Validation of a white-matter specific dual MR/optical contrast agent for high-resolution imaging

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INTRODUCTION

High-field magnetic resonance (MR) microscopy is emerging as a tool to study the microstructure of many tissues, including the cytoarchitecture of the human brain. However, the inherent contrast mechanisms in MR are insufficient to resolve fine structural details. This problem may be alleviated by targeted contrast agents, which have the potential to aid identification of neuronal substructures. We have previously reported work on a white-matter specific stain, luxol fast blue (LFB), with a dual role as MR and histology contrast agent [1,2]. LFB has a paramagnetic copper core and improves the contrast-to-noise ratio (CNR) between white matter and cortical layers 5 and 6, more than formalin fixation alone and also more than treatment with MnCl or Gd-DTPA [1].

In this work, we address three remaining questions about this new contrast agent: **1)** the relaxivity of LFB at 14T, **2)** the duration LFB-stained samples should be immersed in lithium carbonate differentiating solution, and **3)** the histological validation of LFB distribution within tissue samples. As LFB is immiscible in water, it is instead combined with ethanol. If ethanol remains in the stained tissue, a chemical shift image will result. The LFB stain is differentiated and made specific for myelin by immersion in a 0.05% aqueous solution of lithium carbonate. In typical histological preparations, tissue slices 10-50 microns thick are immersed in the lithium solution for 5-10 seconds, until gray and white matter can be differentiated. This time must be increased for our sample geometry (~10mm x 10mm x30 mm) and should be long enough for the lithium carbonate to penetrate the sample and displace the ethanol without over-differentiating the tissue.

Previously, we assumed the LFB had penetrated throughout the sample because the change in T1 of white matter had been greater than that of the overlying gray matter. We have now verified the selective distribution of LFB within tissue by means of optical histology. In addition, high-resolution MR images of samples treated with LFB display enhanced vertical bands, which may be radial organizations of neocortical axons.

METHODS

1) Relaxivity measurements: Six concentrations of LFB (Acros Organics, Morris Plains, NJ) (0 mM, 6.5 mM, 13 mM, 39 mM, 65 mM, and 130 mM) dissolved in an ethanol-acetic acid mixture were scanned using a 20-mm birdcage coil in a vertical 89-mm bore 14T scanner (Bruker Avance) with a 100 G/cm gradient coil. An inversion-recovery prepared spin echo sequence was used to measure longitudinal relaxation times. **2) Differentiation duration:** The staining method described by Blackwell et al. [1, 2,], was followed, altering the time of immersion in the lithium carbonate solution. A single-pulse MR spectrum was then obtained in a 10-mm birdcage coil from the *ex vivo* tissue sample surrounded by a susceptibility-matching perfluoropolyether fluid (Fomblin, Solvay Solexis, Thorofare, NJ). **3) LFB distribution:** Imaged tissue was embedded in paraffin and cut into 20 micron-thick slices. One-third of slices was restained for myelin, one-third was counterstained with cresyl violet, and the remaining third was mounted without additional processing. Slides were viewed under a microscope and photographed.

RESULTS and DISCUSSION

1) The relaxivity of LFB at 14T was estimated to be 2.1 s-1/mM-1, which is about half that of gadolinium-DTPA at the same field strength. Although the relaxivity of LFB is lower than gadolinium-DTPA, it has been shown previously to be a more effective contrast agent due to its selective distribution. [1] **2)** After a duration of 30 hours in the lithium carbonate solution, the methyl triplet and methylene quartet of ethanol were eliminated from the NMR spectrum of the *ex vivo* sample. An upper bound on the differentiation duration is still under determination. **3)** MR images of the control and LFB-stained samples appear in Figure 1 A and B, respectively, and a slide without further histological processing appears in Figure 1 C. The region of lowest signal intensity in the MR image of the LFB-stained sample is the white matter, which corresponds to the blue-tinted central region of Figure 1C. This pigment is from the LFB and does not appear in the surrounding gray matter, which is opaque. Upon further inspection of Figure 1B, the uniformity of the inner gray matter, layers 5 and 6, is interrupted by dark speckling and also by fine lines having a perpendicular orientation to the laminae. The speckling could be blood, air pockets, or LFB particulates. The fine lines appear to be the radial fascicles of the cerebral cortex as demonstrated by the higher-resolution optical image in Figure 1C. To the best of our knowledge, this is the first demonstration of radial fascicles with magnetic resonance imaging. LFB and other specific, exogenous contrast agents hold much promise for enhancing the cytoarchitecture in high-resolution, high-field MR imaging.

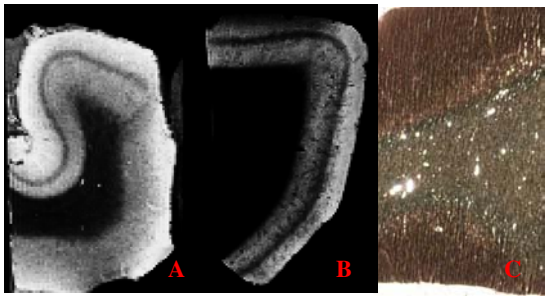

Figure 1: MR images at 14T, 80 micron isotropic resolution of A) control visual cortex and B) LFB-stained visual cortex. Histological slide in C) shows distribution of LFB throughout myelinated fibers.

References

[1] Blackwell, M et al. Proc of ISMRM, 2005. [2] Blackwell, M et al. Proc of Society for Molecular Imaging, 2005.