Diffusion tensor imaging of spinal cord injury: regional differences in white matter damage during the hyperacute phase

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Introduction

Despite many recently reported advancements in the field of traumatic spinal cord injury (SCI), noninvasive measures of ongoing pathophysiologic processes in the injured cord remain elusive, especially during the clinically relevant hyperacute phase (0-6 hours after injury). Detailed spinal cord functional studies have uncovered specific regions of white matter containing long tracts subserving locomotion, micturition, fine digital movements, proprioception, sensation of pain, and other functions affected by SCI. Region specific measurements of axonal injury severity during the hyperacute phase will provide useful diagnostic information for predicting recovery and for evaluating experimental therapies directed at specific functional targets. Recently, diffusion tensor imaging (DTI) derived axial diffusivity ($\lambda_{||}$) was shown to be a sensitive biomarker for axonal injury in spinal cord white matter[1]. Present studies suggest that noninvasive measurements of $\lambda_{||}$ can detect gross regional differences in white matter injury following hyperacute SCI in mice.

Methods

Twenty eighteen-week-old female C57BL/6 mice (Harlan-Sprague Dawley, Inc. Indianapolis, IN) were anesthetized with ketamine (80mg/kg) and xylazine (10mg/kg) intraperitoneally. After dorsal laminectomy at the T12 vertebral level, mice received 0.3mm (n = 5), 0.6mm (n = 5), or 0.9mm (n =5) contusive SCIs utilizing a modified OSU impactor (1.7 mm round impactor tip, 0.9 mm impact distance, 0.15 m/s impact speed). Five mice received sham laminectomy only. The surgical site was closed in layers with 4-0 Vicryl and nylon sutures. Injections of enrofloxacin (2.5mg/kg) and lactated ringers were administered subcutaneously.

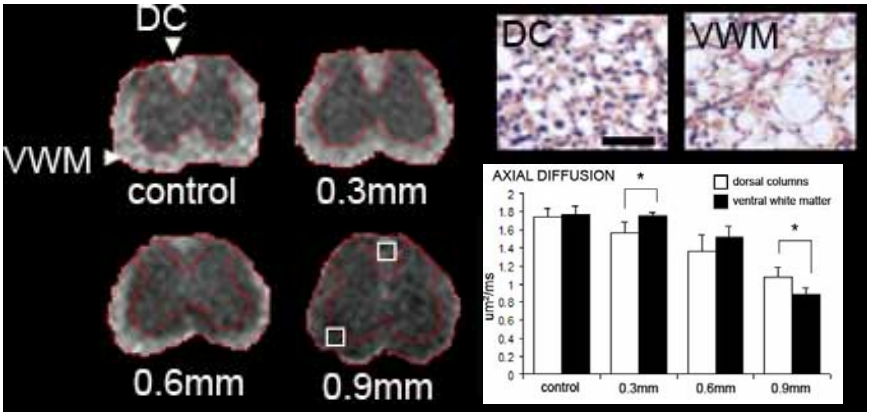
For DTI data collection, mice were anesthetized with isoflurane and placed into an Oxford Instruments 200/330 magnet (4.7 T, 33cm clear bore) equipped with a 15 cm inner diameter, actively shielded Oxford gradient coil (18G/cm, 200μs rise time). A conventional spin-echo imaging sequence was modified by adding Stejskal-Tanner diffusion weighting gradients. The spin echo time (TE) = 38 ms, time between application of gradient pulses (Δ) = 20 ms, and diffusion gradient on time (δ) = 7 ms were fixed for all experiments. The repetition time (TR, ~1.2s) was varied according to the period of respiratory cycle (~270ms). Three different image slices were collected during every breath. Images were obtained with diffusion sensitizing gradients applied in six orientations: (Gx,Gy,Gz) = (1,1,0), (1,0,1), (0,1,1), (-1,1,0]), (0,-1,1), and (1,0,-1). Two diffusion-sensitizing factors, or b values, of 0 and 0.785 ms/μm^2 were used. Eight scans were averaged per k-space line with field of view 1×1 cm^2 and data matrix 128 × 128 (zero filled to 256 × 256). Each multi-slice DTI data set (total of 9 slices) covering T11 – T13 of the cord was obtained starting immediately after the impact procedure. Average acquisition time was 3 hours. The eigenvalues (λ1, λ2, and λ3) and eigenvectors of the diffusion tensor, the trace of the diffusion tensor (Trace), $\lambda||$, radial diffusivity, and relative anisotropy were derived from diffusion-weighted images using software written in Matlab (MathWorks, Natick, MA, USA).

Immediately following the scan, animals were euthanized and perfused with 4% paraformaldehyde. Standard Bielschowsky silver stains were performed to evaluate axonal integrity.

Results

Present studies demonstrate a rapid decrease in white matter $\lambda_{||}$ following hyperacute SCI corresponding to primary axonal injury as evidenced by standard silver staining (see figure). Axonal injury appeared to extend outward from the central canal in a radial pattern that involved progressively more white matter with each increase in injury grade. This pattern is well-documented following standardized impactor injuries and ultimately leads to functional deficits proportional to the degree of damaged white matter[2].

$\lambda_{||}$ varied regionally with increasing injury displacement. Following mild (0.3mm) displacement, $\lambda_{||}$ measurements demonstrated significantly more severe damage in DC compared to VWM (p = 0.010). Severe displacement (0.9mm) resulted in significantly worse injury in the VWM (p = 0.012), consistent with reports of more pronounced axonal damage in ventral white matter, especially the lateral columns, following dorsal contusive injuries[3].



Axial diffusion (λll) maps obtained from the epicenter of sham, mild (0.3 mm), moderate (0.6 mm) and severely (0.9 mm) injured spinal cords and representative silver staining of observed DC and VWM axonal injury differences 4 hrs. after 0.9mm injury (scale bar = 25μm). Quantitative regional differences in axial diffusion are presented in the lower right box.

Discussion

The initial hours following human and experimental SCI represent an important and largely understudied gap in basic science knowledge that results in part from a lack of noninvasive measures of spinal cord pathophysiology. Spinal cord contusion injuries result in rapid stretch of compact bundles of axons within the white matter. Depending on the velocity of the impactor tip and degree of tissue displacement, axons experience a range of tensile forces that lead to stretch or disruption as spinal cord tissue extrudes longitudinally from the center. During mild displacement, DC are primarily affected. After severe dorsal displacement, maximum axonal loss occurs in VWM tracts and increases exponentially from the dura centrally into the gray matter[3]. Disruption of spinal cord microvasculature results in variable degrees of intraparenchymal hemorrhage and edema. This accounts for the predominantly central pattern of necrosis and tissue loss documented extensively in the literature.

These studies demonstrate for the first time that region-specific noninvasive assessments of spinal cord white matter injury severity can be obtained in mice during the clinically important hyperacute phase (0-6 hours) after SCI using MRI. Cross-sectional regional DTI-derived measurements of $\lambda_{||}$ in DC and VWM varied with increasing cord displacement in a pattern consistent with spinal cord viscoelastic properties.

References **1.** Kim, J.H. et al. *Neurobiol Dis* 21, 626-32 (2006) **2.** Ma, M., Basso, D.M., Walters, P., Stokes, B.T. & Jakeman, L.B. *Experimental Neurol* 169, 239-54 (2001) **3.** Blight, A.R. & Decrescito, V. *Neuroscience* 19, 321-41 (1986)

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