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

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Introduction. Typical malignant gliomas consist of three parts: (1) an inner necrotic core, (2) a shell of viable tumor, and (3) an outer zone of infiltrating tumor cells. The inner core and the shell of viable tumor are readily detectable by current imaging techniques. However, the failure of surgical resection and radiation therapy, the primary treatments for gliomas, are often attributed to inadequate detection of the outer zone of infiltrating tumor cells by standard imaging techniques. Treatment outcome may improve if more localized therapies could be prescribed to the appropriate outer zone of invasion. A technique that might offer promise in detection, DWI, is unique in that it reflects an ensemble average of the underlying microscopic structure. In biological environments, the chosen diffusion time (τ) determines the degree to which the ensemble of protons survey the microscopic structures. Currently, mainstream clinical diffusion-weighted MR is limited to τ > 25 msec. Diffusion weighting using τ > 20 msec generally agree [1,2,3]. However, this agreement diminishes when τ falls below 20 msec. [4,5,8]. 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 previously proposed that diffusion-weighted images and/or ADC maps obtained for short τ may improve the sensitivity of DWI to tumor cell invasion [8]. To further optimize these techniques we evaluated the effects of echo time on short diffusion time experiments.

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, separation of the bipolar lobes and echo times were each independently varied. *Rat Study*. Nine male Sprague-Dawley rats were inoculated, intracerebrally, with 10^5 (10μL) C6 glioma cells, which were modified to express the DsRed fluorescent protein, and imaged 13 to 15 days post-inoculation. Rectal temperature was monitored and maintained at 37°C ± 0.5°C.

Images were acquired with a round single turn RF coil. Four series of DW images were collected. For Set A, a set of bipolar SE DW images were acquired with **TE=50 msec**, TR=2 sec, Δ=δ=10 msec, **$\tau \cong$ 6.66 msec**, b-values = 300 (1x) and 1500 (4x) s/mm^2. Set B was collected identically to A but with **TE=70 msec**. For Set C, a set of bipolar SW DW images were acquired with **TE=26 msec**, TR=2 sec, Δ=δ=10 msec, **$\tau \cong$ 2.66 msec**, b-values = 300 (1x) and 1500 (4x) s/mm^2. Set D was collected identically to C but with **TE=70 msec**. From these data diffusion maps and diffusion-weighted T2 maps were created. For comparison, a MSME sequence (TE = 10, 20, 30 …160 ms, TR = 2000 ms) was used to collect the standard T2 maps. Immediately following imaging, rats were sacrificed, brains were extracted, sectioned into 5 um thick slices, stained with hematoxylin and eosin, and fluorescent images obtained, Figure 2.

Figure 1: Constant time diffusion maps from two (day 14) C6 inoculated SD rats.

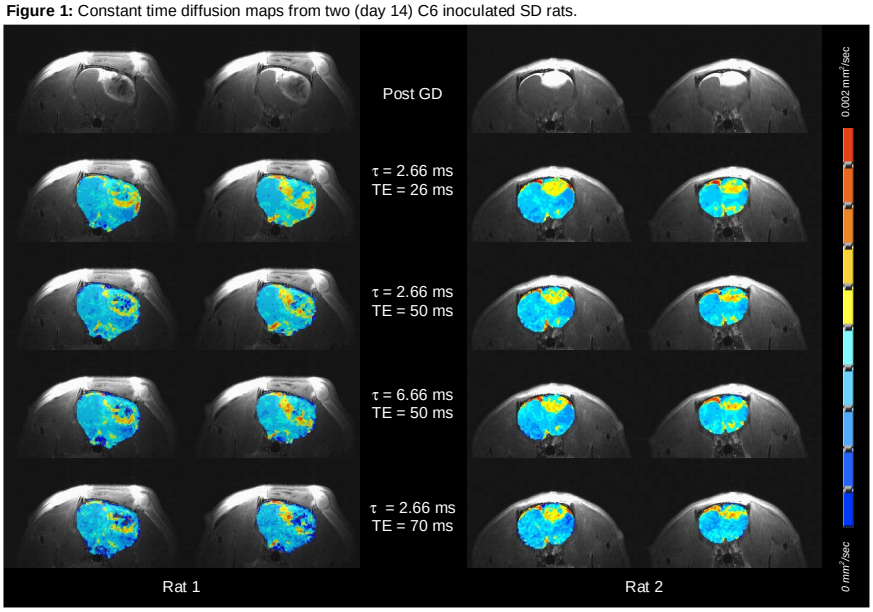


Figure 2: Fluorescent images of DsRed C6 glioma invasion taken from histological sections of Rat 2 (depicted in Figure 1). Note the pattern of C6 glioma invasion.

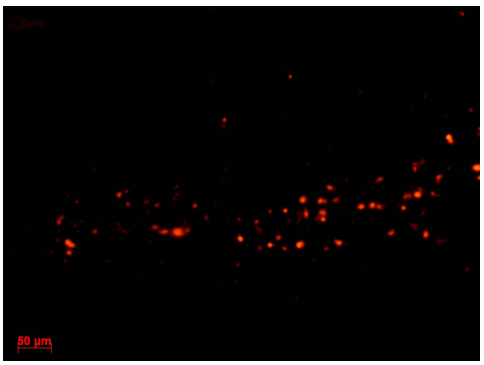
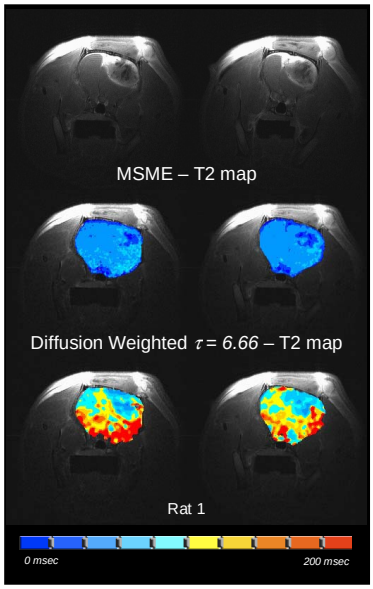


Figure 3: Standard and short time diffusion weighted T2 maps of Rat 1 (depicted in Figure 1).



Results and Discussion. Apparent diffusion coefficient (ADC) maps overlayed on T_1 weighted post-contrast images from two representative rats are shown in Figure 1. The tumor inoculation site can be seen above the right hemisphere. As seen in Figure 1, the spatial distribution and reported ADC values depend on the choice of τ and TE. This is consistent with previous studies that demonstrate a longer T_2 and a larger diffusion coefficient in nucleus compared to 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. Comparing the standard T_2 maps to the diffusion-weighted T_2 maps suggests that τ = 6.66 represent a compartment with longer T_2 values A shorter T_E may allow for a more equal contribution from all compartments, both short and long T_2 compartments, to the diffusion signal. This suggest that short time diffusion with a longer T_E may be more representative of invading tumor cells. This hypothesis will be further tested with more 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.

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