Early measures of ADC response to radiotherapy and/or sunitinib in a murine intracranial model of human glioblastoma multiforme

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Introduction: MRI can monitor cell density, necrosis, and vasogenic edema by quantification of the apparent diffusion coefficient (ADC) (1, 2). As physiological hallmarks of glioblastoma multiforme (GBM) include hypoxia, necrosis, angiogenesis and vasogenic edema, ADC has demonstrated utility in measuring early response to radiotherapy and predicting clinical outcomes (3). Sunitinib (SU) is a multi-targeted tyrosine kinase inhibitor (TKI) that acts as an anti-angiogenic agent (AA), which may impact tumour necrosis and edema. Pre-clinical studies have shown that combining AA with radiation (RT) enhances cell killing (1). In the setting of evaluating these therapies, DWI has promise as an early quantitative imaging biomarker of response to therapy. This study evaluated the possible association of early measures of ADC response with tumor growth following treatment with SU and/or RT in an intracranial murine model of human GBM.

Methods: Sixty six to 8-week old NOD SCID mice had intracranial (IC) injection of 2 x 10^5 U87 glioma cells in the right frontal lobe (1mm anterior, 2mm lateral to bregma at 3mm depth from the dura). A multi-parametric MRI evaluation of 16 mice, including DWI, was performed every 3-4 days starting day-7 post-IC injection. T2-weighted (RARE) imaging on day 7 post-IC injection confirmed tumor presence and baseline volume to facilitate stratification to treatment arm: (1) control - placebo (2) RT alone – RT + placebo (3) SU alone - oral gavage SU (4) RT + SU. Radiation 8Gy was delivered in 1 fraction to the right hemi-brain on day 8 post-IC injection. Sunitinib treatment consisted of 0.8mg in 0.4 mL CMC solution and placebo consisted of 0,4mL CMC delivered by oral gavage each morning for 7 weekdays (9 days total, days 8-17 post-IC injection). MRI scanning used a 7-Tesla Bruker BioSpec 70/30 with the B-GA12 gradient coil, 7.2cm linear volume transmitter, and murine slider bed with head coil. Sessions included 2D-RARE for anatomy (TE/TR=72/5000ms), DW-EPI (b=1000s/mm^2, 3-directions, 9 EPI shots, TE/TR=24/3000ms) and contrast-enhanced T1-weighted RARE (TE/TR=8/1200ms), each with consistent slice prescription and image resolution (125x125x500-µm voxels). Contrast delivery (15µl Gd-DTPA) was accomplished manually using a precision 50µl-volume 27-G Hamilton syringe and tail vein cannula. The contrast-enhanced scan was started at 5-minutes post injection. Imaging processing and manual ROI segmentation used MIPAV (NIH, Bethesda, MD). Tumor volumes were drawn on T1w-RARE images as the integrated region of signal enhancement. Tumor ROIs were applied directly onto ADC maps, to extract mean and standard deviation ADC via histogram analysis. An additional control ROI was manually delineated at the contra-lateral brain.

Results: Based on day 7 post-IC T2w images, 5 mice were excluded from further analysis, as they had no visible tumour. Control mice became symptomatic by day 14 and both RT alone and control mice were euthanized after imaging day 14, whereas RT+SU and SU-only arms were imaged until day 21 then euthanized. Control animals demonstrated exponential tumor growth between days 10 and 14 whereas each of the treatment arms demonstrated tumour growth delay through towards day 14 (less than 3.3mm^3). The Rad+SU arm demonstrated continued benefit at day 21 compared to the SU-only arm (RT+SU: 9.5±7.2mm^3; SU: 48±29mm^3). Quality assurance evaluation of ADC identified shifts in global ADC between imaging slices, such that tumor and CL brain ADC were linearly correlated (slope = 1.11±0.12, R=0.83). Consequently, tumor ADC values were quantified as their percentage elevations compared to the contra-lateral brain. Baseline ADC values were 8±5% above CL brain across all animals. From baseline, controls demonstrated moderate ADC elevations through to day 10 (from 5±5% at baseline to 16±4% at days 7 and 10), then elevated to 39±5% at day 14. SU alone maintained ADC elevations within 19±19% through to day 10, elevating to 21±6% and 28±4% at day 21. Radiation-treatment arms demonstrated considerable ADC responses, with the radiation-only arm displaying ADC elevations of 17±3%, 24±6%, 43±10%, and 29±19% at days 3, 7, 10, and 14. The RT+SU arm displayed ADC elevations of 18±8, 25±4%, 28±7%, 40±8%, and 32±3% at days, 3, 7, 10, 14, and 21 post-treatment.

Summary and Conclusions: Both RT trial arms had considerable early elevations in ADC at post-treatment day 3, and ADC remained elevated until substantial tumour growth occurred, whereas sunitinib monotherapy maintained ADC below all 3 other arms. Both radiation arms provided sustained tumor control compared to SU alone, which maintained growth delay only during treatment. Therefore, these early ADC changes may be a useful biomarker of treatment response and growth delay. Multiple mechanisms may be contributing to ADC response differences between RT, SU, and control mice including radiation cytotoxicity, sunitinib-induced cytostasis and vascular changes, necrosis, and tumor vasogenic edema. This complexity motivates parallel investigation of additional MRI biomarkers including T2 and DCE to investigate the etiology of these ADC changes. A diffusion QA phantom will aid in addressing standing bias in ADC methodologies.

References: (1) Mendel et al, Clin Cancer Res, 2003; (2) Oh et al, *J Magn Reson Med*, 2005; (3) Moffat et al, Neoplasia, 2006;

Figure: (A) Representative T1w-RARE and ADC images from a RT-SU animal at baseline (top row) and at day 10 (bottom row); (B) Tumor growth delay (control - circle; SU - down-triangle; RT+SU - up-triangle; RT – square; RT/RT+SU/SU are overlaid through day 15) ; (C) ADC timecourse (same symbols as (b)).