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Anisotropic diffusion of metabolites in peripheral nerve using diffusion weighted magnetic resonance spectroscopy at ultra-high field

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Abstract

Although the diffusivity and anisotropy of water has been investigated thoroughly in ordered axonal systems (i.e., nervous tissue), there have been very few studies on the directional dependence of diffusion of metabolites. In this study, the mean apparent diffusion coefficient (Trace/3 ADC) and fractional anisotropy (FA) values of the intracellular metabolites *N*-acetyl aspartate (NAA), creatine and phosphocreatine (tCr), choline (Cho), taurine (Tau), and glutamate and glutamine (Glx) were measured parallel and perpendicular to the length of excised frog sciatic nerve using a water suppressed, diffusion-weighted, spin-echo pulse sequence at 18.8 T. The degree of anisotropy (FA) of NAA (0.41 ± 0.09) was determined to be less than tCr (0.59 ± 0.07) and Cho (0.61 ± 0.11), which is consistent with previously reported human studies of white matter. In contrast, Glx diffusion was found to be almost isotropic with an FA value of 0.20 ± 0.06. The differences of FA between the metabolites is most likely due to their differing micro-environments and could be beneficial as an indicator of compartment specific changes with disease, information not readily available with water diffusion.

Keywords: Diffusion spectroscopy; Sciatic nerve; DTI; Diffusion tensor imaging

1. Introduction

Magnetic resonance is well established for measuring diffusion properties of water in the human brain and neural tissue. In 1990, the diffusion behavior of water was examined in the cat central nervous system by using diffusion-weighted magnetic resonance imaging with the diffusion sensitization gradients applied in different directions (*X*, *Y*, and *Z*) [1]. Faster diffusion (i.e. greater signal attenuation) was noted parallel (versus perpendicular) to the white matter tracts in cat brain and this was termed anisotropic diffusion. Diffusion anisotropy of water in neural tissue has been examined quite extensively since that date, but particularly after the introduction of diffusion tensor imaging (DTI) in 1994 [2,3], and more recently this fundamental property of water in white matter has been used to perform 3D tractography of the fibers in the brain [4–7]. DTI of tissue water includes contributions from exchanging intra- and extracellular water, which makes compartment specific interpretation of diffusion characteristics rather complex. It has long been shown that reductions in water diffusion are a sensitive indicator of early cerebral ischemia [8], and despite the theory that this decrease is due to a water shift from extra-cellular to intra-cellular space, purely intracellular metabolites also show a reduction of diffusion by a similar magnitude without any compartmental shifts [9–15]. These metabolite diffusion studies are usually interested in either single direction diffusion or rotationally invariant mean diffusivity.

To date, there have only been two published human studies that have studied anisotropic diffusion of intracellular metabolites in tissue [16,17]. One study measured the ADC of NAA parallel and perpendicular to the corpus

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callosum in the normal human brain at 1.5 T and found the ADC parallel to be ~2–3 times greater than the ADC perpendicular [16]; however they examined only two human volunteers and measured only one metabolite, NAA. More recently the full diffusion tensor of gray and white matter in the normal human brain at 3 T was examined [17] and indicated that the fractional anisotropy of tCr (0.66 ± 0.13) and Cho (0.67 ± 0.14) was significantly higher than NAA (0.56 ± 0.13, tCr $p = 0.001$ and Cho $p = 0.001$, paired t-test), which may seem counterintuitive since tCr and Cho are not solely located in the axons (presumably highly anisotropic) as is NAA but rather are also found in environments expected to be more isotropic such as astrocytes and oligodendrocytes [18].

Accurate in vivo DW-MRS measurements are quite challenging due to subject movement, shimming issues, low SNR, and large voxel sizes that include a variety of distinct neural structures. A model system, such as an excised sciatic nerve, eliminates all of those factors. In such a preparation, the nerve is aligned and fixed in position which yields a better shim, much more time available to average, and permits observation of a single well defined structure (a bundle of axons). Also, the size of the nerve allows the use of a spectrometer with a magnetic field strength much higher than used in vivo. The anisotropy of the intracellular metabolites *N*-acetyl aspartate (NAA), creatine and phosphocreatine (tCr), and choline (Cho) have been measured previously in the excised bovine optic nerve at 11.7 T [19]. The ADC values were measured parallel and perpendicular to the long axis of the optic nerve using extremely high b-values (100,000–300,000 s/mm^2), and the diffusion decay curves were fit to a bi-exponential. The diffusion attenuation curves varied with diffusion gradient direction for NAA, tCr, and Cho; however the focus of that study was on q-space analysis at extremely high b-value, and therefore differences in diffusion with direction at lower b-values were not examined. The purpose of this paper was to examine the anisotropic diffusion of the three main metabolites (NAA, tCr, and Cho), as well as any other measurable metabolites, in the excised frog sciatic nerve, with the ultimate goal to relate this information to the anisotropic nature of the metabolite diffusion in the white matter of the human brain.

2. Methods

2.1. Nerve sample preparation

All sciatic nerve samples were taken from adult *Xenopus laevis*, the African clawed frog, which had been housed in an aquatic environment at room temperature. In total, nine nerve samples from five frogs were used in this study. Following euthanasia in MS-222 (3-aminobenzoic acid ethyl ester), ~3 cm segments of nerve were removed and placed in an oxygenated physiological buffer (112 mM NaCl, 3.0 mM KCl, 1.6 mM $MgSO_4$, 3.0 mM $CaCl_2$, 5.0 mM glucose, 3.0 mM HEPES). The perineurial sheath surrounding the nerve was removed from all nerve samples. The nerve samples were immersed in Fluorinert (FC-77, Sigma-Aldrich Canada Ltd. Oakville, Ontario, Canada) and were aligned along the Z-axis of the magnet by tying thread to one end and pulling the nerve (and Flourinert) into a small capillary tube (1 mm inner diameter) after which both ends were sealed with parafilm. The capillary tube was then oriented within a Wilmad 535P-5 mm NMR tube along the Z-axis and was surrounded by 99% D_2O (required for the lock signal, Fig. 1). All nerve samples were placed in the NMR between 45 min and 3 h of extraction.

2.2. NMR experiments

An 800 MHz (18.8 T) Varian Inova NMR spectrometer running VNMRJ 1.1D equipped with a XYZ-gradient HCN 5 mm probe with a maximum gradient strength of ~30 G/cm along the X and Y axes and ~60 G/cm along the Z-axis, was used for all diffusion experiments. A diffusion-weighted spin-echo pulse sequence with 5.0 ms diffusion gradient pulses (δ) and a diffusion gradient separation (Δ) of 20.0 ms yielded a diffusion time ($\Delta - \delta/3$) of 18.3 ms. Sequence parameters were as follows: TE = 30 ms, TR = 3 s, SW = 12,000 Hz, number of complex points = 24,002. Water suppression was achieved using a water selective 90° shaped Gaussian pulse prior to the hard 90 in the spin-echo pulse sequence. The gradient strengths of the X, Y, and Z gradients were calibrated based on a Cr diffusion coefficient of 0.80×10^{-3} mm^2/s at 20 °C [20] using a 600 μL phantom containing NAA (3 mM), Cr (3 mM), and Cho (1 mM). Seven separate calibrated b-values were measured for each direction (227, 539, 903, 1107, 1444, 1710,

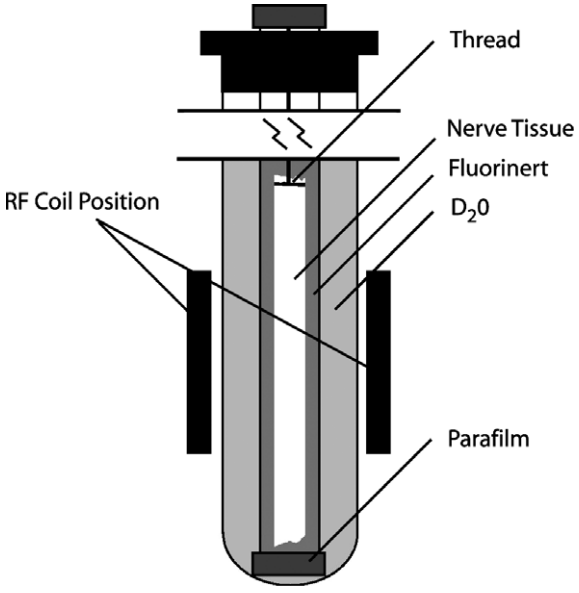

Fig. 1. Diagram of the setup for the 5 mm NMR tube. The excised sciatic nerve of the frog was immersed in a fluorinated compound to prevent dehydration and placed in a 1 mm inner diameter capillary tube, which was aligned parallel to the Z-axis of the magnet. The capillary tube was surrounded by 99% D_2O, which was required for the lock signal.