Oxidative Phosphorylation Assay

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Tradename: AC ExoVitalize

Code: 60193

CAS #: 7732-18-5 & 8016-20-4 & 90244-99-8 & 123465-35-0 (or) 8002-43-5

Test Request Form #: 10233

Lot #: N230508B

Sponsor: *Active Concepts, LLC; 107 Technology Drive Lincolnton, NC 28092*
Study Director: *Maureen Drumwright*
Principle Investigator: *Daniel Shill*

Test Performed:
Oxidative Phosphorylation (Mitochondrial Membrane Potential) Assay

Introduction

Cellular metabolism can occur with (aerobic) or without (anaerobic) oxygen to generate ATP (adenosine triphosphate), the end molecular byproduct of metabolism. The stored energy within ATP is harvested and utilized by cells to maintain homeostasis as it is required for cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. Aerobic metabolism, also known as oxidative phosphorylation, occurs within the mitochondria, generates a large amount of ATP, and is the primary source of cellular energy transduction. One driving force of oxidative phosphorylation is the mitochondrial membrane potential (ΔΨm), which represents the transmembrane potential of hydrogen ions. Maintaining ΔΨm is necessary as the proton flux from cytosol to matrix is harnessed to generate ATP. This electrochemical gradient is not only utilized for ATP synthesis but is also an indicator of mitochondrial function and health as prolonged depressions in ΔΨm reduce cell viability. Specifically, unregulated mitochondrial membrane potentials produce excessive amounts of oxidative stress which lead to an acceleration of DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation.

Accordingly, a Mitochondrial Membrane Potential Assay was conducted to assess the *in vitro* effect of **AC ExoVitalize** to stimulate oxidative phosphorylation in dermal fibroblasts. Activating this biological process maintains cellular homeostasis and vitality.

Assay Principle

The Mitochondrial Membrane Potential Assay is based on the detection of the ΔΨm in cells. The lipophilic and cationic JC-10 dye selectively enters mitochondria and concentrates in the mitochondrial matrix where it fluoresces red. The JC-10 dye reversibly changes color from green to orange as membrane potentials increase due to shifts in emitted light from 520 nm to 570 nm, expressed as modifications in the JC-10 from monomeric to J-aggregate, respectively. As ΔΨm increases, ATP synthesis also increases, thus ΔΨm can be utilized as a surrogate measurement of oxidative phosphorylation.

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Materials

A. Kit:	JC-10 Mitochondrial Membrane Potential Assay Kit (Abcam; ab112134)*
B. Incubation Conditions:	37°C, 5% CO_2, and 95% relative humidity (RH)
C. Equipment:	Forma Humidified Incubator; ESCO Biosafety Laminar Flow Hood; Synergy HT Microplate Reader; Pipettes; Light Microscope
D. Cell Line:	Normal Neonatal Human Dermal Fibroblasts (ATCC; PCS-201-010)*
E. Media/Buffers:	Fibroblast Basal Medium (PCS-201-030)*; Fibroblast Growth Kit (PCS-201-041)*; Ethanol
F. Reagents:	100x JC-10 Dye Solution (ab112134)*; Assay Buffer A (ab112134)*; Assay Buffer B (ab112134)*; Phosphate Buffered Saline (PBS); Antimycin A (Sigma Aldrich, A8674)*
G. Culture Plate:	96 Well Black Side/Clear Bottom Tissue Culture Treated Microplates
H. Other:	Sterile disposable pipette tips

**Or suitable alternatives, subject to change without notice based off vendor availability*

Methods

Human neonatal dermal fibroblasts were seeded into a 96-well tissue culture microplate and grew to 80%-90% confluency in complete media (CM). CM and 0.01% and 0.1% concentrations of **AC ExoVitalize** in CM were added to cells and placed at 37°C. Following a 24-hour incubation, the media in all wells was removed and cells were washed once with PBS. Next, 200 pM of Antimycin A, initially dissolved in ethanol and further diluted in CM, was added to all wells, except control and treated wells which received CM. Following a 30-minute incubation at 37°C, the media in all wells was removed and cells were washed once with PBS. Next, 50 µL of the JC-10 Dye Solution was added to all wells and the entire plate was incubated at 37°C for 30 minutes. Lastly, 50 µL of Assay Buffer B was added to all wells and fluorescence measurements were taken at Excitation/Emission wavelengths of 490/525 nm and 540/590 nm.

Regarding experimental controls, CM alone was regarded as a baseline value, and Antimycin A (a known a complex III inhibitor of the mitochondrial electron transport chain) was utilized as a negative control to reduce oxidative phosphorylation. All conditions were tested in duplicate. A ratio analysis of the fluorescent intensities (525/590) was performed. The ΔΨm data is expressed as a percent of the baseline control (CM) and calculated by the following equation:

$$\Delta\Psi m\ (\%\ of\ CM) = \frac{525/590\ Ratio_{Sample}}{525/590\ Ratio_{CM}} \times 100$$

Results

The data obtained from this study met criteria for a valid assay and the controls performed as anticipated. Compared to untreated fibroblasts, Antimycin A (200 pM) reduced mitochondrial membrane potential. Fibroblasts treated with **AC ExoVitalize** at 0.01% and 0.1% demonstrated increased ΔΨm compared to untreated fibroblasts.

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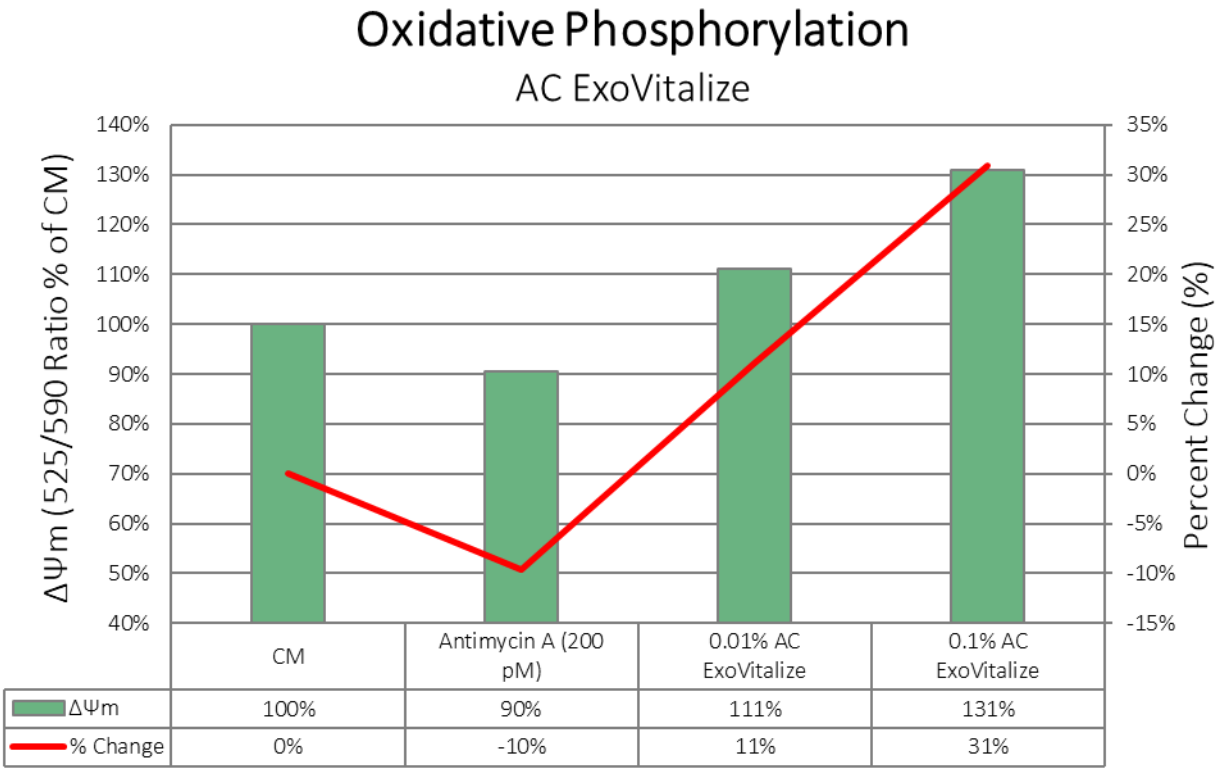

Figure 1. The effect of **AC ExoVitalize** on ΔΨm in dermal fibroblasts.

Discussion

As shown in Figure 1, fibroblasts incubated with Antimycin A, a known oxidative phosphorylation disruptor, exhibited a 10% reduction in ΔΨm compared to untreated fibroblasts. These data demonstrate ΔΨm in fibroblasts is dynamic and oxidative phosphorylation can be manipulated with exogenous compounds.

Conversely, fibroblasts treated with **AC ExoVitalize** at 0.01% and 0.1% demonstrated 11% and 31% increases in ΔΨm compared to untreated fibroblasts, respectively. These data demonstrate **AC ExoVitalize** increases ΔΨm and oxidative phosphorylation in fibroblasts.

In summary, oxidative phosphorylation is a biological process that generates ATP to assist in cellular migration and proliferation, molecular transportation across membranes, and the biosynthesis of cellular components. A necessary component of oxidative phosphorylation is the maintenance of ΔΨm given prolonged reductions in this electrochemical gradient are indicative of poor mitochondrial health. Increasing oxidative phosphorylation not only maintains ΔΨm, but also reduces the formation of reactive oxygen species which augments the ability of dermal cells to reduce characteristics of cellular aging (e.g. DNA mutation, cellular senescence, advanced glycation end products, protein oxidation, and collagen degradation). Collectively, these data indicate **AC ExoVitalize** stimulates oxidative phosphorylation, demonstrated by increases in mitochondrial membrane potential, which assists in the maintenance of cellular homeostasis, vitality, mitochondrial function, and attenuate the physical signs of cellular aging.