Serial multiparametric MRI in study design and response evaluation of radiation and antiangiogenic therapy in an intracranial murine glioblastoma model

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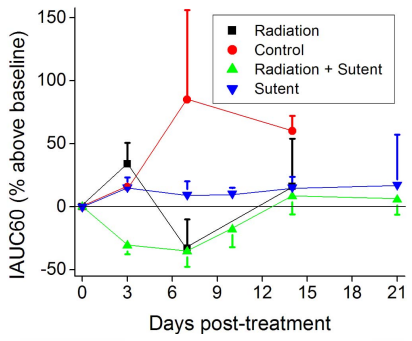
Introduction: A major challenge in preclinical studies of intracranial mouse tumour models is the ability to non-invasively identify mice with established tumours, measure tumour size for stratification and quantitate response to therapy.[1] The evaluation of new anti-angiogenic agents pose further challenges in evaluating treatment response to these cytostatic agents, which may have early vascular effects with little change in tumour volume. Sunitinib (SU) is a multi-targeted tyrosine kinase inhibitor (TKI) that acts as an anti-angiogenic agent (AA).[2] Pre-clinical studies have shown that combining AA with radiation (RT) enhances cell killing.[3] This study utilized serial micro-MRI to select mice with established intracranial tumours at baseline, measure tumour size and growth, and measure vascular changes with serial DCE-MRI following treatment with radiation and/or sunitinib in order to identify potential early imaging biomarkers that predict for tumour growth delay.

Methods: Sixty 6 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 ant, 2mm lat to bregma at 3mm depth from the dura). T2-weighted (RARE) imaging on day 7 post-IC injection confirmed tumor presence and baseline tumor size to facilitated stratification to treatment arm: (1) control - placebo (2) RT alone – RT + placebo (3) SU alone - oral gavage SU (4) RT + SU. Radiation 8 Gy was delivered in 1 fraction to the right hemi-brain on day 8 post-IC injection. Sunitinib treatment consisted of 0.8 mg in 0.4 mL CMC solution and placebo consisted of 0.4mL CMC delivered by oral gavage for 7 weekdays (treatment days 1-9). MRI scanning utilized a 7-Tesla Bruker BioSpec 70/30 with the B-GA12 gradient coil, 7.2cm linear volume transmitter, murine slider bed, and murine head coil. Four mice from each arm had serial imaging sessions, which included: (1) 2D-RARE anatomical imaging (TE/TR=72/5000ms; 125x125x500-µm voxels); (2) T1 was quantified using a saturation-recovery RARE (SR-RARE) technique in 5 contiguous 500 µm slices (TE=14ms; TR=450, 700, 1000, 1500, 3000, 5000ms; in-plane resolution 250x250µm); (3) DCE-MR (TE/TR=2.3/39.1ms; flip angle 35 degrees; matched spatial resolution and slice prescription to SR-RARE; temporal resolution 2.5seconds per repetition of the slice set; 100 repetitions) with contrast delivery (0.38mmol/kg Gd-DTPA) by manual injection over 6 seconds via a tail vein cannula, utilizing a precision 50µl-volume 27-G Hamilton syringe, started after 6 baseline images. (3) contrast-enhanced T1-weighted RARE anatomical imaging (TE/TR=8/1200ms) with matched slice prescription and image resolution to 2D-RARE, started at 5-minutes post contrast injection. Image processing and manual segmentation utilized MIPAV (NIH, Bethesda, MD). Tumor ROIs were delineated as the integrated region of signal enhancement across each multi-slice T1w-RARE acquisition. A ROI was delineated at the slice with maximal tumour diameter on DCE-MRI and propagated across all time points. Signal intensity curves for each ROI was generated on MATLAB. Initial area under the curve at 60 sec (iAUC60) were extracted for each mouse from each time point. Images with out-of-plane motion artifact were excluded from the analysis. The sagittal artery was consistently identified as the AIF and signal time-intensity data was captured. ROIs were also copied onto SR-RARE images for T1 quantification using a 3-parameter fit of monoexponential recovery.

Results: Based on day 7 post-IC T2w images, 5 mice were excluded from further analysis as tumour was not visualized. The mice with tumours were allocated to treatment arms such that average baseline tumour volumes for all arms were between 0.54 $\pm$ 0.30mm^3 and 0.61 $\pm$ 0.31mm^3, not statistically significantly different. Of the 16 mice followed for imaging, one SU-only mouse died following oral gavage and one RT only mouse died during urine collection. Of note, no mice expired with imaging. 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 sacrificed. Serial imaging demonstrated exponential tumour growth in control animals. Each of the treatment arms demonstrated tumour growth delay until day 14 (less than 3.3mm^3), but by day 21 the Rad+SU arm demonstrated longer tumour growth delay than SU alone (RT+SU: 9.5±7.2mm^3; SU: 48±29mm^3). The iAUC60 values increased day 3 post-RT in the RT alone arm. Sunitinib resulted in slightly increased iAUC60 by day 3 and then remained stable throughout the duration of sunitinib. In the SU + RT arm, there was an evident early drop in iAUC60 30.1$\pm$6.8% in all mice, which rose after the sunitinib treatment was completed. The iAUC60 increased and became more variable with tumour growth. Based on measures from 9 mice, tumor T1 was noted to be significantly lower compared to contralateral normal brain (2280 +/- 160 (n = 9) vs 2559 +/- 138, p=0.0003), to support future quantitative perfusion analysis.

Summary and Conclusions: This study demonstrates feasibility of using multiparametric micro-MRI to overcome the common challenges of intracranial mouse tumour models. Baseline T2w images were used to include only mice with visible gross tumour and to stratify mice to treatment arms based on baseline tumour size. Serial MRI images were then used to quantitate tumour growth and vascular changes on DCE-MRI (iAUC60) with radiation and/or sutent treatment. Following both RT and SU monotherapy, iAUC60 increased by day 3. The rise in iAUC60 after RT is consistent with previous studies of acute radiation injury to brain vascularity.[4] The rise in iAUC60 following SU alone has been noted in a recent study of renal cell carcinoma and is contrary to the effects of pure VEGF blockade.[5] Importantly, the combination of the two treatments resulted in a significant decrease in iAUC60 at day 3 and this reduction in iAUC60 was maintained throughout the duration of sunitinib treatment. The subsequent increase in iAUC60 occurred with tumour growth, as it was seen with all other groups with tumour growth. These early changes in vascular parameters measured by DCE-MRI may be useful early biomarkers for treatment response, predicting subsequent tumour growth delay and survival. Further analysis will progress to a quantitative perfusion modeling analysis with incorporation of measured AIF and T1.

(1)



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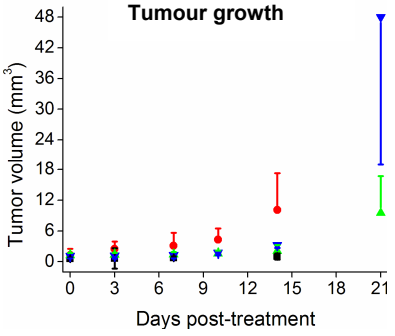

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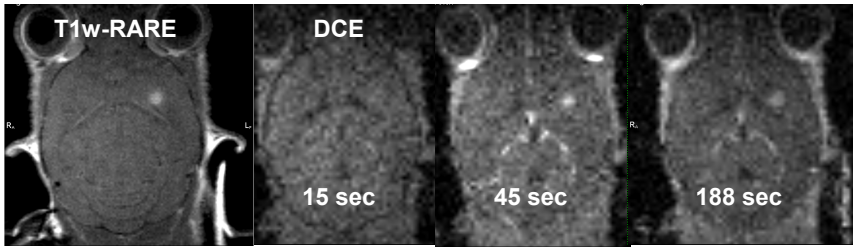

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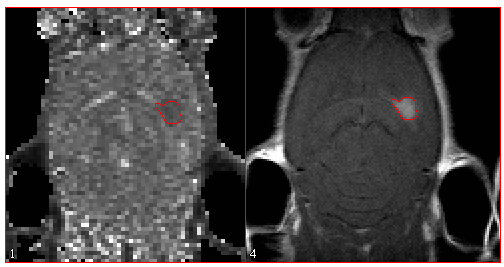

Figures: (1) Percent change in iAUC60 over time (2) Tumour growth delay (3) Representative DCE and post-contrast T1w-RARE images from a RT+SU animal at baseline (4) Representative quantitative T1 map and post-contrast T1w-RARE from RT + SU animal at baseline

References: [1] Bock – Neoplasia 2003 [2] Schueneman – Cancer Res 2003 [3] Wachsberger - Clin Cancer Res, 2005 [4] Olsson – Neuropath and Applied Neurobiol 2008 [5] Hillman – Neoplasia 2009