Mitochondrial Transplantation into the Injured Spinal Cord Improves Cellular Respiration

Jenna Gollihue\textsuperscript{1} • Samir Patel, PhD\textsuperscript{1} • Charles Mashburn, MS\textsuperscript{2} • Khalid Eldahan\textsuperscript{1} • David Cox, MS\textsuperscript{2} • Patrick Sullivan, PhD\textsuperscript{3} • Alexander Rabchevsky, PhD\textsuperscript{1}

\textsuperscript{1}Physiology, University of Kentucky • \textsuperscript{2}Spinal Cord and Brain Injury Center, University of Kentucky

**Background:** Traumatic spinal cord injury (SCI) results in excitotoxicity, excessive reactive oxygen and nitrogen species (ROS/RNS) production, and necrotic cell death which contribute to development of secondary pathophysiological cascades. Many branches of these cascades such as ROS/RNS production, glutamate-induced excitotoxicity, calcium dysregulation, decreased ATP production, compromised bioenergetics, and apoptotic cell death can stem from mitochondrial dysfunction.

**Hypothesis:** We hypothesized that mitochondria transplanted after SCI can be taken up in vivo by host cells and replace damaged endogenous mitochondria, providing a multi-mechanistic approach to restore cellular bioenergetics.

**Methods:** Mitochondria were isolated either from soleus rat muscle or PC12 cells in which the mitochondria were transgenically-labeled. Adult female Sprague Dawley rats received a severe spinal contusion injury, followed immediately by injections of isolated mitochondria around the injury site. 24 hours after transplantation, animals were euthanized and tissues were processed for either 1) isolated mitochondrial respiration assays or 2) histological analyses.

**Results:** Oxygen consumption rates of injured tissues transplanted with 100ug mitochondria showed significantly increased respiration after 24hr compared to 50ug, 150ug, or vehicle-treated injury alone. Further, we compared the effects of transplanting either cell culture-derived or muscle-derived mitochondria on bioenergetics and found that both transplants showed significantly increased State III-driven respiration at the 100ug dosage compared to vehicle-treated injured, reaching almost 90% of sham levels. We also visually identified transplanted transgenically-labeled mitochondria in both naive and injured cords and found instances of co-localization within a variety of host cell types by 24 hr. Mitochondria had a higher propensity to be taken into macrophages and endothelial cells of the spinal cord, with fewer instances of incorporation evident in astrocytes and oligodendrocytes.

**Conclusions:** Mitochondrial transplantation after spinal cord injury increases overall respiration in the acutely contused cord in a dose-dependent manner. Further, mitochondria were successfully taken into host cells of different types. Current studies are quantifying phenotypic distribution over time post-injury as well as examining the effects of transplanting mitochondria from both sources on tissue sparing and long-term functional recovery.

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