Monitoring stem cell therapies of spinal cord injury using quantitative T2 relaxation.

G. J. Stanisz[1], S. Karimi-Abdolrezaee[2], N. Lobaugh[1], W. Oakden[3], V. Gretka[1], E. Efetkharpour[2], G. Hawryluk[2], J. Wang[2], and M. Fehlings[2]

[1]Imaging Research, Sunnybrook HSC, Toronto, ON, Canada, [2]Neurosurgery, Toronto Western Hospital, Toronto, ON, Canada, [3]Medical Biophysics, University of Toronto, Toronto, ON, Canada

INTRODUCTION

In animal models of spinal cord injury (SCI), a combinational therapy using transplantation of exogenous adult neural precursor cells, delivery of a cocktail of growth factors and anti-inflammatory/neuroprotective drug minocycline (1) leads to increased survival and integration of transplant, remyelination of injured axons at the injury site, and improved neurological recovery. To translate similar therapies into the clinic, it is critical to evaluate, non-invasively and at multiple time points, this process of tissue repair and regeneration. In this study, we used a rat model of SCI to demonstrate the feasibility of using quantitative T2 relaxation to distinguish between regenerating and pathological tissues when using neural precursor cell- based therapies.

METHODS

Six Wistar rats were used in this study. The aneurysm clip compression model of SCI was used (1). A midline incision was made at the thoracic area (T5–T9), and skin and superficial muscles were retracted. The rats underwent a T6–T8 laminectomy and received a 23 g clip (Walsh, Oakville, Ontario,Canada) compression injury for 1 min at the level of T7 of the spinal cord. Adult neural precursor cells (ANPC) were isolated from yellow fluorescent protein (YFP)-expressing transgenic mice [strain 129-Tg(ACTB-EYFP)2Nagy/J; The Jackson Laboratory, Bar Harbor, ME]. The cells were diluted in the growth medium (50 x 10^3/µl) and used for cell transplantation. Transplantation of ANPCs in to the injured spinal cord was performed two weeks after initial injury. Using a Hamilton syringe, a total volume of 8 µl of cell suspension, containing 3–4x10^5 live cells, was injected into the dorsal spinal cord, next to the midline. Between two and four intraspinal injections were made 2 mm rostrally and 2 mm caudally to the injury site. To enhance the survival of the transplanted cells, a mixture of growth factors including PDGF-AA (1µg/100µl; Sigma), bFGF (3µg/100µl; Sigma), and EGF (3 µg/100 µl; Sigma) in a solution containing aCSF, BSA (100 µg/ml), and gentamycine (50µg/ml) was infused for 7 d using a catheter connected to an osmotic minipump (model 1007D, 0.5 µl/h; Alzet, Cupertino, CA). The catheter (300 µm O.D.) was implanted intrathecally at the area of transplantation. Following MRI, animals were sacrificed and immunohistochemistry was performed (1). For MRI, rats were anesthetized (ketamine-xylazine), placed in an MR-compatible head restraint and scanned at 3T (GE Signa) using a custom-designed surface coil. Images were acquired starting at 7 days and up to 60 days post-injury. A set of axial proton density (PD) and T_2-weighted images were simultaneously acquired (TR 2500ms, TE 30/80ms, BW 10.42/5.68, matrix 128x128, FOV 8cm, slice thickness 4mm, NEX 0.5) to determine the locations of injury. A single slice, qT_2 pulse sequence (2) was used (sagittal slice,TE=10ms, matrix 128x128, FOV 6cm, slice thickness 2mm, NEX 6, 96 echoes collected). Analysis of the T_2 relaxation decay curves was performed using the Gaussian model of T_2 decay (3). This model assumes that the T_2 spectrum has three different components, each with a Gaussian distribution on a logarithmic time scale. The T_2 spectrum shows the relative signal amplitude per logarithmic interval as a function of T_2 relaxation. The typical ROI of injured spinal cord consisted of ~15 voxels. For comparison, regions of interest (ROI) proximal to SCI were chosen. Finally, the maps of the location of long T2 component were generated for individual voxels.

RESULTS

In all untreated animals, immuno-histopathology showed the processes of demyelination and axonal loss at the site of SCI. Spinal cords of rats treated with ANPC exhibited the presence of myelin basic protein and new myelin formation around healthy axons. In both cases the proximal portion of spinal cord was normal. Quantitative T2 spectra are shown in Fig.1

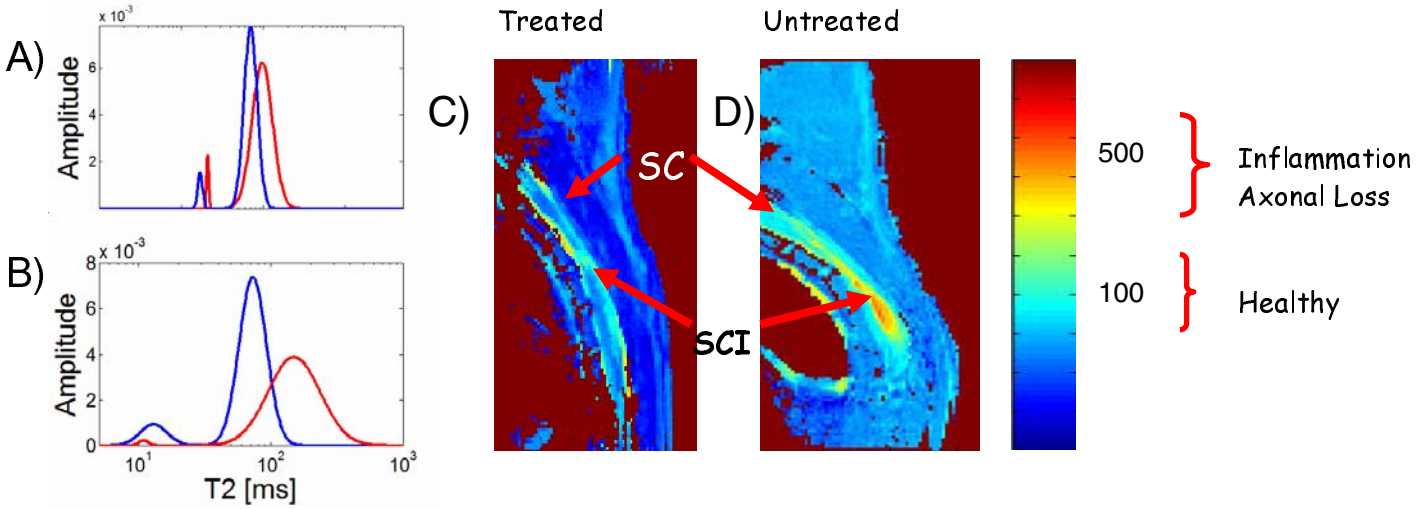

Figure 1. *T2 spectra of treated (A) and untreated (B) spinal cord for proximal (blue) and injured (red) portions of spinal cord, five weeks post injury. Maps of the long T2 component position (C,D) are also presented. SC – proximal portion of the spinal cord. SCI – site of spinal cord injury. In all cases, the T2 spectra consisted of two components,*

In proximal portions of spinal cord, the Myelin Water Fraction (MWF, the area of short T2 component) was 10 ± 2% and location of the intra/extracellular water (I/EW) component was 70 ± 10 ms). Injured portions of untreated spinal cords underwent demyelination (MWF < 3%) and a significant increase in the I/EW peak position (120 to 500 ms) six weeks post injury. The T2 spectra of stem cell treated spinal cords resembled normal spinal cord – the MWF was on average 8 ± 2% and the position of I/EW peak was between 80 and 110 ms. In this experiment, SNR (~30) was sufficient to calculate the long T2 component maps, but reliable calculations of myelin water fraction at the voxel level would require SNR level greater than 60.

DISCUSSION & CONCLUSIONS

The results of this study demonstrated that quantitative T2 is capable of depicting remyelination following neural stem cell therapies in the spinal cord. Untreated SC exhibited substantial loss of myelin (decrease in the short T2 component) and inflammation/axonal loss (increase in the position of the long T2 component). T2 spectra of treated spinal cord resembled normal tissue. Stem cell therapies resulted in remyelination of axons and reduced the degree of inflammation. MRI results were also confirmed by immunohistochemistry.

REFERENCES:

1) S.Karimi-Abdolrezaee et al, 2006, J Neurosci, 26 (13): 3377-3389, 2) Whittall KP et al MRM, 2002. 47: 403. 3) Webb, S. et al MRM, 2003. 49: 638