Intracellular Contributions to MR Diffusion Contrast in Stroke: Intraneuronal Viscosity and Neurite Beading

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Introduction. A rapid drop in the water apparent diffusion coefficient (ADC) in ischemic brain tissue is a well-known phenomenon [1]. Biophysical parameters that could affect water ADC include: (i) trans-membrane water exchange rates/membrane permeability, (ii) glial and neuronal cell-type-specific intracellular viscosities, (iii) extracellular tortuosity and fluid viscosity, (iv) intracellular and extracellular volume fractions, (v) intracellular restrictions/hindrances imposed by tissue microarchitecture (e.g., neurite beading), and (vi) temperature. Recently, neurite beading has been hypothesized to make a significant contribution to the ADC changes observed in stroke [2]. In the current work, the pre- and post-ischemic diffusion characteristics of the intraneuronal metabolite N-acetylaspartate (NAA) are described in terms of a biophysical model that takes into account contributions from parameters ii and v listed above.

Methods. All protocols were approved by the Washington University Animal Studies Committee. Female Sprague-Dawley rats aged 10-12 weeks were used in this study. Animals were anesthetized with 1.75% isofluorane in O_2 and immobilized in a stereotactic head holder. Brain temperature was varied by blowing warm air into the magnet bore and adjustment of water circulating through a pad placed underneath the animal. MR measurements were performed using a 4.7-T Agilent/Varian DirectDrive™ small-animal imaging system. A diffusion-weighted PRESS sequence (TR = 2 s, TE_{tot} = 144ms, 32 averages, 16 b values, b < 20 ms/μm^2, voxel size = 6 × 6 × 6 mm^3) employing half-sine-shaped diffusion gradient waveforms was used for measurements at a 50 ms diffusion time. Diffusion behavior was investigated at shorter diffusion times, from 1.5 to 3.75 ms using an oscillating gradient version of the DW-PRESS sequence. The rather large spectroscopy voxel consists primarily of gray matter wherein the macroscopically-averaged orientation of cylindrical cellular processes is random [3]. Water-suppression was not employed and individual FIDs were phase and frequency aligned before co-addition to correct for possible shifts due to slight head motions. Global cerebral ischemia was induced in-magnet by injection of a lethal dose of Euthasol via i.p. catheter.

Data Modeling. The resulting time-domain diffusion-weighted MRS data were modeled using Bayesian signal analysis software (http://bayesianananalysis.wustl.edu/index.html) to estimate amplitudes and frequencies of H_2O and the prominent ^{1}H metabolite resonances--NAA, Cr_{tot}, Cho_{tot}. Brain temperature was estimated based upon the chemical shifts of water and these metabolites [4]. Metabolite diffusion-attenuation data with t_{diff} = 50ms were modeled according to the 3-D cylinder model in Eqn. [1] (see Ref [5]). Relevant parameters include $D_\perp$ (the apparent diffusion coefficient perpendicular to the cylinder axis), $D_{||}$ (the "free" diffusion coefficient down the cylinder axis), and a coefficient β that accounts for diffusion kurtosis down the cylinder axis. The symbol Φ(x) denotes the error function of the argument x. To provide a basis for comparison between our results and the significant body of literature that has investigated pre- and post-ischemic metabolite diffusion at b ≤ 3 ms/μm^2, the low b-value subset of data points was fit as a single exponential decay, described by an ADC. An alternate approach, using the short-diffusion-time data (t_{diff} ≤ 2.629 ms), was employed to estimate metabolite free diffusion coefficients from diffusion-time-dependence of the ADC. In this treatment, porous media theory [6,7] was applied according to Eqn. [2], wherein S/V is the pore surface to volume ratio and c′ ~ 1.93 is a first-order correction term to account for the finite duration of the sine-wave oscillating gradient [7,8]. For water diffusion data (t_{diff} = 50 ms), a statistical model was employed to estimate the most probable apparent diffusion coefficient, D_m, fit to a truncated Gaussian distribution of ADCs [9].

$$S(b) = S_o \cdot \exp(-b \cdot D_\perp) \cdot \sqrt{\frac{\pi}{4 \cdot b \cdot [D_{||} \cdot (1 - \beta \cdot b \cdot D_{||}) - D_\perp]}} \cdot \Phi\left(\sqrt{[D_{||} \cdot (1 - \beta \cdot b \cdot D_{||}) - D_\perp] \cdot b}\right) \quad \text{Eqn. [1]}$$

$$ADC(t_{diff}) = \underbrace{D_o}_{\text{y-int ercept}} - \underbrace{c' \cdot \frac{4}{9\sqrt{\pi}} \cdot \frac{S}{V} \cdot D_o^{3/2} \cdot \sqrt{t_{diff}}}_{\text{slope}} + \ldots \quad \text{Eqn. [2]}$$

Results. NAA pre- and post-ischemia group-averaged (mean ± sd) diffusion-attenuation data are presented in Fig. 1 for the 50 ms diffusion time. Bayesian modeling according to Eqn. [1] produces estimates for pre-ischemia diffusion parameters of $D_{||}$ = 0.38 ± 0.02 μm^2/ms, $D_\perp$ = 0.02 ± 0.01 μm^2/ms, and a kurtosis parameter, β of essentially zero (0.02 ± 0.03). Post-ischemia, $D_{||}$ decreases by ~ 16% to 0.32 ± 0.02 μm^2/ms while β increases to 0.06 ± 0.03. $D_\perp$ is essentially unchanged at 0.01 ± 0.01 μm^2/ms. Treatment of the *in vivo* diffusion-time-dependent data according to porous media theory (Eqn. [2]) yields an estimated free diffusion coefficient of 0.36 ± 0.05 μm^2/ms for NAA, in remarkable agreement to $D_{||}$ estimated from the biophysical model of Eqn. [1]. $D_{m,H2O}$ = 0.89 μm^2/ms pre-ischemia, which decreases to 0.57 μm^2/ms post-ischemia.

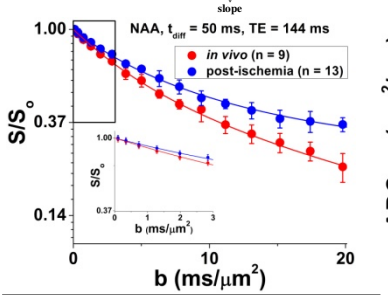

Figure 1. Semi-log plot of group-averaged NAA diffusion data for T_{NMR} in the range from 36 – 38 °C. **Inset:** Fits of b < 3 ms/μm^2 data to $S(b) = S_o \cdot e^{-b \cdot ADC}$ yield a pre-ischemia ADC for NAA of 0.122 ± 0.004 μm^2/ms vs. 0.097 ± 0.003 μm^2/ms post-ischemia, a 20% decrease.

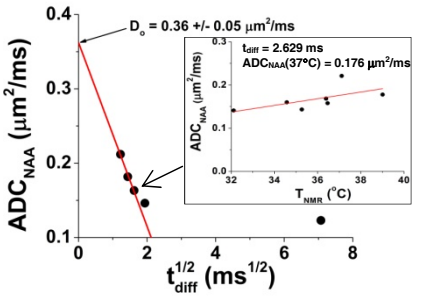

Figure 2. Treatment of the diffusion-time-dependent ADC of NAA according to Eqn. [2]. **Inset:** Measurements at each diffusion time were measured at a range of brain temperatures to provide a best estimate for ADC(t_{diff},37°C) used in the fit shown in the main figure panel.

Discussion/Conclusions. The estimated diffusion parameters for the intraneuronal metabolite NAA suggest an ~ 19% increase in neuronal intracellular viscosity post-ischemia. The modest increase in the NAA-diffusion kurtosis term is consistent with neurite beading [5] (known to occur in the neuronal dendritic tree in brain ischemia [9]). Neither the post-ischemia increase in neuronal intracellular viscosity nor the modest increase in the NAA-diffusion kurtosis term appears to be of sufficient magnitude to serve as the dominant underlying biophysical genesis of the 36% decrease in water diffusion ($D_{m,H2O}$) post-ischemia.

References. 1. *Am. J. Neuradiol.* **11**:423-429 (1990). 2. *PNAS* **107**: 14472-14477 (2010). 3. *MRM* **52**: 1052-1059 (2004). 4. *MRM* **60**: 536-541 (2008). 5. *NMR Biomed* **23**: 661-681 (2010). 6. *NMR Biomed* **8**: 297-306 (1995). 7. *JMR A* **121**: 187-192 (1996). 8. *MRM* **55**: 75-84 (2006). 9. *MRM* **50**: 664-669 (2003). 9. *J Neurosci* **28**: 11970 (2008).