Diffusion Anisotropy Reflects Progression of Spinal Cord Contusion Injury in Mice

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Introduction

Diffusion tensor imaging (DTI) derived parameters show great sensitivity to pathology of central nervous system white matter[1]. However, concerns have been raised regarding the effects of modest signal to noise ratio (SNR) upon the derived DTI eigenvalues[2]. Herein, we report relative anisotropy (RA) measurements of paraformaldehyde fixed mouse spinal cord ventrolateral white matter at various SNR. We then compare RA between *in vivo* and *ex vivo* mouse spinal cord ventrolateral white matter and gray matter. Finally, we present RA measurements *in vivo* and *ex vivo* of residual ventrolateral white matter in contusion injured mouse spinal cord at one and seven days post injury (DPI).

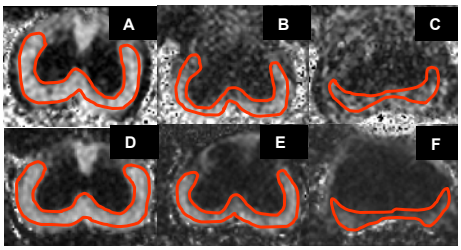

Fig. 1 Representative RA maps (A - *in vivo* normal, B – *in vivo* one DPI, C – *in vivo* seven DPI, D – *ex vivo* normal, E – ex vivo one DPI, F – *ex vivo* seven DPI). Red lines mark ROI containing residual white matter.

Methods

Five 16-week-old female C57BL/6 mice were employed for control group *in vivo* and *ex vivo* DTI measurements. For the lesion group, eight 16-week-old female C57BL/6 mice underwent severe contusion injury (2.0 mm impactor tip diameter, 0.9 mm displacement, impact time 20 ms) at vertebral segment T13 with an electromagnetic driven impactor. Injured animals were separated into two groups (n=4 for each) and *in vivo* DTI measurements were performed at one and seven days post injury (DPI). *In vivo* images were acquired with modified Stejskal-Tanner spin-echo diffusion-weighted sequence with diffusion-sensitizing factors b = 0 and .785 μm^2/ms while respiratory gating. Immediately following *in vivo* measurements, mice underwent left ventricle cardiac perfusion fixation procedure with 4% paraformaldehyde and *ex vivo* DTI measurements were conducted with the same parameters as *in vivo* with b = 1813 μm^2/ms to compensate for the decreased *ex vivo* ADC.

Results and Discussion

In vivo and *ex vivo* RA maps of normal and contusion injured mouse spinal cord (Fig.1) showed excellent contrast between gray and white matter even on injured cord. Region of interest (ROI) assessments were performed based on RA maps. There was no statistically significant difference in the *ex vivo* RA of ventrolateral white matter at a wide range of SNR (Table 1). *In vivo and ex vivo* RA (SNR = 50) from the gray and ventrolateral white matter of normal mice spinal cord was not statistically different (Table 2). Both *in vivo* and *ex vivo* measurements showed white matter / gray matter RA ratio of ~3.2. A slightly greater RA in T12 than T13 and L1 is also seen (Table 2).

ROI analysis of *in vivo* and *ex vivo* RA values from the residual ventrolateral white matter comparing the injured with the control was performed. In contusion injured white matter, both *in vivo* and *ex vivo* RA showed similar pattern of injury induced changes. The RA of residual ventrolateral white matter decreased over time whether examined *in vivo* and *ex vivo* after contusion injury. The impact site (T13) showed the lowest RA, presumably reflecting severe damage. The two adjacent sites (T12 and L1) showed insignificant decrease in RA at one DPI suggesting limited damage followed by significantly decreased RA at seven DPI reflecting significant progression of white matter injury toward sites adjacent to the impact epicenter. In summary: DTI SNR of 40 – 130 yields equivalent RA in mouse spinal cord ventrolateral white matter. Further, RA is equivalent whether determined *in vivo* or *ex vivo* (SNR = 50). The present study demonstrates that *in vivo* DTI of mouse spinal cord can be achieved at sufficiently high resolution. RA reflects progression of injury in the cord temporally and spatially.

SNR	RA (T12)	RA (T13)	RA (L1)
40 ± 1	0.94 ± 0.02	0.90 ± 0.02	0.88 ± 0.03
50 ± 2	0.94 ± 0.01	0.90 ± 0.03	0.89 ± 0.04
65 ± 1	0.94 ± 0.01	0.90 ± 0.03	0.89 ± 0.03
75 ± 1	0.94 ± 0.01	0.89 ± 0.03	0.89 ± 0.03
130 ± 5	0.92 ± 0.02	0.88 ± 0.02	0.86 ± 0.02

Table 1. RA of mouse spinal cord ventrolateral white matter at different SNR determined *ex vivo* (n=5).

RA (SNR=50)	*In vivo* White matter	Gray matter	Ratio (WM/GM)
T12	0.96 ± 0.05	0.29 ± 0.05	3.29±0.16
T13	0.91 ± 0.04	0.28 ± 0.04	3.30±0.09
L1	0.89 ± 0.04	0.27 ± 0.04	3.33±0.12
RA (SNR=50)	*Ex vivo* White matter	Gray matter	Ratio (WM/GM)
T12	0.94 ± 0.04	0.21 ± 0.04	3.23±0.05
T13	0.89 ± 0.05	0.20 ± 0.05	3.17±0.13
L1	0.89 ± 0.06	0.19 ± 0.06	3.14±0.09

Table 2. RA of normal mouse spinal cord gray and ventrolateral white matter (n=5).

References

1. Song *et al.*, *Neuroimage*, 20:1714-1722 (2003).
2. Bastin *et al.*, *Magn. Reson Imaging*, 16(7): 773-785 (1998).

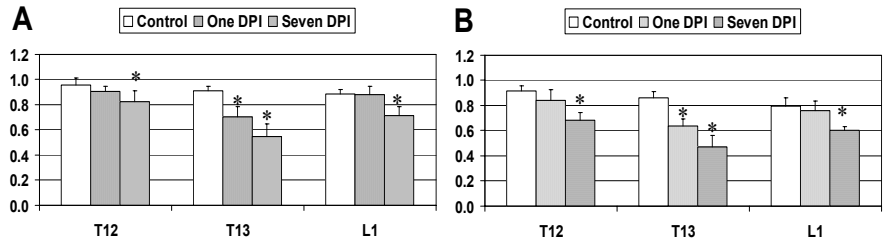

Fig. 2 *In vivo* (A) and *ex vivo* (B) RA of residual ventrolateral white matter from both control (white bar) and contusion injured mice at one (vertical lined bar) and seven (horizontal lined bar) DPI. T13 is the impact site. Asterisk denotes significantly different RA compared to control ($p < 0.05$).