Magnetic Resonance Imaging and Mathematical Modeling of Progressive Formalin Fixation of the Human Brain

C. J. Yong-Hing[1], A. Obenaus[2], R. Stryker[3], K. Tong[4], G. E. Sarty[5]

[1]Diagnostic Imaging, University of Alberta, Edmonton, Alberta, Canada, [2]Department of Radiation Medicine, Loma Linda University, Loma Linda, California, United States, [3]Division of Nephrology, Department of Medicine, University of Ottawa, Ottawa, Ontario, Canada, [4]Department of Radiology, Loma Linda University, Loma Linda, California, United States, [5]Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Introduction

Post-mortem magnetic resonance (MR) imaging is widely used for pathologic diagnosis and research. The temporal changes in T_1 relaxation, apparent diffusion coefficient (ADC) and proton density (PD) values of human brain tissue after formalin fixation have never been formally reported. Several investigators have observed but never adequately explained a lighter band of tissue, apparently related to the diffusion of formalin into the brain(1). If not taken into consideration, such changes may lead to artificial results and misdiagnosis. This study investigates how MR appearance and parameters of brain tissue change with formalin fixation over time and finds a plausible physical explanation of the lighter "formalin band'' seen in T_1 weighted images by fitting the data to a mathematical model of formalin diffusion.

Materials and Methods

Coronal MR images of three human brains were acquired before and during formalin fixation at multiple time points with a 1.5T imager using T_1-weighted, multiple echo T_2-weighted, and water diffusion weighted sequences. T_1 relaxation maps, PD maps, T_2 relaxation maps, and water diffusion (ADC) maps were calculated. Regions of interest (ROIs) were plotted on the PD images or T_2 maps in gray and white matter then propagated to the remaining two maps (Fig. 1). ROI map values were calculated and the mean map value across all three brains at each depth was calculated for both gray and white matter. The area fixed by formalin appeared lighter than adjacent tissue on the coronal T_1-weighted images (T_R = 600, uncorrected for gain). This formalin band was outlined, as was the entire brain (Fig. 2). The ratios of the areas of the formalin band to the rest of the brain were plotted over time to quantify the formalin band progression. The area ratios were converted to normalized radius values between 0 and 1 (Fig. 4A). Approximating the brain as a solid sphere, using a diffusion coefficient of one, Fick's law of diffusion was used to model diffusion of formalin into the brain. To compare the measured data to this model, model time was matched to experimental time on the basis of normalized radius value for each measured point (Fig 4B). Assuming Fick's law of diffusion was being followed in the experiment, a linear regression, $t_1 = t/D$ where t_1 represents the model time and t represents the measured time, was used to compute D, the diffusion coefficient of formalin into the brain.

Results

A qualitative difference was only observed on the T_1-weighted images where formalin-fixed brain appeared as a lighter band that became progressively thicker with time as the formalin fixed deeper tissues. Fig. 3 illustrates how the signal was reduced for all measured parameters except ADC, which remained constant. The location of the inner boundary of the formalin band followed the steepest formalin concentration gradient in the model. The exponential growth of the formalin band to an asymptote, calculated from a least squares curve fit to the ratio of formalin band area to the area of the rest of the brain, was determined to be $m = -0.66 \exp[-0.02\, t] + 0.66$ (correlation, $r = 0.988$, $p < 0.0001$), while a similar fit to the same data presented as normalized radius (Fig. 4A) was $m = -0.58 \exp[-0.02\, t] + 0.58$. The formalin band approached an asymptote of 0.58 and not zero. Similarly, the normalized radius position of the zero of the second derivative of the model formalin concentration (corresponding to the maximum concentration gradient) converged to a similar asymptote. This similarity shows that, at least to first order, the formalin fixation may be modeled by simple diffusion and that the edge of the observed formalin band corresponds to the maximum formalin concentration gradient. The diffusion coefficient of formalin into the brain was 5.26×10^{-4} normalized radii per hour (Fig. 4B).

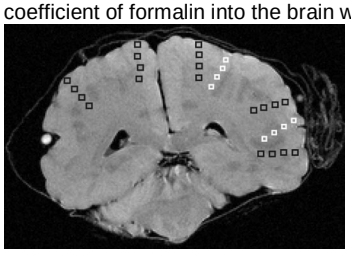

Fig. 1

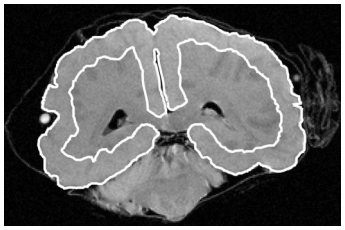

Fig. 2

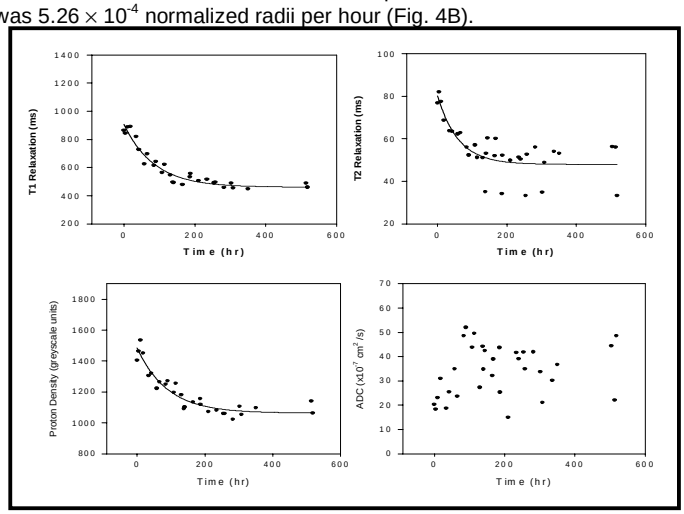

Fig. 3

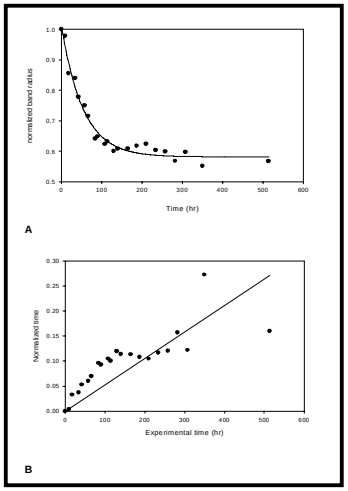

Fig. 4

Discussion

The reduction in T_2 relaxation is in keeping with previously reported results(1). We also found significant reduction in T_1 relaxation and PD signals with progressive formalin fixation and quantified the change in the light band of formalin-fixed tissue, previously described as artifact(1). The inner boundary of the formalin band is at a location consistent with a mathematical model of the steepest part of the formalin concentration gradient. In our model, the diffusion rate of formalin into the brain was 53 mm^2/hour and the brain is not fully fixed until 15 weeks. Furthermore, the attenuation of T_1 relaxation, T_2 relaxation and PD is ongoing during fixation. Investigators who image formalin-fixed brains need to be aware of these MR signal changes. Given the very long time to complete fixation, investigators should consistently report the duration of fixation at the time MR images are acquired and should consider the effects of incomplete fixation in order to avoid artificial results, misinterpretation and misdiagnosis.

References

1. Blamire AM, Rowe JG, Styles P, McDonald B (1999) Optimizing imaging parameters for post mortem MR imaging of the human brain. Acta Radiol 40(6): 593-7

Disclosure

Grant support for G.E.S. and A.O. from the Saskatchewan Health Services and Utilization Research Council (HSURC) and the Canadian Institutes for Health Research (CIHR).