Axonal Injury Is Detected By Postmortem DTI Before Fixation (But Not After)

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Introduction

Axial ($\lambda_{\parallel}$) and radial ($\lambda_{\perp}$) diffusivities derived from diffusion tensor imaging (DTI) have been demonstrated as surrogate markers of axonal and myelin damage in the central nervous system. Recently, the reduced sensitivity of *ex vivo* $\lambda_{\parallel}$ in detecting axonal damage in fixed tissue has been reported (1, 2). It is hypothesized that structural changes caused by formalin fixation reduces the sensitivity of *ex vivo* $\lambda_{\parallel}$ to detect the axonal injury. In this study, the comparison of *in vivo* and *ex vivo* DTI was conducted on live and dead (without formalin fixation) mouse brain. After *in vivo* DTI measurements, euthanasia was performed *in situ* by over dose of inhalant anesthetics, and postmortem DTI was conducted. The current findings suggest that $\lambda_{\parallel}$, $\lambda_{\perp}$, and diffusion anisotropy are sensitive to detect the underlying pathology in mouse optic nerve both *in vivo* live and *in situ* after death.

Materials and Methods

Retinal ischemia

Six male Swiss Webster mice, 6 – 8 weeks of age, underwent the retinal ischemia preparation (3). 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 one hour. 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. At 14 days after the ischemia, optic nerves were examined using *in vivo* DTI.

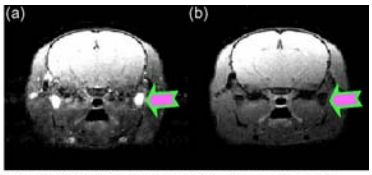

Fig. 1 T1WI (a) before and (b) after euthanasia.

Diffusion Tensor Imaging

Data were acquired using spin-echo diffusion weighted imaging sequence with TR 1.6 sec, TE 50 msec, Δ 25 msec, δ 8 msec, NEX 4, 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. At the conclusion of in vivo DTI, euthanasia was performed by increasing the isoflurane to 7%. A T1-weighted spin-echo image (T1WI with TR 0.5 s and TE 20 ms) was conducted to confirm the death by checking the blood vessel signals. After the death, DTI was repeated twice at 3 and 6 hrs after death. Relative anisotropy (RA), $\lambda_{\parallel}$, and $\lambda_{\perp}$ were measured in control and injured optic nerves and compared between the measurements *in vivo* live and *in situ* after death. Paired t-test was performed, and $p < 0.05$ was considered significant.

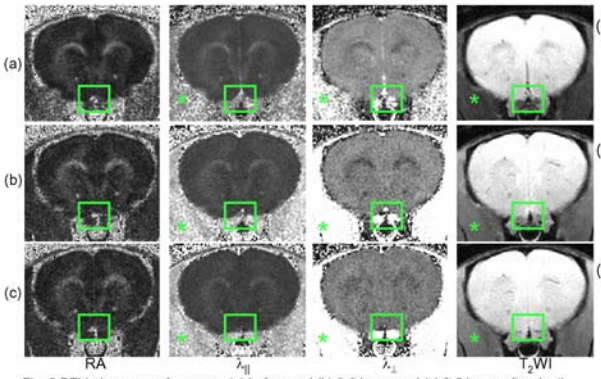

Fig. 2 DTI index maps of a mouse (a) before and (b) 0-3 hours and (c) 3-6 hours after death.

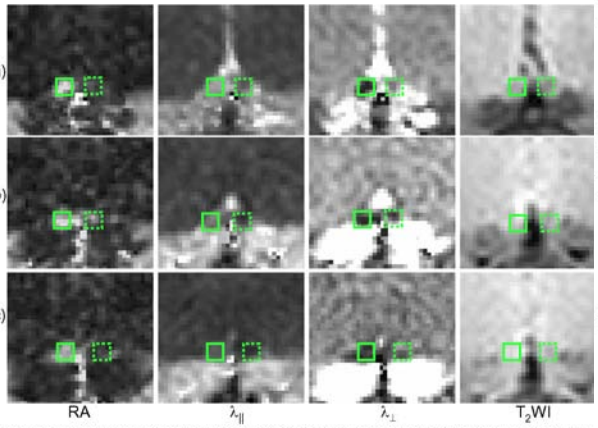

Fig. 3 DTI index maps of control (solid rectangles) and injured (dashed rectangles) optic nerv (a) before and (b) 0-3 hours and (c) 3-6 hours after death.

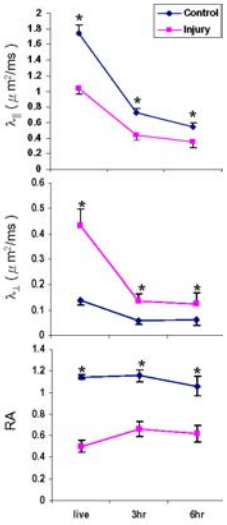

Fig. 4 Measured $\lambda_{\parallel}$, $\lambda_{\perp}$, and RA

Results

The euthanasia is confirmed by the stop of blood flow examined by T1WI (Fig. 1). The bright blood vessel becomes dark suggestive of the cessation of blood flow. The DTI maps of the mouse brain *in vivo*, and *in situ* 0-3 and 3-6 hours after death are shown in Fig. 2. Comparable RA maps are observed among all time points. The image contrasts in $\lambda_{\parallel}$, $\lambda_{\perp}$, and T2WI between the brain and muscle (* in Fig. 2) changed after death. The expanded view of the optic nerve (Fig. 4) shows that injured optic nerve with reduced RA, reduced $\lambda_{\parallel}$, and increased $\lambda_{\perp}$ comparing with those of the uninjured controls throughout all time points examined. The quantitative measurements (Fig. 4) reveal that $\lambda_{\parallel}$ and $\lambda_{\perp}$ in both control and injured optic nerves decreased 50-70% after death. However, significant differences in between injured and control optic nerves are preserved.

Discussions and Conclusions

In this study, DTI was conducted on live and dead mice without fixation. Although the diffusion coefficients decreased about 60% in both control and injured optic nerves after death, the lesion contrast is preserved. The present data suggests that without the effect of formalin fixation the sensitivity of $\lambda_{\parallel}$ to the axonal damage is not affected. The effect of tissue fixation on DTI parameters requires further examination before the use of fixed tissue as the model system for investigating *in vivo* morphology and pathology.

References

(1) Sun et al., MRM, 2005; 53: 1447 – 1451.
(2) Sun et al., MRM, 2005; in press.
(3) Song et al., Neuroimage, 2005; 26: 132 – 140.
(4) Song et al., Neuroimage 2003; 20:1714-22.