Morphological and metabolic characterization of a new model of spinal cord injury without reloading using ^{1}H MRI and ^{31}P NMR spectroscopy

C. Baligand[1], R. S. Vohra[2], F. Ye[2], J. Keener[3], W. Lim[2], S. C. Forbes[2], P. K. Shah[2], P. Bose[3,4], G. A. Walter[1], F. Thompson[3,4], and K. H. Vandenborne[2]
[1]Physiology and Functional Genomics, University of Florida, Gainesville, Florida, United States, [2]Physical Therapy, University of Florida, Gainesville, Florida, United States, [3]North Florida/South Georgia Veterans Health System of Florida, Gainesville, Florida, United States, [4]Departments of Physiological Science and Neurology, University of Florida, Gainesville, Florida, United States

INTRODUCTION: Contusion spinal cord injury (SCI) animal models are widely used to study muscle atrophy and loss of muscle function after neural disruption and muscle unloading. However, spontaneous recovery has been observed within a few weeks in rodents, which has been partly attributed to muscle reloading with free activity. On the other hand, cast immobilization is another well charcterized model of disuse (1, 2). In this study, we used MR imaging and spectroscopy to non invasively characterize a novel model of contusion spinal cord injury without muscle reloading. We demonstrated that cast immobilization can be used in combination with severe SCI to produce a profound and prolonged muscle atrophy. We characterized this model non invasively at 11.1T using volumetric MRI and ^{31}P spectroscopy to follow muscle cross sectional area (CSA) and assess bioenergetics, respectively. The severity of the spinal cord lesion was assessed with microscopy MRI at 17.6T and standard histology.

METHODS: ***Animals***. All measures were performed in 16 to 19-week old female Sprague-Dawley rats (n=54, average body weight = 288 ± 16 g). ***Spinal cord injury***. Spinal cord contusion injuries were produced using New York University impactor as previously described (3). A 10-g weight was dropped from a 50-mm height onto the T8 or T9 segment of the spinal cord, which was exposed by laminectomy, and locomotor function was assessed weekly using the 21 Basso-Beattie-Bresnahan (BBB) locomotor scale (4). ***Cast immobilization (IMM)***. Lower body joints were fixed at resting angles. The casting tape encompassed the caudal fourth of the body (superior to tail). A thin layer of padding was placed underneath the cast in order to prevent abrasions. ***Experiment design***. Animals were divided into 4 groups for weekly follow-up: controls (CTR; n=12), spinal cord injury only (SCI; n=13), 2 weeks cast immobilization only (IMM; n=8) and SCI combined with 2 weeks of cast immobilization (SCI+IMM; n=7). After in situ assessment of the soleus contractile properties, all hindlimb muscles and spinal cord tissue were taken for histology. ***MR acquisitions***. Measurements were performed at 11.1 T (horizontal) and 17.6T (vertical) (Bruker, Paravision 3.0.2, Paravision 4.0). **Lower limbs 3D-imaging:** 3D GE T1 weighted images of both legs were separately acquired using a home-build quadrature ^{1}H surface coil (470 MHz). The coil was designed to allow proper positioning on casted legs. Imaging was performed pre-surgery and weekly until 3 weeks post-surgery using the following parameters: 1s sech pulse, TR/TE= 115/4.5 ms; FOV: 25x25x25 mm3, matrix size: 256x256x64. **^{31}P dynamic spectroscopy**: A subgroup of 6 SCI and 8 CTR were used for quantification of energy metabolites at rest and assessment of mitochondrial oxidative capacities 1 week post-SCI. Data were collected with a 1.0 x 1.5 cm oval ^{31}P (190.5 MHz) surface coil, placed over the belly of the gastrocnemius. For shimming and localization, a 3-cm ^{1}H surface coil was placed on the side of the leg, orthogonal to the ^{31}P coil. The sciatic nerve was electrically stimulated using subcutaneous needle electrodes (Grass Instruments, West Warwick, RI) with repeated 1 ms pulses (1 Hz, supramaximal voltage ~15-20 V). ^{31}P spectra were acquired at rest for 2 min, during exercise (6 min) and recovery (12 min) (50 µs square pulse, TR = 2 s, SW= 10 kHz, 8000 complex data points, NA=5). **In vitro spinal cords 3D-imaging:** 3D high-resolution T1 weighted imaging of tissues in fluorinert was performed in a 750 MHz vertical Bruker magnet using a 5 mm coil (TR/TE: 100/10ms; FOV: 2.5x2.5x10mm3;matrix: 128x128x256). **Data analysis:** Segmentation of the triceps surae muscle was manually performed in the Osirix software and maximal CSA (CSA_{max}) was defined as the average of the 3 highest consecutive CSA. ^{31}P spectra were integrated in Xwin NMR (Bruker) after line broadening of 8 dB, phase and baseline correction. All data were corrected for saturation. PCr recovery was fitted to a mono exponential and results expressed as the time constant τPCr (s).

RESULTS: Contusions resulted in severe injuries as shown by low BBB scores (week1: 1, week2: 3.5, week3: 4.5), and spinal cord MRI. Images revealed severe atrophy of the spinal cord and a lost of contrast between white and grey matter at the injury site. Muscle atrophy reached 26 ± 4% 1-week post-surgery, then recovered up to 22 ± 6% 3-weeks post-surgery in SCI (Fig. 1). IMM alone resulted in 35 ± 4 % after 2 weeks. Importantly, in SCI rats, IMM not only prevented spontaneous recovery but resulted in further loss of muscle mass (42 ± 7 % at week3). Soleus specific tetanic force did not show any difference between SCI, IMM and CTR rats. In vivo exercise led to ~40% a PCr depletion in CTR and 60% in SCI and to minor acidosis (CTR: 7.02 ± 0.01; SCI: 6.98 ± 0.02). PCr recovery was slower in SCI rats (τPCR= 129± 15 s) as compared to CTR (96 ± 8 s), as shown in Fig. 2.

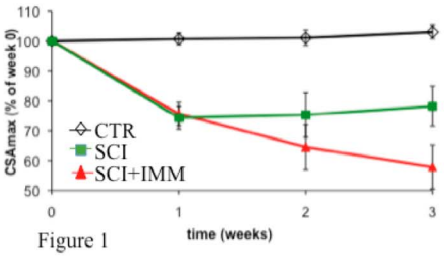

Figure 1

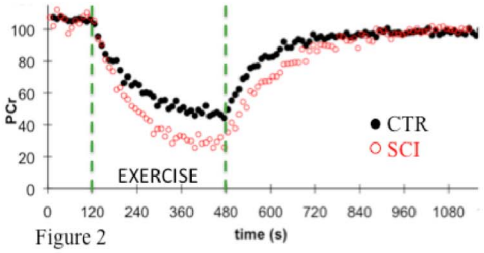

Figure 2

CONCLUSION: We demonstrated that cast immobilization could prevent spontaneous muscle mass recovery after contusion SCI. In addition, preliminary results showed a slower rate of mitochondrial ATP production after 1 week SCI, which may reflect the decrease in SDH activity previously found in rats and cats model (5, 6). This methodology provides a relevant model of muscle disuse and non-invasive tools for the assessment rehabilitation strategies such as locomotor training and drug therapies.

References: (1) Pathare, et al. NMR in Biomed 2008, (2) Frimel, et al. Muscle Nerve 2005, (3) Liu, et al. Spinal Cord 2008, (4) Basso, et al. J Neurotrauma 1995, (5) Gregory, et al. Spinal Cord 2003, (6) Jiang, et al. J Neurotrauma 1990.