TACSM Abstract

The Evaluation of Nox2 Role in Microgravity–Induced Skeletal Muscle Atrophy

AMIN MOHAJERI, KHALED MOUSTAFA, & JOHN LAWLER.

Redox Biology & Cell Signaling Lab; Department of Health & Kinesiology; Texas A&M University; College Station, TX

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Advisor / Mentor: Lawler, John (jml2621@tamu.edu)

ABSTRACT

Long term exposure to the outer space microgravity weightlessness subjects the skeletal muscle fiber dysfunction, which is characterized by fibrosis, cellular remodeling and loss of physical performance. Under mechanical unloading condition, profound and progressive muscle fibers atrophy is developed. It would furthermore increase the risk of our astronauts' injury upon returning to a gravitational field (e.g., Earth or Mars). **PURPOSE**: Skeletal muscles are dynamic mechanical and metabolic machines with the capability of sensing and proportionally adapting to the alterations in the surrounding mechanical loading through the regulation of proteins synthesis/degradation rate. Prolonged exposure to mechanical unloading is associated with increased production of reactive oxygen species (ROS), and the translocation of nNOSμ from the sarcolemma in the skeletal muscle. In addition to mitochondria which is known to be a source of ROS production during unloading, the sarcolemmal NADPH oxidase-2 (Nox2) is, also, found to be increased in unloaded muscles. Furthermore, nNOSμ translocation, perturbations in heat shock proteins (e.g. HSP70), and antioxidant enzyme (e.g., MnSOD) have been implicated in in skeletal muscle fiber atrophy. Down- and up-stream pathways involved in the assembly of Nox2 subunits are not fully understood. Nuclear factor erythroid 2-related factor 2 (Nrf2) is a leucine zipper protein and transcription factor which is reported to be responsible for providing cells with protection under stress through the upregulation of antioxidant proteins (e.g., heme oxygenase-1, MnSOD, etc.) and the suppression of ROS production, It would further contribute to the alleviation of Nox2 upregulation, nNOS translocation, and muscle fiber atrophy. **METHODS**: 4-month old F344 rats were assigned into 3 groups: ambulatory control (CON), hindlimb unloaded (7 days) + 5 mg/kg/day scrambled sequence (HU), and hindlimb unloaded (7 days) + 5 mg/kg/day peptidyl Nox2 inhibitor gp91dstat (HUG). At the conclusion of the study, the soleus muscles were collected and frozen in isopentane cooled in liquid N2. Samples were fractionized for western blot analysis. In addition, HSP70, MnSOD, Nrf2, and HO-1 antibodies were employed, as well as nNOS translocation marker for immunofluorescence. **RESULTS**: Exposure to mechanical unloading significantly reduced protein abundance of HSP70, MnSOD, and NrF2. While no protective effect of gp91dstat treatment was observed in HSP70 and MnSOD levels, Nrf2 level was significantly protected during unloading when Nox2 was inhibited, suggestive of the potential for preservation of antioxidant protection. Furthermore, the nNOS sarcolemmal activity, which was reduced in the hindlimb unloading study group, was increased with the use of peptidyl Nox2 inhibitor Our data suggest that HO-1 protective effect against unloading-induced atrophy could be more efficient than that of MnSOD. Although, it remains unsolved, it is a focus of future investigations. **CONCLUSION**: This study provides the first evidence that elevated Nox2 can play a causal role in mechanotransduction and cellular remodeling which occur in unloaded skeletal muscle fiber.