Post-mortem MRI of Human Brain Hemispheres: Effects of Formaldehyde Fixation on T2 Relaxation

R. J. Dawe[1], D. A. Bennett[2], J. A. Schneider[2], S. K. Vasireddi[1], and K. Arfanakis[1]

[1]Department of Biomedical Engineering, Illinois Institute of Technology, Chicago, IL, United States, [2]Rush Alzheimer's Disease Center, Rush University Medical Center, Chicago, IL, United States

Introduction: Postmortem MRI of the human brain allows for invasive examination of the tissue specimen immediately following the MR scan, a practice that is not possible with living subjects. An increasing number of researchers have exploited this opportunity, correlating MR results from cadaveric brains with the findings of histological examination[1]. Ultimately, these correlations may provide a means of using MRI to probe the tissue microstructure in the brains of living patients, allowing for clinical diagnosis of various neurological diseases. Although postmortem imaging of the human brain has potential advantages in research, it also presents new challenges that have not been dealt with for in vivo imaging. In particular, the MRI-sensitive properties of postmortem tissue can change rapidly as a result of decomposition, chemical fixation, or changes in water content, causing misinterpretation of MRI results[2]. The purpose of this study was to characterize the T_2 relaxation changes that occur in cadaveric human brain hemispheres that have been chemically fixed by immersion in formaldehyde. The findings of this work suggest that it takes up to three months for T_2 values of deep tissue to stabilize after death and fixation of the brain hemisphere.

Methods: Within 5 hours postmortem, five human brain hemispheres were removed from cadavers, immersed in 4% formaldehyde solution, and refrigerated at 4° C for at least three days. While still immersed in formaldehyde solution, the hemispheres were imaged on a weekly basis for three months using a 3.0-T GE MRI scanner (General Electric, Waukesha, WI), with a follow-up scan at six months postmortem. A 2D fast spin echo sequence with two echo times was used to acquire proton density weighted and T2-weighted images, in sagittal slices through the hemispheres. The following parameters were used: TR=3.6 s, TE1=13.0 ms, TE2=52.0 ms, FOV=16×16 cm, slice thickness=1.5 mm, acquisition matrix=256×256 zero-padded to 512×512, NEX=6. Total scan time was 31 minutes. T_2 maps were generated for each scan by calculating T_2 values on a voxel-by-voxel basis. For each subject, the T_2 volumes were normalized to the T_2 volume from the first timepoint using non-rigid body registration. Regions of interest (ROIs) were selected at locations near the surface (in grey matter) and in deep tissue (in white matter) for each hemisphere (Fig. 2C) and copied to the normalized versions of the T_2 volumes from all timepoints. The mean T_2 value and standard deviation of each ROI were calculated, and plots of T_2 versus time were constructed. A least squares approach was used to fit the data to a model of T_2 values as a function of time. The times at which T_2 reached a minimum and a plateau (95% of asymptotic value) were estimated. Additionally, T_2 difference maps were created by subtracting one T_2 volume from a T_2 volume of an earlier timepoint.

Results: No significant change was observed in the mean T_2 values of ROIs located near the surface of the hemispheres during the six months of this study (Fig. 1). Instead, these T_2 values appeared to randomly fluctuate from week to week. It is apparent that the T_2 values of ROIs near the surface of the hemispheres decreased from an in vivo value[3] of approximately 100 ms to the observed value of approximately 40 ms, but this reduction occurred prior to the first scan. In contrast, ROIs selected near the middle of each hemisphere exhibited a reduction in T_2 from approximately 55 ms to 30 ms in the first 2 weeks of scanning (Fig. 1). T_2 values then gradually rose to a plateau of approximately 40 ms over a period of 4 to 8 weeks. The changes in T_2 over time were well described by functions of the form $T_2 = A \cdot \exp(-k_1 \cdot t) + B \cdot [1 - \exp(-k_2 \cdot t)]$, where t is time postmortem. Student's t-tests revealed that in the deep tissue, the initial decrease was statistically significant in the deep tissue of all five hemispheres ($p < .001$), as was the subsequent gradual increase ($p < .002$). These results were also visible in the T_2 difference maps (Fig. 2).

Discussion: The T_2 decrease that occurred at locations near the surface of the hemispheres prior to the first scan and was observed in deep tissue in the first few scans can be attributed to protein cross-linking induced by formaldehyde[4]. The cross-links confer rigidity to the tissue, reducing the mobility of water molecules[5] and reducing T_2 values. In tissue near the surface of the hemispheres, the sharp T_2 decrease occurs within days after death, but at deep locations, up to 46 days are required for T_2 to reach a minimum. This delay can be explained by the fact that the fixative agent takes longer to reach deep locations because of greater distance as well as consumption of formaldehyde molecules. We hypothesize that even after some formaldehyde reaches deep locations of the hemisphere, the fixative may not be present in sufficient quantity to cause abundant protein cross-linking. Therefore, additional decomposition continues and allows water molecules to become more mobile, leading to increases in T_2 at these deep locations. Of the five hemispheres examined in this work, a maximum of 96 days postmortem were required for T_2 values to stabilize at the deepest locations (Table 1). Therefore, in studies that combine histology and postmortem MR imaging of human brain hemispheres, it is crucial to take into account the time dependent and location dependent variations of the MR properties of the tissue.

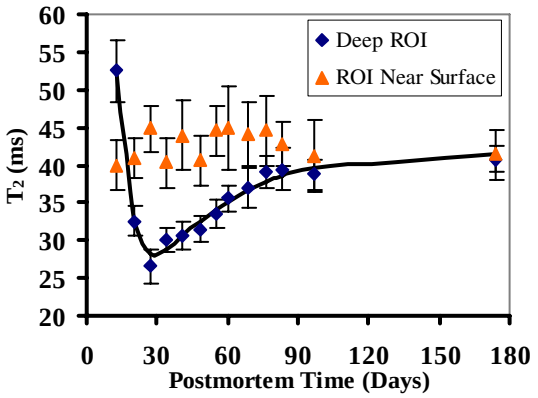

Figure 1. Typical T_2 timecourses for ROIs selected near the surface of the hemispheres (▲) (in grey matter) and in deep tissue of the hemispheres (♦) (in white matter). The solid line represents the least squares fit of the function $T_2(t) = A \cdot \exp(-k_1 \cdot t) + B \cdot [1 - \exp(-k_2 \cdot t)]$. Error bars show the standard deviation of the measurements.

Hemisphere	Time to T_2 Minimum	Time to Stable T_2	Asymptotic T_2
1	29 days	96 days	41.5 ms
2	46	77	31.7
3	32	78	39.9
4	23	56	46.1
5	32	88	36.0
Average	32 ±9	79 ±15	39.1 ±5.5

Table 1. For each hemisphere, the postmortem times for T_2 to reach a minimum and stable value (95% of asymptote) are shown. The asymptotic value of T_2, or the value to which T_2 stabilized after 6 months postmortem, is also shown.

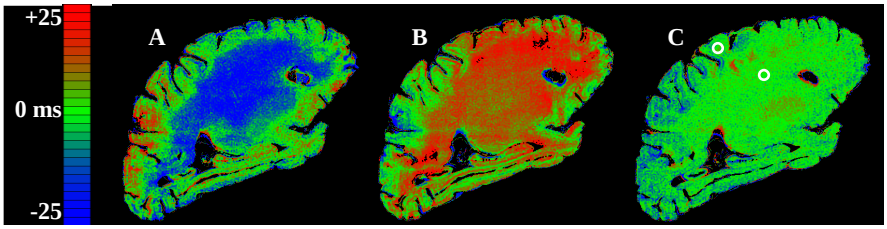

Figure 2. T_2 difference maps between (A) 13 and 27 days postmortem, (B) 27 and 76 days, and (C) 76 and 174 days. Areas of T_2 decrease, increase, and no change are indicated by blue, red, and green, respectively. The white circles in (C) represent typical ROIs at locations near the surface of the hemisphere and in deep tissue.

References:

[1] Bobinski M, et al., Neuroscience 2000;95:721-725. [2] Yong-Hing CJ, et al., MRM 2005;54:324-332. [3] Wansapura JP, et al., JMRI 1999;9:531:538. [4] Puchtler H and Meloan SN, Histochemistry 1985;82:201-204. [5] Kennan RP, et al., J Magn Reson 1996;110:267-277.