Probing intracellular compartments in normal brain and brain tumor using short diffusion times

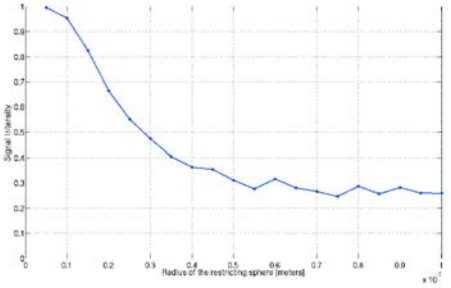
D. E. Prah[1], E. S. Paulson[1], F. Wu-Zimmer[1], and K. M. Schmainda[1,2]

[1]Department of Biophysics, Medical College of Wisconsin, Milwaukee, Wisconsin, United States, [2]Department of Radiology, Medical College of Wisconsin, Milwaukee, Wisconsin, United States

Introduction. A hallmark of the malignant glioma is local tumor cell invasion. Often the failure of surgical resection and radiation therapy, the primary treatments for gliomas, are attributed to tumor invasion. Treatment outcome may improve if more localized therapies are prescribed to areas of invasion. Standard imaging techniques inadequately detect invasion. DWI is unique in that it reflects an ensemble average of the underlying microscopic structure. In biological environments, the chosen diffusion time determines the degree to which the ensemble of protons survey the microscopic structures. Currently, mainstream clinical diffusion-weighted MR is limited to diffusion times greater than 25 msec. Diffusion weighting using diffusion times greater than 20 msec generally agree [1,2,3]. However, this agreement diminishes when diffusion times fall below 20 msec. [4,5]. One explanation is that diffusion weighting becomes less sensitive to restrictive boundaries as the diffusion time decreases, thus less sensitive to potential differences in the intracellular environment. Given that the intracellular compartments of tumor cells vary both in quantity and size due to their high metabolic and mitotic activity compared to normal tissue, we propose that diffusion-weighted images and/or ADC maps obtained for short diffusion times may improve the sensitivity of DWI to tumor cell invasion.

Materials and Methods. All studies were performed on a Bruker Biospec 30 cm 9.4T using local gradient coils capable of achieving maximum gradient strengths of 40 G/cm per channel. *Pulse sequence*. Short diffusion times were achieved by incorporating isotropic diffusion weighting into a standard spin echo (SE) pulse sequence using a pair of balanced bipolar gradients positioned around the refocusing pulse. The effects of eddy currents were assumed negligible after measurements of ethanol and water phantoms revealed no parameter dependent significant differences in the calculated diffusion coefficient from literature values. Diffusion times, gradient amplitudes, and separation of the bipolar lobes were each independently varied. *Simulation*. Ensembles of protons were uniformly distributed within impermeable spheres of radius, R. For each time step, each proton's new position was normally distributed assuming the mean and standard deviations were equal to the mean radial displacement calculated using the Einstein Relation, $<x>=(6D\tau)^{(1/2)}$, and an assumed diffusion coefficient of 2.0E-3 mm^2/sec. The position of a proton that traversed the radius of the sphere was recalculated until it fell within the sphere. Accumulated phase and corresponding signal attenuation was calculated for each sphere using the above-mentioned DW sequence with a constant diffusion time of 2.66 msec. *Rat Study*. Three male Sprague-Dawley rats were inoculated, intracerebrally, with 10^5 (10μL) C6 glioma cells and imaged 14 days post-inoculation. Rectal temperature was monitored and maintained at 37°C ± 1°C. Three series of DW images were collected. For Set A, a standard clinical SE DW images were acquired with TE=59.1 msec, TR=2 sec, δ=25 msec, Δ=30 msec, **$\tau \cong$ 22 msec**, b-value = 1000 s/mm^2. For Set B, a set of bipolar SE DW images were acquired with TE=49.1 msec, TR=2 sec, Δ=δ=10 msec, **$\tau \cong$ 6.66 msec**, 20 b-values ranging from 0 to 1760 s/mm^2. For Set C, a set of bipolar SW DW images were acquired with TE=25.1 msec, TR=2 sec, Δ=δ=10 msec, **$\tau \cong$ 2.66 msec**, 20 b-value ranging from 0 to 1760 s/mm^2. Images were acquired with a round single turn RF coil. Immediately following imaging rats were sacrificed, brains were extracted, sectioned into 7 um thick slices, and stained with hematoxylin.

Figure 1: DW signal from a proton pool restricted with a sphere



Results and Discussion. The simulation results, shown in Figure 1, demonstrate that as the size of the compartment decreases (or t increases) the DW signal is attenuated thereby resulting in an an underestimation of the actual unrestricted diffusion coefficient. This further suggests a decrease in sensitivity of the DW signal to smaller intracellular compartments. Apparent diffusion coefficient (ADC) maps overlayed on T_2 weighted (b=0 s/mm^2) images from one representative rat are shown in Figure 2. The tumor inoculation site can be seen above the right hemisphere. The first row shows ADC maps collected with standard clinical imaging parameters (i.e. b=1000, t=22ms). Hemtoxylin stained sections, not shown, suggests a much larger area of tumor invasion than depicted in Row A. The second and third rows show ADC maps obtained using diffusion times of 6.66 and 2.66 msec, repectively. Thus, the spatial distribution and reported ADC values depend profoundly on the choice of t and TE. Notice from the underlay that the DW images had different T_2 weighting due to the differences in echo times. Previous studies have suggested that the nucleus has a longer T_2 and a larger diffusion coefficient than the cytoplasm [7]. Furthermore, proliferating tumor cells are known to have increased nuclear to cytoplasmic ratios, thus suggesting longer intracellular diffusion times and T_2s of proliferating tumor cells. These characteristic diffusion and T_2 values may explain the results shown in Figure 2. The results in row B obtained with t = 6.66 ms / TE=59.1 ms show larger areas of high ADC compared to the results shown in Row A, obtained for longer t=22 ms, and the same TE (59.1 ms). For the results in row C, at shorter t (2.66 ms) and shorter TE (25.1 ms) the area of high ADC is again different. In this case the shorter TE may be allowing for a more equal contribution from all compartments, both short and long TE compartments, to the diffusion signal. Thus this latter result may be most representative of the true distribution of invading tumor cells. This hypothesis will be tested with the planned validation studies where the MRI results will be compared to the spatial distribution of fluorescing C6 tumor cells.

Conclusion. The results of this study demonstrate an important dependence of diffusion results on the choice of the diffusion experimental parameters. The proper choice of these parameters may enable us to design a diffusion experiment that is optimally sensitive to invading tumor cells.

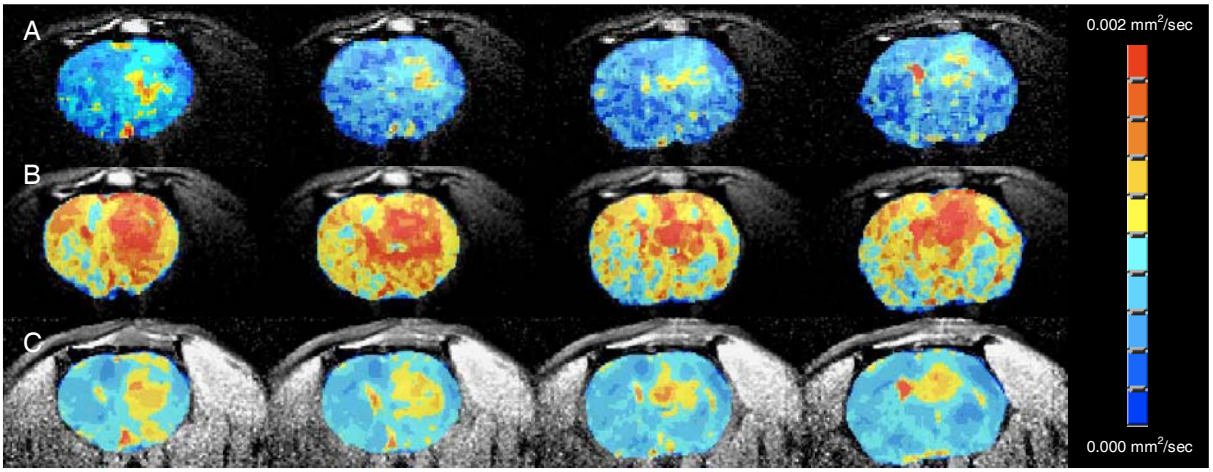


Figure 2: ADC maps from A) the standard SE clinical sequence, τ = 25 msec, B) the bipolar SW DW sequence, τ=6.66 msec, and C) the bipolar SW DW sequence, τ=2.66 msec

References:

(1) Moonen CT et al. MRM 1991;19:327-332.
(2) van Gelderen P et al. MRM 1994;31:154-163.
(3) Le Bihan D et al. Neuroreport 1993;4:887-890.
(4) Helmer KG et al. NMR Biomed. 1995;8:297-306.
(5) Niendorf et al. MRM 1994;32:672-677.
(6) Does MD, Parsons EC, Gore JC. MRM 2003;49(2):206-15.
(7) Schoeniger et al. JMR Series B 1994;103:261-273.