Postmortem Interval Alters the Water Relaxation and Diffusion Properties of Nervous Tissue – Implications for High Resolution MRI of Human Autopsy Samples

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INTRODUCTION

High-resolution MRI characterizations of diffusion anisotropy in formaldehyde-fixed autopsy tissue samples may better characterize cytoarchitecture in normal and injured human nervous tissue than *in vivo* samples. Unfortunately, logistics and patient family needs dictate that autopsy tissues are usually obtained following a 4+ hour postmortem interval (PMI). The stigmata of PMI can be seen in routine histology of these samples, but the impact of cellular autolysis that occurs during the PMI on MRI contrast mechanisms remains poorly understood. Previous studies have characterized the impact of fixative on the MRI properties of nervous tissue [1]. This study tested the hypothesis that PMI significantly alters MRI of neuronal tissue by characterizing the impact of PMI's from 0 - 36 hours on the T_1, T_2 and water diffusion properties of rat cortical slices. Water diffusion was analyzed using a two-compartment model with exchange that estimated the apparent diffusion coefficient (including tortuosity effects) in the extracellular space (ADC_{ex}), the mean restriction size (a), the transmembrane exchange rate (k) and the intracellular magnetization fraction (V_{in}). This study also compared differences in these MRI properties between cortical slices that were perfusion- or immersion-fixed in 4% formaldehyde.

METHODS

Coronal rat cortical slices were procured from 5 rats [1], then left undisturbed inside sealed, humidified chambers until being removed and immersion-fixed in a >50:1 volume excess of 4% formaldehyde in isotonic PBS (pH 7.4, 300 ± 1 mOsm/kg) at postmortem intervals of 0, 2, 4, 8, 12, 24 or 36 hrs. In addition, rat cortical slices were prepared from 3 transaortic perfusion-fixed rats. Slices were stored for 10+ days prior to MRI data collection, then washed for 12 hrs in PBS and placed into a multi-slice perfusion chamber [2] for MRI using a 14.1-T vertical, narrow-bore magnet with a 10-mm Helmholtz pair coil. Diffusion measurements at 5 diffusion times (T_d) along with T_1 and T_2 measurements [1] were acquired with low in-plane resolution (128 x 64 matrix, 1.5 cm x 1.5 cm FOV) to improve signal-to-noise. Diffusion measurements employed a standard PGSE sequence for 12 diffusion-weighted images (*b*-values = 7 - 15,000 s/mm^2) at T_d's of 10, 20, 30, 45 and 60 ms (δ = 3 ms) (NEX = 2, TR = 1.5 s, TE ranging from 23.3 to 72.3 ms). T_1 and T_2 measurements employed partial saturation (TR = 150 ms - 10 s) and multi-echo sequences (TE = 10 - 300 ms) respectively [1]. Scan time per treatment group was approximately 200 min. A two-compartment diffusion model with trans-membrane water exchange that assumes restricted diffusion in the intracellular space and extracellular water diffusion mediated by tortuosity [3] was fitted to the MRI data. Model, T_1 and T_2 fits were compared statistically using a 1-way ANOVA and Tukey multiple comparisons tests.

RESULTS

Multiecho, saturation recovery and diffusion MRI data from rat cortical slices had excellent mean SNR (e.g. 16.1 ± 1.1 for diffusion-weighted MRI with T_d/TE = 60/72.3 ms, *b* = 15005 s/mm^2). Further, the mean difference between experimental data and the fitted two-compartment model was less than 1%. Several differences between perfusion- and immersion fixation of rat cortical slices were noted; mean rat cortical slice proton density, T_1 and T_2 were 30%, 8% and 21% higher respectively ($P < 0.001$) in immersion-fixed samples (Fig. 1). Further, compared to perfusion fixed slices, fixation by immersion reduced a by 16%, k by 35%, V_{in} by 20% (all, $P < 0.001$) and ADC_{ex} by 16% ($P = 0.064$)(Table 2). Slice T_1 and T_2 also both increased significantly with immersion fixed slices of lengthening PMI (Fig. 1), where the majority of the changes occurred within the first 4 hours (13% and 34% respectively) ($P < 0.001$). After 24 hrs, immersion-fixed slice T_1 and T_2 had increased from baseline by 20% and 52% respectively ($P < 0.001$). Water diffusion also changed significantly with increasing PMI (Table 1). At 4 hours, k decreased 26% ($P < 0.001$) and V_{in} had increased 25% ($P = 0.002$). Compared to baseline, 24 hrs PMI demonstrated a 38% increase in ADC_{ex}, a 26% increase in k and a 39% increase in V_{in} ($P < 0.001$).

DISCUSSION

Because all human autopsy tissues are formaldehyde-fixed prior to study, MRI data from unfixed rat cortical slices were not collected for these experiments, but the impact of different chemical fixatives compared to viable, unfixed rat cortical slices have been described previously [1]. To maintain clinical validity, the experimental conditions were designed to mimic the most likely conditions of human nervous tissue obtained for high resolution MRI postmortem studies i.e. fixed in 4% formaldehyde with a varying length of PMI. It was surprising that simply switching from perfusion to immersion fixation at 0-hours PMI significantly increased the T_1 and T_2, while significantly decreasing the mean restriction size (a), the transmembrane exchange rate (k) and the intracellular magnetization fraction (V_{in}) of rat cortical slices – these changes may relate to the rapid and complete penetration of fixative when perfused through tissue vasculature and to the potential agonal changes that occur during slice/tissue procurement when tissue is immersion-fixed. Significant changes were also noted to the relaxation and diffusion properties of rat cortical slices with increasing PMI. These changes are best attributed to the ischemic conditions experienced by tissue after cessation of perfusion, which ultimately lead to autolytic biochemical cascades like the activation of proteolytic enzymes such as calpain or caspases.

This study demonstrated important MRI contrast changes to nervous tissue procured and chemically-fixed following a donor's demise, and suggests human autopsy samples may have circumscribed validity for *in vivo* MRI, even with relatively short PMIs. Further, the MRI properties of nervous tissue fixed in 4% formaldehyde differ significantly depending on whether the tissue is perfusion-fixed or immersion-fixed. These differences should be considered when comparing perfusion-fixed nervous tissue samples from animal models of disease to MRI data collected from immersion-fixed human autopsy samples or clinical data.

ACKNOWLEDGEMENTS

1. Shepherd et al. ISMRM 13:619 (2005). 2. Shepherd et al. MRM 48:565-569 (2002). 3. Li et al. MRM 40:79-88 (1998). Funded by NIH RO1 NS36992 & P41 RR16105. Thanks to Dan Plant for technical assistance.

Figure 1: T_1 and T_2 of immersion-fixed tissue at 0, 4 and 24 h PMI, and for perfusion-fixed tissue [mean ± SD]

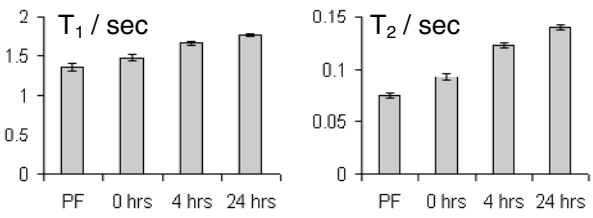

Table 1: Fitted model parameters for immersion-fixed tissue at 0, 4 and 24h PMI, and for perfusion-fixed tissue [mean ± SD]

Postmortem interval	N	ADC_{ex} (μm^2/ms)	a (μm)	k (s^{-1})	V_{in} (no units)
PF	8	0.62 ± 0.10	2.4 ± 0.3	140 ± 14	0.45 ± 0.10
0 hrs	8	0.52 ± 0.04	2.0 ± 0.1	91 ± 3	0.36 ± 0.03
4 hrs	8	0.60 ± 0.07	2.1 ± 0.2	68 ± 3	0.45 ± 0.02
24 hrs	7	0.72 ± 0.03	2.2 ± 0.1	67 ± 2	0.50 ± 0.01
1-way ANOVA		<0.001	0.003	<0.001	<0.001