Prospects for quantitative imaging of myelin with dual-echo short inversion time 3D UTE MRI

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Introduction

Myelin is a lipid bilayer sheath encasing axons that enhances nerve conduction efficiency. Malformation or loss of myelin is at the core of many neurodegenerative disorders[1]. At present, there are few alternatives to destructive histologic methods to directly assess myelin. While MRI relaxometry and diffusion methods can indirectly assess myelin, they only detect myelin-associated water and not myelin itself. The short T_2^* of myelin protons and the presence of strong long T_2^* signals in white matter (WM) have thus far prevented direct myelin imaging with MRI. In this work, we examine the feasibility of ultra-short echo time (UTE) MRI to directly image myelin in purified myelin extracts and excised rat spinal cords (SC). NMR is first used to identify and characterize the MR signal from myelin. A dual-echo short inversion-time UTE sequence (de-STUTE) based on Ref. 2 is then used to suppress the long T_2^* signal and image a rat SC.

Methods

Rat and bovine SC samples were harvested from Sprague-Dawley rats (Charles River Labs) and a local butcher. A sucrose gradient method[3] was used to extract myelin from SC tissue using both WM and grey matter (GM). This method has been shown to preserve the bilayer structure of myelin. The myelin extract was suspended in D_2O (99.9% D, Sigma-Aldrich). Bovine myelin extract was used to prepare myelin/D_2O mixtures with varying myelin concentration (1.24-17.36 mg/ml). All spectroscopic and imaging experiments were performed on a 9.4 T vertical bore spectrometer/micro-imaging system (DMX-400, Bruker Instruments).

^{1}H NMR spectra were obtained for a freshly excised rat thoracic SC immersed in Fomblin (Sigma-Aldrich), as well as bovine and rat myelin extracts and samples with varying myelin concentration. Rat and bovine myelin extract was also dissolved in an organic solvent mixture to acquire high-resolution ^{1}H, and ^{31}P and ^{13}C proton-decoupled NMR spectra for identification of lipid components.

The rat thoracic SC section was imaged using a 2D de-STUTE sequence (128x128, FOV ~2cm). A 5ms adiabatic inversion pulse and 500ms inversion time were used to selectively invert and null long T_2^* signal. A 2D ramp-sampled UTE image with hard pulse excitation was then acquired with TE=10μs. Following a refocusing gradient, another 2D UTE image was acquired with TE=1200μs. A magnitude subtraction of the long TE image from the short TE image suppressed any residual long T_2^* signal.

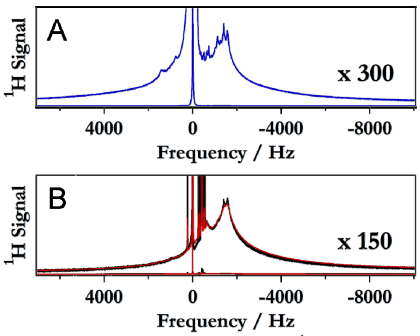

Fig 1. Full-scale and magnified ^{1}H spectra of intact rat SC (A) and myelin extract (B) for rat (red) and bovine (black). Spectra centered on water frequency. Note intact SC needed higher magnification to see broad peak.

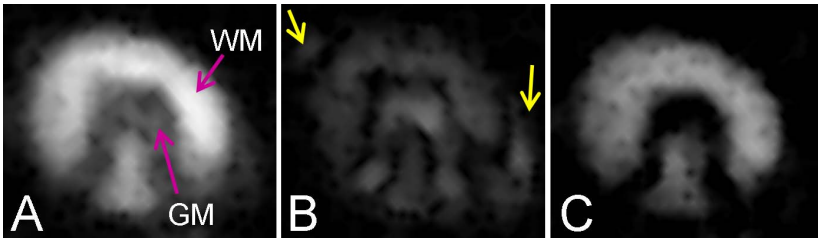

Fig 2. Linearly interpolated 2D de-STUTE images of excised rat thoracic SC section. A) TE=10μs. GM and WM are as indicated. B) TE=1200μs. Yellow arrows show residual surface water. C) Magnitude difference image of B from A.

Results and Discussion

Fig. 1 shows ^{1}H NMR spectra for intact rat SC and rat/bovine myelin extracts. The intact rat SC spectrum highlights the dominant water peak that would mask any myelin signal without long T_2^* suppression. The myelin extract spectra show residual sucrose and water peaks. The water peak is reduced as the myelin extract is mixed in D_2O. All three spectra share a non-Lorentzian, broad resonance (linewidth ~1700Hz) whose center is shifted ~3.5 ppm upfield from the water peak, which is consistent with the chemical shift of methylene protons in lipids. High-resolution ^{1}H, ^{31}P and ^{13}C spectra of myelin extract (not shown) are consistent with the galactolipid, phospholipid, and cholesterol constituents of myelin[4] and show negligible contributions from proteins. This strongly supports that the short T_2^* signal of SC consists predominantly of myelin lipids.

From its line width, T_2^* of myelin was estimated to be 100-200μs. Therefore, ramp-sampled UTE MRI should detect this signal with TE=10μs. Figure 2 shows de-STUTE images from a rat thoracic SC section. The short TE image shows good contrast between WM and GM. The long TE images shows a significant decrease in overall signal intensity implying that the short TE image contains a substantial amount of short T_2^* signal. The dark boundary between GM/WM and SC/water is the result of partial volume averaging of adjacent regions with different T_1s leading to destructive interference near the null time. The magnitude difference shows excellent suppression of GM and residual water outside the SC. The short T_2^* signal resides entirely in WM, which suggests that myelin is being imaged. Figure 3 shows ^{1}H spectra of various concentrations of myelin extract. The peak integrals are highly correlated with myelin extract concentration. The data highlight the potential of de-STUTE to quantify myelin concentration using a reference. Further work is needed to develop a 3D de-STUTE sequence and construct a reference phantom with similar relaxation properties to myelin analogous to the approach in Ref. 5.

Conclusion

This work examined the potential for de-STUTE to directly image and quantify myelin. NMR results indicates that the short T_2^* signal of SC is predominantly myelin lipids. de-STUTE images exhibit a short T_2^* signal present only in WM, which suggests the constituent imaged is indeed myelin.

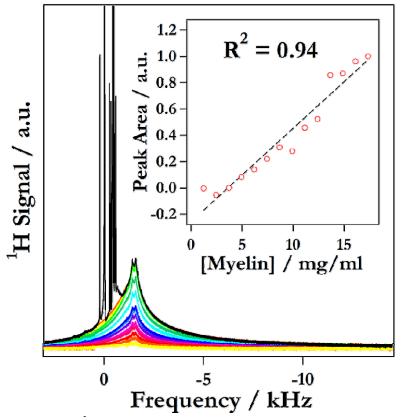

Fig 3. ^{1}H spectra for a series of bovine myelin extract concentrations. Residual sucrose and water peaks (black) were removed using a bi-exponential fit of the broad peak. Inset: Fitted broad peak area vs myelin concentration.

References: 1. van der Knaap, MS et al, *Magnetic Resonance of Myelin, Myelination, and Myelin Disorders*, Springer-Verlag (1995). 2. Waldman, A et al, *Neuroradiology*, **45**:887 (2003). 3. Larocca, JN et al, *Curr. Protoc. Cell Biol.*, 3.25.1 (2006). 4. Husted, C et al, *MRM*, **29**:168 (1993). 5. Techawiboonwong, A et al, *Radiology*, **248**:824 (2008). **Acknowledgements:** NIH T32 EB00814