The water apparent diffusion coefficient, but not T2, in ex vivo brain tissue is affected by previous exposure to alkaline pH

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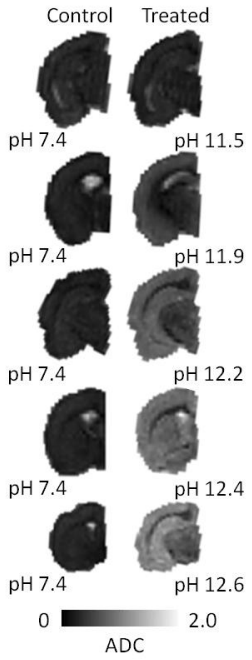
Introduction: Experiments with postmortem brain tissue are useful for studying the biophysical determinants of MRI-based contrast observed in vivo (1,2) as well as complementing other experimental techniques that utilize postmortem tissue, such as histological characterization (3). These applications of ex vivo MRI could benefit from improved understanding of the determinants of physical properties (spin relaxation, diffusion, etc.) in postmortem tissue, as well as further characterization of the dependence of these molecular properties on common laboratory tissue processing procedures (4). Here we show that exposure of tissue to alkaline conditions can induce dramatic (>2-fold) and irreversible changes in the water apparent diffusion coefficient (ADC). Interestingly, the biophysical changes that underlie this effect on the water ADC do not influence water ^{1}H transverse relaxation.

Methods: Normal adult rat brain tissue was used for this study (n = 6. Note: Some animals contributed two data points in Fig. 2). Animals were given an i.p. injection of 0.5 mL euthasol (Butler-Schein Animal Health Supply, Dublin, OH). Heparin (0.01 mL/10 mL), with phosphate buffered saline (1x PBS), was injected into the left cardiac ventricle until the fluid of the right cardiac atrium was clear. Paraformaldehyde (4%, approximately 35 mL, pH 7.4) was then injected into the left cardiac ventricle. The brains were extracted and placed in paraformaldehyde (4%, approximately 30 – 45 mL) for 24 hours. Samples were then transferred to 1x PBS, pH 7.4 at 4°C for at least 24 hours.

Figure 1



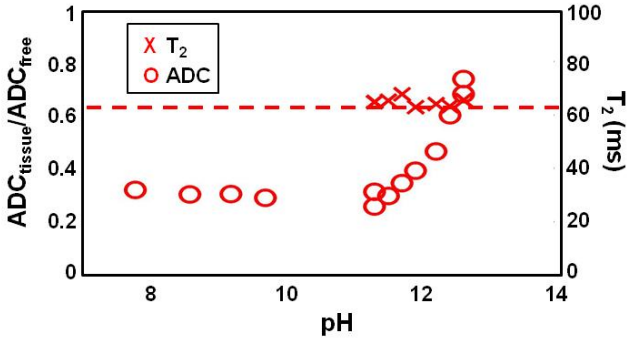
Brains were sectioned coronally at 3 evenly spaced intervals from the rostral to caudal extent of the brain (resulting in 4 coronal sections). In any given section, the two hemispheres were separated. One hemisphere was treated with experimental buffer solution, the other hemisphere served as a control. Glycine/NaOH buffers were created ranging from pH 7.8 to pH 12.6. Tissue slices were treated for 24 hours, and then returned to 1xPBS, pH 7.4 and allowed to equilibrate for at least 24 hours at 4°C prior to scanning.

A multi-slice spin-echo pulse sequence incorporating a Stejskal-Tanner diffusion sensitization gradient pair was used to acquire DTI data, specifically ADC measurements, on an 11.7 Tesla magnet (Bruker, Germany) interfaced with a 9 cm inner-diameter magnetic field gradient coil insert. Scan parameters were as follows: TR = 9600, TE = 42 ms, FOV = 5.12x2.56 cm, Matrix = 128 x 64, Voxel size = 0.4 mm^3, Averages=1, Directions=3 (along the x, y, and z coordinates in the laboratory frame). The b value was 2500 s/mm^2, δ = 12 ms and Δ = 20.958 ms. One scan was acquired with b=0. The intensity values of each voxel were averaged for the diffusion-sensitized images to generate the $s^{ave}(b)$ image, and ADC was calculated by solving the signal attenuation equation: $ADC=-\ln(s^{ave}(b)/s(0))/b$. T_2 was measured from additional images acquired with TE values of 18, 36, 54 and 90 ms.

Results: In Figure 1, coronal views of treated hemispheres are pictured for a subset of the 13 buffers of increasing basicity, paired with corresponding neutral-buffer-exposed controls. Those sections treated with highly alkaline buffer show higher ADC than controls. Figure 2 displays increasing ADC (relative to the ADC water in the buffer bathing the tissue) with increasing basicity of buffer treatment. In contrast, T_2 did not depend on buffer treatment (dashed line indicates the average T_2 measurement for the control tissue), suggesting this effect is specific to the ADC.

Conclusions: These data suggest that varying the pH of buffers and fixatives used in ex vivo tissue preparation can have a significant impact on measurements of ADC. This effect is potentially realized through modifications to biological membranes. Exposure to moderately alkaline pH is a commonly-used technique for extracting membrane-associated proteins (5), and more extreme basic pH is associated with increased membrane porosity (6). While further research is necessary to determine what elements of tissue are being affected by alkaline exposure, this research provides an avenue toward developing further insight into the determinants of physical parameters observable by MRI.

Figure 2



References: **1.** Sun et al. Magn Res Med (50) 2003 **2.** Sun et al. Magn Res Med (53) 2005 **3.** Dortch et al. Magn Res Med (64) 2010 **4.** Shepherd et al. Magn Res Med (62) 2009 **5.** Okamoto et al. Curr Protoc Cell Biol 5.4.1 – 5.4.17 2001 **6.** Cartaud et al. J Cell Biol (90) 1981