction (decreased ATP production, increased reactive oxygen species production, induction of apoptosis)
- ***DRP1 is a therapeutic target***



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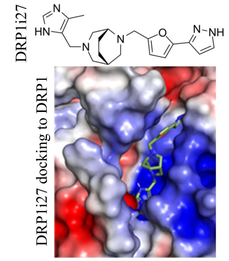
Mitochondrial Therapeutics II: DRP1i27

- DRP1i27 is a small-molecule inhibitor of human DRP1
- Molecular docking: DRP1i27 binds the GTPase domain of DRP1 and forms hydrogen bonds with key residues including Gln34 and Asp218
- DRP1i27 binding to the DRP1's GTPase site inhibits its hydrolysis activity, reducing mitochondrial membrane constriction and subsequent fission
- DRP1i27 is a *potential* mitochondrial-directed therapeutic
- DRP1i27 is experimental and not FDA approved for use in humans



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Experimental Protocol

Cells: HLMVECs
Grown to confluence
Passages 3-6

Therapeutics: None
Elamipretide (SS31), 20 nM
DRP1i27, 5 μM

Exposures: Normobaric Normoxic (NB-NO)
*incubator conditions
Hyperbaric Hyperoxic (HBO)
* 4.8 ATA 4.8 ATA
* 4.8 ATA
*99.1% O_2 / 0.9% CO_2 gas mix
*30 min ascent time

Metabolic Measurements:
*Respiration – Seahorse Analyzer

Dynamics Measurements:
*Fluorescence Microscopy
*Image analysis:
*Mitochondrial Motility
*Mitochondrial Potential
*Mitochondrial Size / Number / Fragmentation
*Intracellular Mitochondrial Distribution
**Intracellular Distribution of Cellular Bioenergetic Capacity*

Other Measurements:
*Western Blots – ETC protein levels and DRP1 levels
*ELISA – Cardiolipin levels

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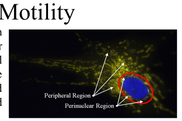
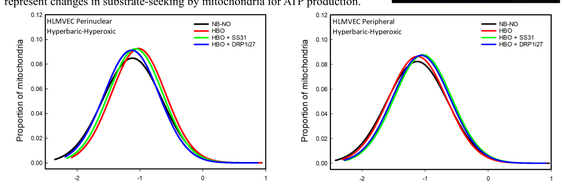
Results: Drug Effects on Mitochondrial Motility

Mitochondrial motility follows a log-normal distribution in all exposures and in both the perinuclear (within 3 μm of the cell nucleus) and peripheral intracellular regions. Both Elamipretide (SS31) and DRP1i27 renormalize mitochondrial motility in the perinuclear region under O_2Tox-inducing conditions and increase motility in the peripheral region. These leftward (decreased motility) and rightward (increased motility) shifts of the curves are statistically significant and represent changes in substrate-seeking by mitochondria for ATP production.





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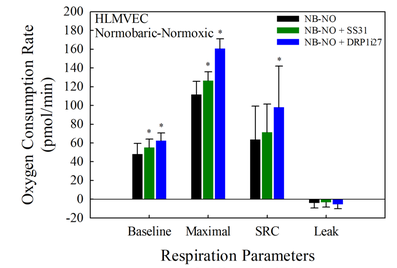
Results: Drug Effects on Respiration at Control Conditions

Both Elamipretide (SS31) and DRP1i27 cause small yet significant increases in baseline respiration under normobaric-normoxic (control) conditions. Presence of either of the therapeutics also increases maximal respiration. However, the spare respiratory capacity (SRC) is only significantly elevated with inclusion of DRP1i27. Proton leak is independent of drug exposure under these control conditions. The results indicate that each of the drugs has some baseline positive effect to enhance cellular respiration in the absence of any toxic challenge.



* P < 0.05 compared to NB-NO

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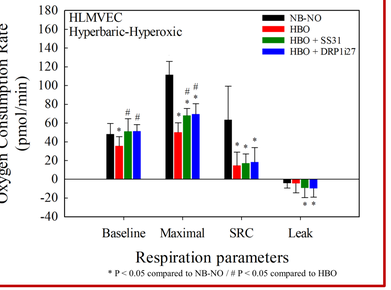
Results: Drug Effects on Respiration After HBO Exposure

Hyperbaric-hyperoxic exposure decreases baseline and maximal respiration and causes a significant fall in SRC. Both Elamipretide (SS31) and DRP1i27 preserve cellular respiration function by maintaining baseline respiration at control levels despite the O_2Tox-inducing exposure. The drugs lead to partial preservation of maximal respiration in the face of hyperbaric-hyperoxic effects, although full normalization to control levels is not achieved. SRC is unaffected by either drug under hyperbaric-hyperoxic exposure whereas proton leak is increased with drug exposure under these conditions. These results indicate that each of the drugs has significant positive effects to maintain cellular respiration at control levels despite the toxic challenge.



* P < 0.05 compared to NB-NO / # P < 0.05 compared to HBO

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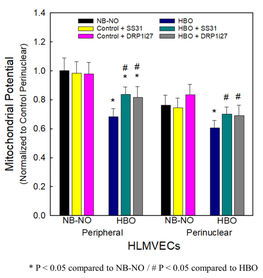
Results: Drug Effects on Inner Membrane Potential $\Delta\Psi_m$

Mitochondria maintain an electrical and chemical gradient, $\Delta\Psi_m$, across their inner membrane to generate cellular energy. Maintenance of this proton gradient is the driving force of the ETC. Protons flow back across the membrane through ATP synthase, physically generating the ATP required for almost all cellular processes. The mitochondrial inner membrane potential also acts as a barometer of cell health. When the potential collapses, the cell recognizes it is damaged and initiates programmed cell death (apoptosis) to prevent harm to the wider organism.

Hyperbaric-hyperoxic exposure produces a marked fall in mitochondrial inner membrane potential compared to controls throughout the cells. However, both drugs function to partially (cell periphery) or fully (perinuclear) renormalize $\Delta\Psi_m$. Maintenance of $\Delta\Psi_m$ in the perinuclear region helps to assure ATP delivery to the cell nucleus despite toxic challenge.



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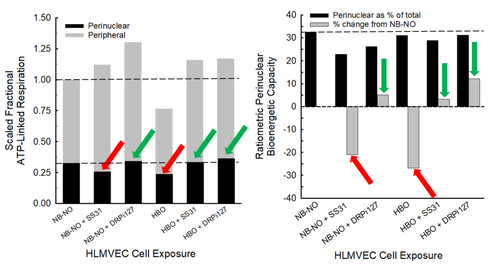
Results: Drug Effects on Bioenergetics Partitioning in Cells

For controls, SS31 and DRP1i27 both increase total cell energy but it is distributed differently within cells. With hyperbaric-hyperoxic exposure cell energy in both the periphery and perinuclear regions is diminished. Each of the drugs leads to a total increase in energy as well as increased energy availability in the perinuclear region (e.g., green arrows).



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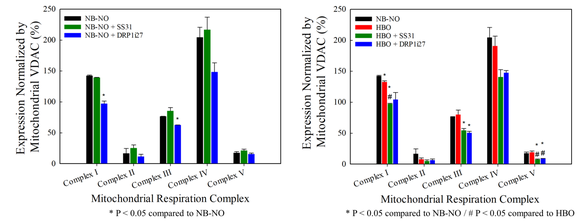
Results: Drug Effects on Respiratory Complex Protein Levels

Mitochondrial respiratory complex levels are generally unaffected by the presence of Elamipretide (SS31), with Complexes I and III reduced by DRP1i27 under control conditions. Hyperbaric-hyperoxic exposure decreases Complex I levels. The addition of Elamipretide (SS31) further reduces Complex I levels, while both drugs further reduces Complex III and IV levels from baseline.



* P < 0.05 compared to NB-NO
* P < 0.05 compared to NB-NO / # P < 0.05 compared to HBO

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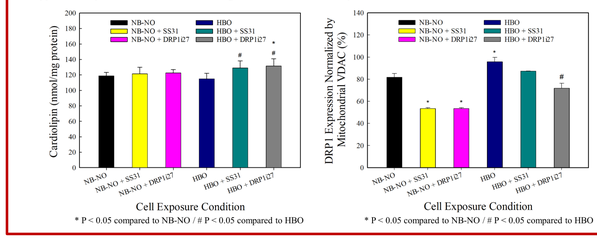
Results: Drug Effects on Cardiolipin and DRP1 Levels

Neither Elamipretide (SS31) nor DRP1i27 has any effect on cardiolipin levels under normobaric-normoxic conditions. Both drugs increase resultant cardiolipin levels after hyperbaric-hyperoxic exposure, even above the control value (DRP1i27). DRP1 expression is decreased by both drugs but is increased following hyperbaric-hyperoxic exposure. Addition of both drugs renormalizes DRP1 expression to control levels.



* P < 0.05 compared to NB-NO / # P < 0.05 compared to HBO
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Conclusions

- ✓ Elamipretide (SS31) and Drp1i27 generally **enhanced** overall cellular bioenergetics in HLMVECs at baseline
- ✓ Elamipretide and Drp1i27 **preserved** bioenergetic function in HLMVECs otherwise susceptible to O_2Tox-induced injury
- ✓ In the face of O_2Tox induction, Elamipretide and Drp1i27 **renormalized** mitochondrial motility in the perinuclear region of HLMVECs toward control levels
- ✓ Elamipretide and Drp1i27 **increased** mitochondrial inner membrane potential $\Delta\Psi_m$ in HLMVECs from low levels caused by O_2Tox exposure
- ✓ Elamipretide and Drp1i27 **increased** perinuclear bioenergy availability beyond baseline (control) levels in HLMVECs subjected to O_2Tox-induced insult
- ✓ Targeted mitochondrial therapeutics have clinical potential in O_2Tox prevention

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