Temporal Changes in Axial and Radial Diffusivities in a Rat Model of Wallerian Degeneration in the Spinal Cord

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Introduction: Wallerian degeneration (WD) in the CNS takes place after injuries to the axon or neuronal body. WD is common in patients with various neurological disorders, including multiple sclerosis. In recent years, diffusion tensor imaging (DTI) has been widely used in detection and monitoring of WD in patients and animal models. Early reports (2,3) suggested that injury to the axon and myelin in the spinal cord alter axial diffusivity ($D_{\|}$) and radial diffusivity ($D_{\perp}$) respectively. It has been shown that axial diffusivity correlated strongly with density of healthy axons in the spinal cord white matter (4). To further investigate the relationships between these DTI parameters and pathology in WD, we examined the temporal changes of these parameters in rat spinal cords after dorsal root axotomy using *ex vivo* high resolution DTI. The animal model is a classic model of WD in the spinal cord, and has been extensively studied by histology. It generates well localized lesion without complications such as edema and breaking down of blood tissue barrier as in spinal cord injury. The affected axons in the spinal cord will mostly be limited to the axons in the severed dorsal root, if secondary degeneration can be ignored. The pathological events after axotomy include rapid axonal degeneration (completed within 3 days), microglial activation (~20 days) and slow clearance of myelin (more than 90 days) (1).

Method: Total 28 rats were used in this study with 4 rats for each of seven time points after left L_2-L_6 dorsal root axotomy (no injury, 16 hours, 38 hours, 3 day, 7 day, 14 day, and 30 day after axotomy). Rats were perfusion fixed with 4% PFA. Ex vivo 3D MRI was performed on an 11.7 T MR system at 28°C, as described in (4). For each specimen, six diffusion weighted images (b=700 s/mm^2) and two non-diffusion weighted (b=50 s/mm^2) image were acquired with a multiple spin echo sequence (TEs = 27/37/47/57 ms, TR = 0.6 s, NA=2, Δ = 14 ms, δ = 6 ms, resolution = 0.1 x 0.1 x 0.1 mm^3. Diffusion tensors were calculated using a Log-linear fitting method, along with FA, $D_{\|}$ and $D_{\perp}$. Currently, histological analysis is still ongoing.

Results: A wedge shaped lesion in the dorsal column first became visible in the $D_{\|}$ map at 38 hours after axotomy (Fig. 1) in the lumbar spinal cord, and spread to the thoracic spinal cords at day 3. From day 3 to day 30, the relative sizes of the lesions remained the same in the $D_{\|}$ maps. Lesions became visible in FA and $D_{\perp}$ maps at day 3, and acquired similar shape as the lesions in the $D_{\|}$ maps at day 7. No apparent lesion was detected in T_2-weighted images throughout the 30 day period. Values of FA, $D_{\|}$ and $D_{\perp}$ in the lesions were measured by regions of interest manually defined in the $D_{\|}$ maps. $D_{\|}$ and FA of the lesions decreased rapidly during the first 3 days, with significant decrease in $D_{\|}$ at 38 hours and in FA at day 3 with respect to values from the contra-lateral side ($p < 0.05$, nonparametric rank sum test). $D_{\perp}$ of the lesions increased significantly during the first 3 days ($p < 0.05$, nonparametric rank sum test). After day 3, $D_{\|}$ of the lesion remained stable, while $D_{\perp}$ in the lesion showed a small but significant increase ($p < 0.05$, nonparametric rank sum test) (Fig. 2).

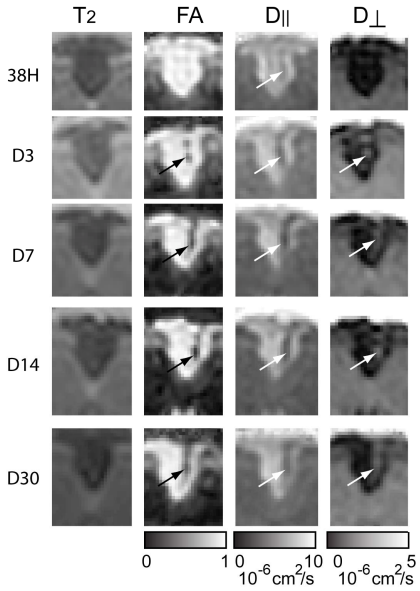

Fig. 1: T_2, FA, axial diffusivity ($D_{\|}$) and radial diffusivity ($D_{\perp}$) maps of Wallerian degeneration in the L_2 dorsal column of the rat spinal cord at different stages after dorsal root axotomy. The arrows indicate the locations of

Discussions: The results suggest that early axonal degeneration in the spinal cord white matter in WD could be detected within 38 hours in the $D_{\|}$ maps. The observed spatial progression of lesion and temporal changes in $D_{\|}$ correlated well with the well known granular disintegration of the cytoskeleton of the axons, which spreads from the site of injury at 3 mm/hour and is complete along the entire spinal cord in 3 days (1). The mechanisms behind the observed temporal changes in $D_{\perp}$, however, remain unclear. Possible explanations of the initial rapid increase in $D_{\perp}$ of the lesion within 3 days after axotomy include formation of myelin ovoids and/or enlarged axonal spaces. The slow increase in $D_{\perp}$ after the initial 3 days may be caused by the gradual myelin clearance. In summary, high resolution DTI can provide quantitative information on the temporal and spatial distribution of axonal degeneration in this model. While $D_{\|}$ is sensitive to early axonal degeneration in the spinal cord white matter, the relationship between $D_{\perp}$ and pathology is complex and needs further investigations.

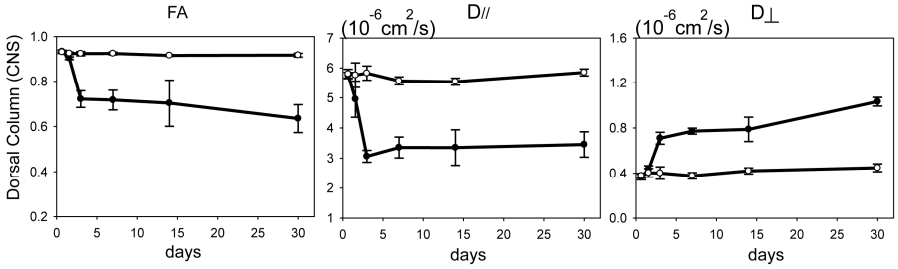

Fig. 2: Temporal changes in FA, axial diffusivity ($D_{\|}$) and radial diffusivity ($D_{\perp}$) in the lesion at L_2 (black circles) and corresponding area in the contra-lateral dorsal column (white circles).

References: 1. George R. et. al., Experimental Neurology 129, 225-236 (1994) **2.** Song S.K. Neuroimage 20:1714-1722 (2003). **3.** Kim, J.H. et. al., Magnetic Resonance in Medicine 58:253-260 (2007) **4.** Deboy, C.A. et. al. Brain 130(8):2199-210 (2007)

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