Optimization of Extrinsic Proton Staining Methods for Ex Vivo Cytoarchitectonic Magnetic Resonance Imaging

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Introduction

Gadolinium chelate contrast agents alter relaxation rates as a function of agent concentration, compartment partition coefficients, and water exchange rates and will enhance the conspicuity of tissue types depending on regional properties of these parameters.[1] Use of post-mortem samples for *ex vivo* imaging allows for great flexibility in the concentrations and imaging parameters used to optimize tissue contrast, and the potential increases in SNR and CNR permits ultra-high resolutions approaching the realms of histology and microscopy. Gadolinium has been used previously in *ex vivo* imaging of mouse tissue[2], but the concentrations for viewing cytoarchitectural detail in the brain have not been experimentally optimized. This research investigates the CNR enhancement of gadopentate dimeglumine as a function of concentration for *ex vivo* imaging of human brain tissue.

Methods

A post-mortem human brain was fixed in a formalin solution for approximately three months. Four slices 3 mm in thickness were sectioned from the parietal lobe of the right hemisphere and soaked in one of the following solutions: 1, 5, or 10 mM concentrations of gadopentate dimeglumine (Magnevist, Berlex Laboratories, Inc.) diluted with phosphate-buffered saline, or a control of phosphate-buffered saline. Tissue samples soaked in solution for 5 days, were blotted, and then immersed in Fomblin (Solvay Solexis), a perfluoropolyether substance, which contributes no background MR signal and aids in susceptibility matching. Sections were imaged in a 7T 90-cm magnet (Siemens Medical Solutions, Erlangen, Germany) using a three-turn solenoid transmit-receive coil. Four acquisitions were acquired using a multi-echo FLASH sequence[3] with a flip angle of 10, 20, 30, or 40 degrees. Each scan acquired ten echoes with a TR of 76 ms and a 4.6 ms echo spacing, yielding TE's of 4, 8.6, 13.2, 17.8, 22.4, 27, 31.6, 36.2, 40.8, and 45.4 ms. An isotropic resolution of 200 microns was obtained. A sample slice from the 1 mM concentration is shown in Figure 1. Maps of T1, T2*, and proton densities (PD) were then fit across the images using data from all flip angles and echo times using the maximum likelihood method of Fischl et al.[3]
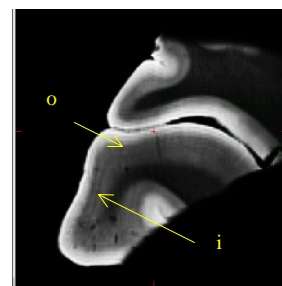
Figure 1: Sample slice soaked in 1 mM gadopentate dimeglumine. Arrows point to outer (o) and inner (i) bands of Baillarger.
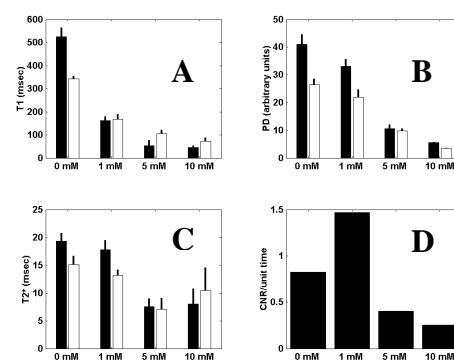
Regions of interest (ROI's) were then drawn within the white matter and gray matter for each concentration used. The contrast-to-noise ratio (CNR) per unit time was calculated for a symmetric echo acquisition with the following equation:

$$CNR = \frac{\overline{GM - WM}}{\sqrt{TR}} g\sqrt{\min(TE - T_{ramp}, TR - TE - T_{ramp})}$$

, where GM and WM represent the mean values of the gray and white matter ROI's, respectively, for all echoes acquired in one acquisition. The noise is proportional to the square root of the receiver bandwidth, which scales as the minimum of $TE-T_{ramp}$ versus $TR-TE-T_{ramp}$, where T_{ramp} is the time required for gradient ramping estimated to be 2 ms. Noise is also reduced by the square root of the number of scans acquired, which is inversely proportional to TR for a given scan time. Measurement parameters of TR, TE, and flip angle were then varied, and new volumes synthesized using the fitted T1, T2*, and PD values. Maximum CNR was calculated for each triplet of TR (5-80 ms), TE (3-78 ms), and flip angle (1-100 degrees), provided that TE<TR-2.1 ms. This procedure was repeated for each contrast agent concentration, and the maximum possible CNR determined.

Results and Conclusion

Figure 2 A-C displays the measured relaxation times and proton densities in gray and white matter for each gadopentate dimeglumine concentration. The CNR comparisons are shown in Figure 2D. Of the four concentrations tested, CNR was a maximum for the 1mM concentration using a TR of 79 ms, TE of 18 ms, and a flip angle of 24 degrees. This prescription yields a PD-T2* weighted image, which is surprising as gadolinium is primarily used for T1 enhancement. In *ex vivo* imaging, the majority of the available cytological and myelological contrast arises from the differences in PD and T2*. Thus, the 1 mM concentration yields the highest CNR for the chosen scanner and sequence because the differences in PD and T2* are preserved while the confounding T1 information is minimized, as shown in Figure 2. The outer and inner bands of Baillarger, tangential nets of myelinated fibers (arrows in Figure 1), are most prominently displayed in the 1 mM images.

Future work will involve modeling these experimental results with a fuller understanding of the laminar differences in partition coefficient and water exchange in fixed tissue as well as the role of ionic versus non-ionic valence structures in contrast distribution.

Figure 2: Bar graphs showing T1 (**A**), PD (**B**), T2* (**C**), and CNR (**D**) as a function of Magnevist concentration. White bar = WM, black bar = GM.

References: 1. Donahue KM et al. J Magn Reson Imaging 1997 Jan-Feb;7(1):102-110. 2. Johnson GA et al. Radiology. 2002 Mar;222(3):789-93. 3. Fischl B, et al., Neuroimage 2004; 23 Suppl 1: S69-84.