In vitro Antioxidant Oxidative Stress Treatment Model in Microgravity
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Introduction: Muscular atrophy, or loss of muscle mass, is prevalent when muscle use is limited by immobilization on earth, or in microgravity when muscle tissue is not exercised by regular loading. Oxidative stress increases with limited muscle use and as astronauts are exposed to ionizing radiation during space travel. This oxidative stress kills muscles cells, causing even further breakdown of the tissue and damage to DNA, increasing cancer risk. We propose that by introducing a variety of antioxidants to combat oxidative stress on murine C2C12 muscle cells that muscular atrophy, ionizing radiation damage, and cell death can be greatly reduced.

Materials and Methods: Several antioxidants (including DL-alpha tocopherol phosphate disodium salt (VE), mesobiliverdin (MBV), and L-glutathione (GSH)) were tested against a uric acid standard in an OxiSelect Total Antioxidant Capacity (TAC) Assay Kit purchased from Cell Biolabs, Inc. MBV had been tested previously at 40μM, 20μM, 10μM, 5μM, and 2.5μM to find toxicity levels. 10μM has been further tested in a rotary cell bioreactor to test its resistance against oxidative stress to C2C12 cells when exposed to cesium-137 radiation, suspended in simulated microgravity, and in a combination of radiation and microgravity. GSH and VE were both tested at concentrations of 400μM, 200μM, 100μM, 50μM, and 25μM, respectively. Prior to the TAC assay, cells were cultured with 10μM MBV and 133μM GSH, each in 2%FBS/DMEM against 133μM H2O2 in separate wells.

Results and Discussion:
Preliminary results of the TAC assay showed that with tuned concentrations of each antioxidant that there is significant potential for use of individual antioxidants against oxidative stress. MBV at 10μM was researched previously to observe whether or not it exhibited qualities for combatting oxidative stress, but quantifiable results have not been found. Based off of the TAC assay, uric acid, MBV, and GSH exhibited highly similar oxidative stress resistance up to 215 copper reducing equivalents (CRE) (at 0.2mM). GSH mimicked uric acid closely to as high as 1000 CRE (at 0.45mM). VE results were skewed due to an inability to fully dissolve the powder into solution. The results for both MBV and GSH are promising possibilities for use against oxidative stress from microgravity and radiation exposure.

Conclusion: Future experimentation with MBV, GSH, and VE will include aseptic cell culture with H2O2 to test how effectively the antioxidants aid in resisting cell death and oxidative stress from radicals. As MBV has already expressed promising results, we assume that at equivalent relative concentrations the GSH and VE will express similar capabilities.

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