FIXATIVE EFFECTS ON THE MR PROPERTIES OF ISOLATED RAT BRAIN SLICES

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Introduction

MRI studies of chemically-fixed tissues are common, yet little is known about how chemical fixation alters the MR properties of tissue. Recent studies on a simple erythrocyte ghost tissue model suggest that aldehyde fixatives, particularly 4% paraformaldehyde, significantly alter mean intracellular residence time [1,2]. In this followup study, we compared the effects of chemical fixation with 4% paraformaldehyde and a modified Karnovsky's solution (2% paraformaldehyde / 2% glutaraldehyde) on the T_1, T_2 and water diffusion properties of rat cortical brain slices. This study demonstrates significant perturbation of nervous tissue microstructure by aldehyde fixation that should be accounted in MRI studies of fixed tissue.

Methods

Vibratome-cut slices of rat isocortex (500 micron thick) were procured from male P30 Long-Evans rats and imaged using a multislice perfusion chamber inside a 600 MHz Bruker spectrometer with a 10-mm birdcage coil [3]. Fresh, or unfixed, slices were imaged promptly in artificial cerebrospinal fluid (300 mOsm/kg, pH 7.4). Chemically fixed slices were immersion-fixed in 4% paraformaldehyde or Karnovsky's solution (2% paraformaldehyde, 2% glutaraldehyde) for 7-10 days, imaged in fixative, then washed 4-5x in PBS (300 mOsm/kg, pH 7.4) over 12 hours and re-imaged. Water diffusion measurements required a pulsed-gradient spin-echo multislice sequence with 12 diffusion-weighted images. T_1 and T_2 were measured in slices with partial saturation and multi-echo sequences. Data were analyzed with a two-pool diffusion model with exchange. Tissue microstructure and MR relaxation properties were thus determined under living, fixed and fixed-washed conditions.

Results

Both 4% paraformaldehyde and Karnovsky's solution fixatives significantly reduced cortical brain slice T_2 ($P < 0.05$) such that data for the two-compartment exchange model were unobtainable from slices in fixative. Washing aldehyde-fixed samples in PBS appeared to restore T_2 to pre-fixation values by removal of excess fixative, but interestingly T_2 was statistically longer than in fresh slices for slices fixed with 4% paraformaldehyde then washed in PBS ($P < 0.05$). Similarly, slice T_1 was reduced from 2.02 ± 0.05 s in unfixed slices to 1.596 ± 0.043 s and to 1.499 ± 0.050 s upon fixation with 4% paraformaldehyde and Karnovsky's solution respectively ($P < 0.05$). Unlike the T_2 effects of free fixative solutions, removal of excess fixative by washing in PBS did not significantly alter the T_1 of fixed slices.

Slices in either aldehyde fixative solution showed significantly increased rates of water exchange between intra- and extracellular compartments and an increase in calculated intracellular diameter after fixation and washing. Slices fixed in Karnovsky's solution and washed showed a significantly decreased intracellular ADC, whereas slices fixed in 4% paraformaldehyde and washed showed an increased intracellular fraction.

Conclusion

Our data demonstrates that the chemical reactions during aldehyde fixation cause permanent alteration to the relaxation and diffusion properties of tissues, as washing fixed nervous tissue samples does not restore their MRI properties to pre-fixative values. This suggests fixative may significantly impact the fractional anisotropy and mean diffusivity measurements of diffusion tensor imaging. Care must thus be taken when extrapolating data from fixed tissue samples to *in vivo* MRI studies. The work has been submitted to the ISMRM (1).

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References

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