A Window for High-Resolution Post-Mortem DTI: Mapping Contrast Changes in Neural Degeneration

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Introduction

High-resolution, post-mortem MRI has provided stunning structural images of cerebral anatomy that are not possible *in vivo* due to motion and rapid imaging artifacts. The reliability and accuracy of post-mortem diffusion tensor imaging (DTI) measurements, however, are not well understood, in part due to the unknown effects of fixation (or lack thereof) on the brain's diffusion properties. In animal studies that used peri-mortem perfusion fixation (rapid introduction of a fixative agent through the capillary beds before death [1]), it was found that mean diffusivity (MD) is altered but fractional anisotropy (FA) is unchanged [2]. With immersion fixation, Pfefferbaum et al. reported an inability to achieve diffusion contrast, though subsequent research with low resolution and thick slices yielded some success, but low diffusion contrast to noise ratio [3,4].

Routine post mortem processing typically involves 24 to 48 hours post-mortem delay before fixation begins. Additionally, brain dead and cerebral ischemia patients are common donors, which may involve an additional 24 to 72 hours of pre-mortem cessation of cerebral vascular perfusion. Because neural tissue degrades relatively quickly *in situ* and *ex vivo* without fixation, DTI contrast is largely lost in these subjects. In cases of highly motivated donors – e.g., those with a terminal condition and a status of "do not resuscitate" and "do not intubate" – it is possible to acquire DTI data within a much shorter time period. But how soon is "soon enough?"

We use a porcine model to study the acute time course (<18 hours) of neural tissue degradation and response to immersion fixation to enable the design of rapid protocols for post-mortem DTI studies.

Methods

Intact cerebra were removed from two porcine specimens (~25 kg, 3-4 months) approximately 1 hour post-mortem. The left frontal lobe from the first specimen was serially MR imaged without fixation or added fixatives (the "degradation study"). The right frontal lobe of the first specimen and both frontal lobes of the second specimen were excised and immersed in lactated Ringers solution in a water bath at 30 °C. At hourly intervals, a 5 mm section was cut from each lobe, immediately immersed in 4% paraformaldehyde (PFA), and refrigerated at 4 °C for 6 weeks, and then MR imaged (the "delayed fixation study"). All procedures were conducted in accordance with institutional animal care and use regulations. DTI was performed on a 11.7T MR scanner at a constant temperature of 30 °C and consisted of sequential 2-D multi-slice, multiple spin echo RARE DTI sequences (four echoes TE= 24.41/34.67/44.93/55.19 ms, TR=2300 ms, two signal averages, nominal resolution 0.18x0.18x1 mm). Diffusion contrast was achieved with a six direction dual gradient diffusion weighting scheme with a b-value of 1000 s/mm^2 [5]. Each DTI sequence required approximately 65 minutes. Minimally weighted (b0) volumes were classified into gray matter (GM), white matter (WM), and cerebrospinal fluid/artifact categories with a semi-automated method. Visualization and statistical analysis of DTI contrasts were computed in Matlab. Histological findings and correlations will be assessed separately.

Results and Discussion

Contrast changes are apparent in both the serial MR scanning of fresh (**Fig. 1A/B**) and fixed specimens (**Fig. 1C/D**). In the degradation study, FA decreased by 0.05 [FA] (9.0%) in WM and 0.02 [FA] (6.2%) in GM, while MD decreased by 0.067x10^{-3} mm^2/s (14.3%) and 0.004x10^{-3} mm^2/s (0.6%) between the first (t=2.97 hrs) and last (t=17.1 hrs) scans. Immersion fixation resulted in a mean FA reduction of approximately 35% in WM and 30% in GM. Simultaneously, mean MD was reduced by approximately 51% in WM and 56% in GM. Between the first (1 hr) and last (13 hrs) fixed sample in the delayed fixation study, FA decreased by 0.07 [FA] (16.0%) in WM and 0.04 [FA] (15.6%) in GM, while MD decreased by 0.022x10^{-3} mm^2/s (9.0%) and 0.03x10^{-3} mm^2/s (10.5%), respectively. A progressive decrease in axial diffusivities (**Fig. 2A)** in both WM and GM leads to reductions in FA and MD. The radial diffusivities are relatively spared.

In the delayed fixation study, the differential loss of anisotropy in GM relative to WM provides an explanation as to why some previous studies have been unable to demonstrate diffusion anisotropy with clinically derived protocols [3]. The within tissue compartment decrease in SNR with delayed fixation indicates that the changes in MD and FA are not uniform, which suggests increasingly uneven tissue degeneration over time even with a short (13 hour) window. Delaying fixation negatively impacts the achievable FA contrast to noise ratio through (1) overall loss of diffusivity, (2) increased loss of diffusivity in WM relative to GM, and (3) increased intra-tissue compartment variability (**Fig. 2B**).

In conclusion, we demonstrate that a practical window of up to 6-10 hours exists where high resolution, post-mortem DTI may be performed while contrasts are relatively preserved. In this time period, the relationship between the parallel diffusivities of GM and WM are relatively intact. Immersion fixation did not well preserve DTI contrasts even in this fairly idealized (thin tissue section) model.

Fig. 1. Fractional Anisotropy

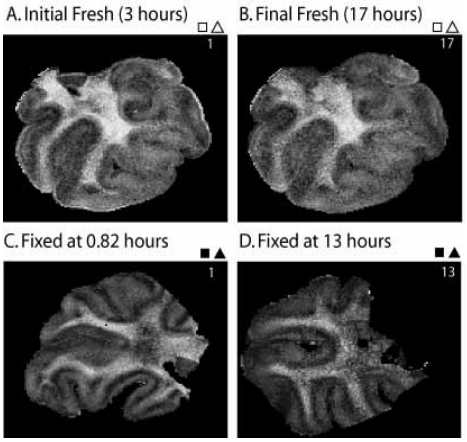


Fig. 2A. Degradation Anisotropies

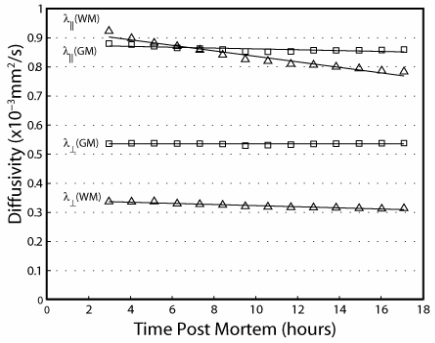
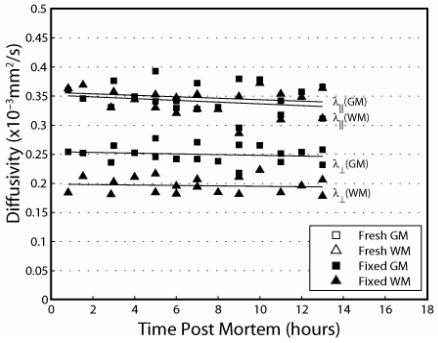


Fig. 2B. Serial Fixation Anisotropies



References: [1] Adickes ED, et al., Arch Pathol Lab Med 1997;121:1199 [2] Sun SW, et al., MRM 2005;53(6):1447 [3] Pfefferbaum A, et al., Neuroimage 2004;21(4):1585 [4] Englund, E., et al., 2004 J Neurol 251(3):350 [5] Pierpaoli C, et al., Radiol 1996;201(3):637