Diffusion Anisotropy Is Not Changed in Formalin Fixed Injured White Matter

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Introduction

Formalin fixation of tissue is a ubiquitous laboratory practice. Diffusion tensor imaging (DTI) of fixed tissue is also widespread in both central nervous system development and pathology. Previously, we demonstrated that the diffusion anisotropy in formalin-fixed normal (1) and acute stroke mouse brain (2) is the same as that observed *in vivo*. However, the Tr(D) differential present *in vivo* between injured and healthy tissues was lost after formalin fixation. There was no apparent injury to the subcortical white matter in the acute stroke model employed. Thus, the effect of fixation on the previously proposed surrogate markers of axon and myelin injury ($\lambda_{\parallel}$ and $\lambda_{\perp}$ respectively) was not evaluated in the injured white matter. To test the utility of $\lambda_{\parallel}$ and $\lambda_{\perp}$ in the fixed, injured white matter, optic nerves of mice undergoing transient retinal ischemia were examined using both *in vivo* and *ex vivo* DTI.

Materials and Methods

Retinal ischemia

Eight male Swiss Webster mice, 6 – 8 weeks of age, underwent the retinal ischemia preparation. Briefly, the intraocular pressure (IOP) of the right eye of each mouse was raised above systolic blood pressure by cannulation of the anterior chamber with a 32-gauge needle connected to a saline reservoir placed above the eye resulting in the applied pressure of 100 – 120 mmHg. The elevated IOP was maintained for 65 minutes. Ischemia was confirmed by ophthalmoscopic observation of the blanched fundus. The contralateral eye, which serves as the control, was not cannulated. Reperfusion started immediately after removal of the cannula. Optic nerves were examined using *in vivo* and *ex vivo* DTI at 3 to 14 days after the surgery to assess the extent of axonal and myelin damages (3).

Diffusion Tensor Imaging

A conventional spin-echo diffusion imaging sequence was employed for acquisition of the required series of diffusion-weighted images (DWI). The *in vivo* DWIs were acquired with TR 0.7 sec, TE 50 msec, Δ 25 msec, δ 10 msec, NEX 16, slice thickness 0.5 mm, field-of-view 3 cm, and data matrix 256×256 (zero filled to 512× 512). Diffusion sensitizing gradients were applied along six directions: [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 (0 and 0.768 ms/μm^2) were used. The same spatial resolution and diffusion weighting parameters were employed for *ex vivo* measurements. Three quantitative indices including RA, axial diffusivity ($\lambda_{\parallel} = \lambda_1$), and radial diffusivity ($\lambda_{\perp} = (\lambda_2 + \lambda_3)/2$) were measured in injured and healthy optic nerves (ON). The change (Δ) of parameters caused by injury is defined as (injured ON value – healthy ON value) / healthy ON value, where values include RA, $\lambda_{\parallel}$ and $\lambda_{\perp}$. Wilcoxon matched-pairs signed-ranks tests were performed to compare in vivo and ex vivo ΔRA, $\Delta\lambda_{\parallel}$ and $\Delta\lambda_{\perp}$. $P < 0.05$ was considered significant difference.

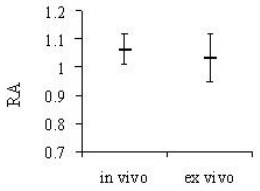

Fig. 1

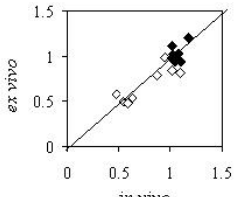

Fig. 2

Results

Consistent with previous study, no significant difference was observed between *in vivo* and *ex vivo* RA of healthy ON as shown in Figure 1 with the slightly larger variance in *ex vivo* than that of *in vivo*. The scatter-plot of *in vivo* and *ex vivo* RA from both normal (solid squares) and injured (open squares) ON is shown in Fig. 2. All data points are distributed close to the line of identity.

ΔRA, $\Delta\lambda_{\parallel}$, and $\Delta\lambda_{\perp}$ are affected by the severity of the injury in the examined ON. The scatter plots between *in vivo* and *ex vivo* ΔRA, $\Delta\lambda_{\parallel}$, and $\Delta\lambda_{\perp}$ are shown in Fig. 3. Based on the paired tests, no statistically significant difference was observed between *in vivo* and *ex vivo* of these indices. However, *ex vivo* $\Delta\lambda_{\parallel}$ has the tendency of underestimating that determined *in vivo*. In contrast, both *ex vivo* ΔRA and $\Delta\lambda_{\perp}$ seem to agree with that determined by *in vivo* measurements more closely.

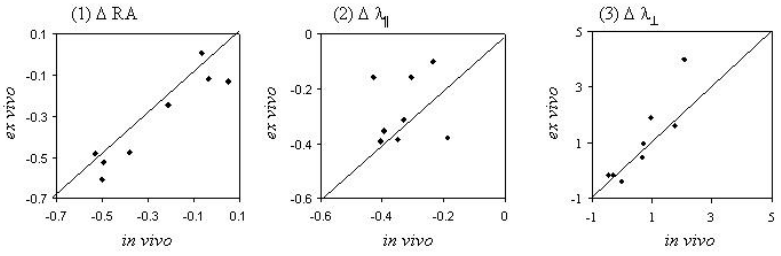

Fig. 3

Discussions and Conclusions

Diffusion anisotropy is preserved in both healthy and injured mouse optic nerves. There is no statistically significant differences observed between *in vivo* and *ex vivo* ΔRA, $\Delta\lambda_{\parallel}$, and $\Delta\lambda_{\perp}$. However, the magnitude of *ex vivo* $\Delta\lambda_{\parallel}$ seems to be much less than those of *in vivo* in a sub-group of animals. The water ADC (mean diffusivity) decreased by 50 – 70% from its *in vivo* values on gray and white matter (1). Thus, it is possible that the significantly reduced ADC measured by *ex vivo* DTI may mask the smaller decrease in $\lambda_{\parallel}$ resulting from injury. This would reduce the sensitivity of using diffusion to detect axonal injury *ex vivo*, similar to the loss of Tr(D) contrast in stroke brain (2). In contrast, the increased $\lambda_{\perp}$ caused by demyelination seems to be preserved well after fixation. Thus, the extent of demyelination assessed by *ex vivo* $\Delta\lambda_{\perp}$ seems still appropriate. In general, *ex vivo* DTI of fixed tissues is a valid practice to investigate white matter pathology. However, the detailed interpretation of the underlying pathology should be practiced with caution.

References

(1) Sun et al., MRM, 2003; 50: 743 – 8.
(2) Sun et al., MRM, 2004; Submitted.
(3) Song SK, et al., Neuroimage 2003; 20:1714-22.