Early Detection of Tumor Treatment Response with Temporal Diffusion Spectroscopy

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Introduction

Diffusion-weighted (DW) MRI is used to characterize microstructural variations associated with tumor proliferation and/or response to therapy. Several clinical and animal studies have demonstrated the utility of DW-MRI techniques for evaluating the efficacy of tumor therapies by monitoring changes in apparent diffusion coefficient (ADC) following treatment (1-3). However, conventionally implemented methods mainly reflect changes in tissue cellularity due to the use of relatively long diffusion times (tens of milliseconds), and therefore, obscure information about structural variations on any smaller or intracellular scale. We have previously reported our development of temporal diffusion spectroscopy, a technique that is capable of probing diffusion times orders of magnitude shorter than those previously reported (4). This technique, also known as the oscillating gradient spin-echo (OGSE) sequence, employs rapid oscillations of the motion sensitizing diffusion gradient and can detect changes in structure at much shorter length scales than conventional methods. Here we show this technique can detect variations in ADC in a 9L tumor model in rats *in vivo* within 24 hours of chemotherapeutic treatment, a time when conventional methods showed no change.

Methods and Results

Both conventional pulsed-gradient spin-echo (PGSE) methods, as well as temporal diffusion spectroscopy methods, were implemented at 4.7T to measure ADC in N = 18 male Fischer 344 rats inoculated intracranially with 9L glioblastoma cells, prior to and following treatment with the anti-neoplastic agent Carmustine (also known as BCNU). Approximately 10 days following inoculation with tumor cells, PGSE images were obtained with δ = 5 ms, Δ = 30 ms, and $b = 0$ and 400 s/mm^2, while temporal diffusion spectroscopy images were collected, at the same b-value, at gradient oscillation frequencies of 120Hz and 240Hz (corresponding to effective diffusion times of 2.1 ms and 1.0 ms, respectively). Other imaging parameters were TR/TE = 2300/75.4 ms, FOV = 32mm x 32mm, matrix = 64x64, slice thickness = 2mm, NEX = 10. Following the initial imaging session, animals were treated with a single 13.3 mg/kg dose (i.p. injection) of Carmustine dissolved in a 10/90% ethanol/saline solution, and scanned 24 hours and 72 hours later using the same imaging protocol. Control animals received an equal volume of vehicle only. Five animals were sacrificed after the imaging session at 24 hours post-treatment, with seven more sacrificed after the session at 72 hours, in order to obtain histological data. Histological sections were then stained with markers of proliferation (Ki-57), apoptosis (Casp-3), and tissue morphology (H & E), and digital images of histological tissue were segmented using a k-means clustering algorithm in order to obtain mean area fractions of stained tissue sections.

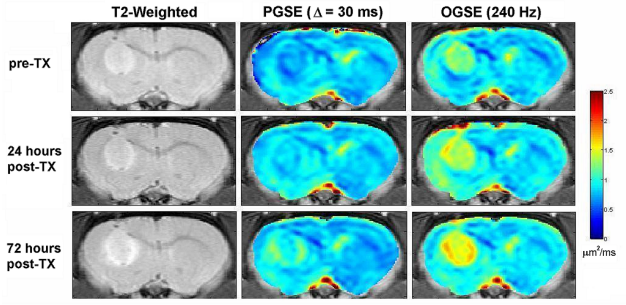

Figure 1. Representative ADC maps of a rat brain bearing a 9L glioma prior to and following treatment with BCNU. OGSE techniques clearly reveal details of tissue microstructure obscured by PGSE methods.

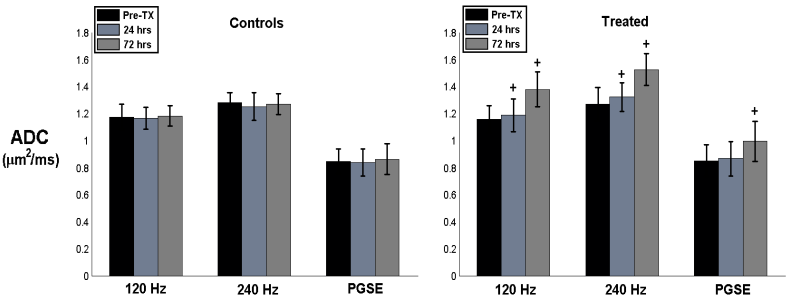

Figure 2. Bar graph showing mean ADC values for both treated and control groups. Error bars represent one standard deviation of the mean. A '+' symbol above data bars represents a statistically significant difference in ADC from pre-treatment values at the $p < 0.05$ level.

Representative ADC maps from both techniques, prior to and following treatment, are shown in Figure 1. While the ADC maps from the PGSE technique show some increase in ADC across time points, the OGSE data at high frequencies both prior to and following treatment reveal much greater details of tissue heterogeneity. Furthermore, OGSE methods were capable of revealing a significant difference in mean ADC between pre-treatment and post-treatment values at 120 Hz ($p = 0.04$) and 240 Hz ($p = 0.02$), while PGSE showed no such effect ($p = 0.13$). Both methods showed significantly different ADC from pre-treatment values at 72 hours post-treatment ($p < 0.05$). Mean ADC values across all animals are shown in Figure 2. Finally, histological data revealed no significant change in the number of proliferating or apoptotic cells between treated and control groups after 24 hours, nor was there any significant difference in extracellular space after 24 hours ($p > 0.05$). However, a significant difference in hemotoxylin stained tissue fraction (representing nuclear size) in tumor regions of interest between treated and controls groups was detected after 24 hours ($p = 0.002$).

Discussion

The early detection of the response of tumors to therapy is important both clinically and in pre-clinical assessments of novel treatments. While conventional PGSE techniques have proven effective in detecting changes in tumor cellularity, such methods are less sensitive to structural variations on an intracellular scale. OGSE methods at moderate frequency, on the other hand, have demonstrated a unique sensitivity to variations in tissue structure prior to gross changes in cellularity, as demonstrated by the ability to detect variations in tissue ADC only 24 hours after therapeutic treatment, as well as by histological evidence which showed no significant differences in cellularity after 24 hours.

References

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Acknowledgements : NIH U24 CA 126588, 1K25 EB005936, 5 RO1 CA109106, and P30 CA68485