NeuroImage 163 (2017) 358–367

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/neuroimage

Towards quantification of myelin by solid-state MRI of the lipid matrix protons

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ARTICLE INFO

Keywords:
MRI
Myelin
UTE
ZTE
Long-T2 suppression
White matter

ABSTRACT

Purpose: Direct assessment of myelin has the potential to reveal central nervous system abnormalities and serve as a means to follow patients with demyelinating disorders during treatment. Here, we investigated the feasibility of direct imaging and quantification of the myelin proton pool, without the many possible confounds inherent to indirect methods, via long-T_2 suppressed 3D ultra-short echo-time (UTE) and zero echo-time (ZTE) MRI in ovine spinal cord.

Methods: ZTE and UTE experiments, with and without inversion-recovery (IR) preparation, were conducted in ovine spinal cords before and after D_2O exchange of tissue water, on a 9.4T vertical-bore micro-imaging system, along with some feasibility experiments on a 3T whole-body scanner. Myelin density was quantified relative to reference samples containing various mass fractions of purified myelin lipid, extracted via the sucrose gradient extraction technique, and reconstituted by suspension in water, where they spontaneously self-assemble into an ensemble of multi-lamellar liposomes, analogous to native myelin.

Results: MR signal amplitudes from reference samples at 9.4T were linearly correlated with myelin concentration ($R^2 = 0.98–0.99$), enabling their use in quantification of myelin fraction in neural tissues. An adiabatic inversion-recovery preparation was found to effectively suppress long-T_2 water signal in white matter, leaving short-T_2 myelin protons to be imaged. Estimated myelin lipid fractions in white matter were 19.9%–22.5% in the D_2O-exchanged spinal cord, and 18.1%–23.5% in the non-exchanged spinal cord. Numerical simulations based on the myelin spectrum suggest that approximately 4.59% of the total myelin proton magnetization is observable by IR-ZTE at 3T due to T_2 decay and the inability to excite the shortest T_2* components. Approximately 380 μm of point-spread function blurring is predicted, and ZTE images of the spinal cord acquired at 3T were consistent with this estimate.

Conclusion: In the present implementation, IR-UTE at 9.4T produced similar estimates of myelin concentration in D_2O-exchanged and non-exchanged spinal cord white matter. 3T data suggest that direct myelin imaging is feasible, but remaining challenging on clinical MR systems.

Introduction

Myelin is an essential biomaterial responsible for electrically insulating axons and thus ensuring efficient neuronal signal transduction (van der Knaap and Valk, 2005). Image-based quantification of myelin has the potential to assess the severity of central nervous system (CNS) abnormalities such as demyelinating disorders and aid in the evaluation of the response to intervention (Paty et al., 2001; van der Knaap and Valk, 2005).

Most current MRI methods for detection of myelin abnormalities use magnetization transfer (MT) imaging or T_2 relaxometry. However, both methods constitute surrogate measures of myelin content, in that they exploit water's chemical or magnetic interaction with the myelin matrix, rather than directly imaging myelin matrix ^{1}H signal. One widely used

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https://doi.org/10.1016/j.neuroimage.2017.09.054
Received 18 June 2017; Accepted 26 September 2017
Available online 28 September 2017

technique is based on measurement of the magnetization transfer ratio (MTR). Schmierer et al. showed positive correlations of MTR with histologically determined axon densities in cadaveric brains from MS patients (Schmierer et al., 2004). Nevertheless, recent data by Vavasour et al. (2011) raise some concerns about the specificity of MTR. In an animal study of murine spinal cord, MTR was found to decrease after experimental demyelination via injection of lysolecithin, but failed to increase after histological evidence of remyelination (McCreary et al., 2009). More recently, bound proton fraction, derived from a quantitative two-pool MT model, has shown to be more predictive of myelin concentration than MTR (Samsonov et al., 2012) but quantitative MT, like MTR, is still a proxy for myelin concentration.

Water in neural tissue exists in several pools, each with characteristic T_2 relaxation times corresponding to their respective microenvironments. T_2 relaxometry relies on quantification of the magnitude of a short T_2 water component (T_2 ~20 ms), the so-called "myelin water," which has been conjectured to arise from water trapped between the myelin bilayers (see, for instance (Laule et al., 2007)). Since the other two long-T_2 water components, cerebrospinal fluid (T_2 ~2 s), and intracellular and extracellular water (T_2 ~100 ms) (Laule et al., 2007), relax much more slowly, they can be separated from myelin water by acquiring and analyzing the Carr-Purcell-Meiboom-Gill (CPMG) signal decay (MacKay et al., 1994). A T_2 spectrum is typically generated by non-negative least squares fitting of an array of exponential functions to the CPMG decay (Whittall and Mackay, 1989), and the myelin water fraction (MWF) then is estimated as the ratio of the area under the T_2 spectrum between the bounds of 20 ms and 50 ms, to the total area under the T_2 spectrum (Whittall et al., 1997). However, there is some evidence that MWF does not always correlate with measured myelin content in normal tissue (Dula et al., 2010). Moreover, estimation of the T_2 spectrum from a superposition of amplitude-weighted exponentials (inverse Laplace transform) is a mathematically ill-posed problem (Epstein and Schotland, 2008) and typically requires high SNR, prior knowledge and appropriate regularization.

Direct detection of myelin would remove the complications of indirect methods (Wilhelm et al., 2012b). However, direct detection is challenging due to the extremely short lifetime of the myelin matrix proton MR signal (Horch et al., 2011) and interference from long-T_2 tissue-water signals. Some of the present authors previously showed the NMR spectral properties of myelin to be consistent with a lamellar liquid crystal, yielding a super-Lorentzian line shape with broad tails extending to about ±20 kHz, resulting in a lifetime of the transverse magnetization on the order of tens of microseconds (Wilhelm et al., 2012b). The work by Wilhelm et al. demonstrates that myelin extract and intact rat spinal cord myelin can be imaged by ultra-short echo time (UTE) MRI (Glover et al., 1992; Robson et al., 2003) on a laboratory 9.4T micro-imaging system. Early during the development of UTE, inversion-prepared UTE was explored at 1.5T to visualize the short-T_2 components purportedly arising from myelin in white matter in the brain of humans (Waldman et al., 2003). More recently, Du et al., using similar approaches, reported relative proton densities of the short-T_2 components of around 3–5% and a signal decay constant of 400 μs s (Du et al., 2014).

In the present work, we further investigated the feasibility of direct myelin imaging, with a focus on quantification, by means of two solid-state imaging techniques. We first examined the potential of long-T_2 suppressed 3D UTE imaging at 9.4T to quantify myelin content in both deuterium oxide (D_2O)-exchanged and non-exchanged (i.e., in the natural ^{1}H-hydrated state) ovine spinal cord, using co-imaged reference samples containing various mass fractions of reconstituted myelin to generate a calibration curve. We then evaluated the performance of an alternative solid-state imaging method (Wu et al., 1999), more recently referred to as zero-echo time (ZTE) MRI (Weiger et al., 2013), given recent evidence that ZTE yields SNR superior to UTE in solid-state ^{31}P imaging with T_2 ~100 μs s (Seifert et al., 2013). Lastly, we examine the feasibility of direct detection and quantification of myelin by ZTE-based methods at 3T under the gradient hardware constraints imposed by a typical clinical imaging system.

Methods

Sample preparation

Myelin was extracted from ovine spinal cord by a sucrose gradient technique, in which the lipid bilayer structure has been shown to be fundamentally preserved (Norton, 1974). Following isolation, the crude myelin was dissolved in a (4:2:1) ternary mixture of chloroform, methanol, and water, to remove residual sucrose contaminants. Dissolution in the ternary mixture inverts the bilayer, thereby releasing embedded proteins, which comprise 30% of total ultrashort-T_2 signal in white matter, and yielding myelin lipids (Wessel and Flügge, 1984). The purified myelin lipids were removed from the chloroform phase using a rotary evaporator. The remaining myelin lipid residue was then re-suspended in distilled water, frozen, and lyophilized to remove all remaining traces of solvent. Finally, the purified extract was suspended in 99.9% D_2O to achieve mass concentrations of 6%, 8%, 10%, 12% and 14%, which serve as reference samples for myelin quantification. The suspended extract self-assembles into liposomes consisting of concentric myelin lipid bilayers separated by thin layers of water (Hope et al., 1986).

Two 36-mm segments of ovine cervical spinal cord were dissected from the neck of a lamb slaughtered four days prior and stored at 4 °C. Before imaging, one segment was subjected to exchange with D_2O in three volumes of 12 mL D_2O-saline each over the course of 72 h, while one was stored in 12 mL of H_2O-phosphate buffered saline. The process of D_2O exchange replaces all native (light) water in the tissue with D_2O, which is invisible on ^{1}H MRI, effectively removing the confounding effects of tissue water on myelin ^{1}H measurement.

Overview of MRI and spectroscopic experiments

Two sets of imaging experiments were performed. First, the quantification accuracy was examined on a laboratory micro-imaging system at 9.4T (Avance III 400, Bruker, Billerica, MA) using a 1000 mT/m 3-axis gradient set and a 25-mm diameter quadrature birdcage radiofrequency (RF) coil. In a second set of experiments, the performance of ZTE-based methods was evaluated at 3T on a clinical imaging system (TIM Trio; Siemens Medical Solutions, Erlangen, Germany) with 40 mT/m maximum gradient strength and a custom-built 4.5 cm-diameter, 8 cm-long, 3-turn transmit/receive solenoidal RF coil constructed of polytetrafluoroethylene (PTFE), which largely eliminates spurious ^{1}H signal.

Both myelin extracts and ovine spinal cord specimens were scanned with UTE and ZTE sequences. The pulse sequence diagrams are shown in Fig. 1. In order to minimize the interference from the non-myelin tissue components, an adiabatic inversion-recovery (IR)-based long-T_2 suppression, with a pass-band below approximately 1 ms, was employed. During the adiabatic preparation pulse, the long-T_2 magnetization of tissue water is inverted ($M_z < 0$), while the short-T_2 magnetization of myelin is saturated (M_z ~0) due to transverse relaxation during the pulse. After an inversion-recovery time (TI), the magnetization of tissue water is nulled (M_z ~0) by longitudinal relaxation, while the magnetization of myelin will have recovered to a positive ($M_z > 0$) and observable level. Prior work in the authors' laboratory showed that adiabatic IR provides highly uniform short-T_2 contrast, achieving highly effective long-T_2 suppression with near-immunity to B_1 inhomogeneity (Li et al., 2012).

At 9.4T, vendor-provided UTE and ZTE sequences were used, with a slight modification to UTE to enable IR preparation. At 3T, the samples were imaged with some of the present authors' previously developed long-T_2 suppressed IR-rZTE-PETRA (point-wise encoding time reduction with radial acquisition; an embodiment of the ZTE sequence designed for clinical scanners) (Li et al., 2017) and ZTE-PETRA (Grodzki et al., 2012) sequences.

In the resulting 9.4T images, due to the severely blurred signal arising