Axial Diffusivity Detects Axonal Injury Live and Postmortem Before but not After Formalin Fixation

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Introduction

Axial ($\lambda_{\parallel}$) and radial ($\lambda_{\perp}$) diffusivities derived from diffusion tensor imaging (DTI) have been demonstrated as potential biomarkers of axonal and myelin damage in the central nervous system. However, reduced sensitivity of ex vivo DTI in detecting axonal damage of formalin fixed tissue have been reported previously (1, 2). In particular, decreased $\lambda_{\parallel}$ in detecting the acute axonal damage of optic nerve (ON) 3 days after the transient retinal ischemia was preserved after the formalin fixation. In contrast, chronic axonal damage (at 14 days after retinal ischemia) detected *in vivo* as a significant decrease of $\lambda_{\parallel}$ in live brains was not seen after formalin fixation. It is unclear whether this sensitivity difference is a result of death or fixation. In this study, DTI indices acquired from live, in situ postmortem, and ex vivo after formalin fixation of the injured ON were compared.

Materials and Methods

Retinal ischemia

Twelve male Swiss Webster mice with 6 – 8 weeks of age were used. One hour transient retinal ischemia was induced in the right eye (3). The left eye served as the control. At 3 and 14 days after the ischemia, 6 mice were randomly selected to perform the DTI in vivo, in situ postmortem, and ex vivo after formalin fixation.

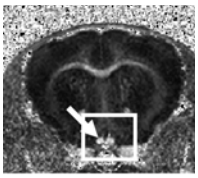

Fig.1 RA of ON

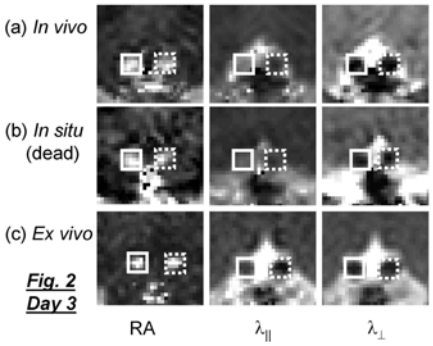

Fig. 2 Day 3

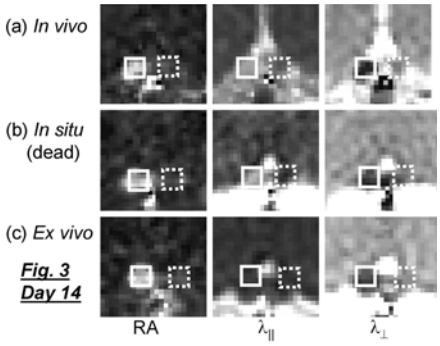

Fig. 3 Day 14

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. 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 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 as evidenced by the decreased iamge intensities of the blood vessels. After the death, DTI with acquisition parameters identical to the previous DTI was repeated twice. Subsequently mouse brains were dissected and immersed in 10% formalin at 4˚C for two weeks and then examined by ex vivo DTI. Relative anisotropy (RA), $\lambda_{\parallel}$, and $\lambda_{\perp}$ were measured in control and injured ON from in vivo, in situ postmortem, and ex vivo DTI respectively. Paired t-test was performed to compare the measurements between injured and normal ON. $p < 0.05$ was considered significant.

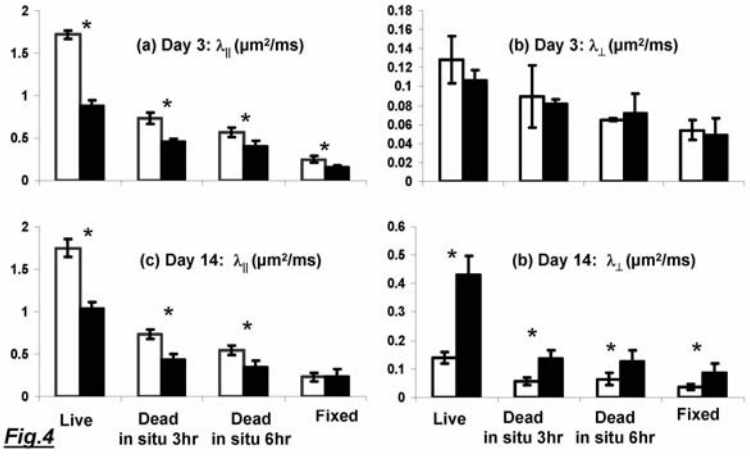

Fig.4

Results

Representative RA maps of ON from a normal mouse was shown in Fig. 1. Maps of RA, $\lambda_{\parallel}$, and $\lambda_{\perp}$ of injured ON from DTI in vivo, in situ postmortem, and ex vivo after formalin fixation were shown in Fig. 2 (3 days after ischemia) and Fig. 3 (14 days after ischemia), where dashed rectangles indicate the injured ON. In Fig. 2, decreased $\lambda_{\parallel}$, suggestive of axonal damage, was seen in vivo, in situ postmortem, and ex vivo. The measurements of regions selected in both injured and normal ON were summarized in Fig. 4. The injured ON exhibited decreased $\lambda_{\parallel}$ and increased $\lambda_{\perp}$ *in vivo* and *in situ* postmortem. However, $\lambda_{\parallel}$ is unable to distinguish the injured from normal optic nerves *ex vivo* after fixation at day 14. Quantitatively, comparing to *in vivo* measurements, $\lambda_{\parallel}$ decreased 58 ± 4% and 69 ± 3% in 3 and 6 hours after death *in situ* in the control eye. Similar decrease in $\lambda_{\parallel}$ was also found in the injured ON. However, after fixation, *ex vivo* $\lambda_{\parallel}$ decreased with different extent between normal and injured ON. As a result, the contrast of $\lambda_{\parallel}$ between injured and normal ON remained at 3 days but not at 14 days after injury. In contrast, *in situ* (3 and 6 hours after death), and *ex vivo* $\lambda_{\perp}$ showed 55 ± 9%, 57 ± 8%, and 73 ± 6% decreases in normal ON and 57 ± 9%, 61 ± 7%, and 75 ± 7% decreases in injured ON comparing to the *in vivo* measurements of the control. Consequently, the increase of $\lambda_{\perp}$ (about 2-fold increments over normal ON) remained *in vivo*, *in situ* postmortem and *ex vivo*.

Discussions and Conclusions

Reduced sensitivity of ex vivo DTI in detecting axonal damage of formalin fixed tissue have been reported previously. This study demonstrated that apparent diffusion coefficients decreased about 60-80% in both control and injured ON after death. The lesion contrast detected as the decreased $\lambda_{\parallel}$ for identifying axonal damage was preserved in the postmortem DTI before but not after formalin fixation.

References

(1) Sun et al., MRM, 2005; 53: 1447 – 1451.
(2) Sun et al., Neuroimage, 2006; 32: 1195-1204.
(3) Song et al., Neuroimage, 2005; 26: 132 – 140.
(4) Song et al., Neuroimage 2003; 20:1714-22.

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