Novel Insights into Intravenous Bradykinin Analogue-Mediated Vasomodulation from Dynamic Contrast-Enhanced MRI of RG-2 Rodent Malignant Gliomas

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INTRODUCTION: There are discontinuities within and between endothelial cells that line the micro-vessels of malignant gliomas resulting in a dysfunctional blood-tumor-barrier (BTB) compared to the normal blood-brain-barrier (BBB). Intravenous infusion of Cereport, a synthetic bradykinin analogue, has been shown by quantitative autoradiography (QAR) to temporarily enhance drug delivery to malignant gliomas (1). Although historically QAR has been utilized to measure tumor vascular parameters during systemic vasomodulation, the major limitations of this method include incompleteness of vascular input function (VIF) shape determination due to manual sampling of blood at only certain specific time points, and inability to acquire tumor tissue concentration data over time in the same animal. Dynamic Contrast-Enhanced MRI (DCE MRI) allows for continuous measurement of change in Gd-DTPA concentration within brain vasculature and glioma tissue over time, and therefore can provide novel insights into bradykinin analogue-mediated changes in tissue vascular parameters during vasomodulation.

OBJECTIVES: Our primary objective was to utilize Gd-DTPA DCE MRI to measure changes in tumor vascular parameters within the same RG-2 rat glioma at baseline and during systemic vasomodulation with short-acting and long-acting bradykinin analogues, Met-Lys-bradykinin (Met-Lys-BK) and Cereport, respectively. Our secondary objective was to evaluate volume effects on measured vascular parameters at baseline and during vasomodulation. For our study we utilized a two-glioma model in which separate areas of rat brain were inoculated with 100,000 and 20,000 RG-2 cells to produce larger and smaller gliomas within each rat brain.

METHODS: All experiments were conducted in accordance with an Animal Study Proposal approved by the NIH Clinical Center Animal Care and Use Committee. The right caudate (anterior) and left thalamus (posterior) of male Fischer rat brain were stereotactically inoculated with either 100,000 or 20,000 RG-2 cells to produce larger and smaller gliomas. The tumor bearing brains of each animal was imaged at post-inoculation day 10-11 following placement of a femoral venous and arterial canulas. Images were acquired on a 3.0-T MR scanner (Philips Intera; Philips Medical Systems; Andover, MA) using a 7-cm small animal solenoid coil. During scanning, rats were kept under isoflurane anesthesia. After a baseline 3D T1W-GRE sequence (TR 8.1 ms, TE 2.3 ms, FA 3°, ST 1 mm [over sampled to 0.5 mm], 16[32] slices, FOV 76.8 mm, MTX 256×256) dynamic scans were acquired at 20 seconds per volume (FA 12°, other parameters same as the baseline 3D GRE sequence). Two sequential 1-hour scans were conducted and intravenous Gd-DTPA (0.10 mmol/kg) was administered over one minute at the beginning of each scan, for acquisition of baseline and treatment tumor concentration curves over 60 minutes, respectively. Three minutes prior to the second Gd-DTPA administration a Normal Saline (NS, Treatment Group 1), Met-Lys-BK (Treatment Group 2), or Cereport (Treatment Group 3) infusion was begun and infused for 15 minutes. All image data were analyzed using Analysis of Functional Neuroimages (http://afni.nimh.nih.gov/) and custom-built MatLab (Mathworks, MA) scripts. Following image acquisition the dynamic scans were motion corrected and co-registered to the initial scan using 3Dvolreg (AFNI). Whole tumor, contra-lateral brain, and superior sagittal sinus (VIF) ROI masks were drawn manually using signal intensity dynamic scan data. The average ROI signal intensity data were converted to concentration curves in MatLab using baseline T1 map without contrast, previously measured molar T1-relaxivity of Gd-DTPA (4.05 $mM^{-1}s^{-1}$) and measured animal hematocrit.

DATA AND STATISTICAL ANALYSES: The VIF shape was assessed qualitatively. Non-model (wash-in, wash-out, mean maximum concentration, time duration at mean maximum concentration, and AUC) and 2-compartment 3-parameter model-based (K^{trans}, K_{ep}, V_e, V_p) tumor vascular parameters, at baseline (1st measurement) and following treatment (2nd measurement), were calculated from Weiner-filtered dynamic scan concentration curve data (2). For statistical analyses of rat brains containing 2 tumors (all except 2 brains) the multiple measurements of any given vascular parameter outcome from each rat were considered as correlated observations without imposing any particular covariance structure (i.e. unstructured). Across and within treatment groups, linear mixed effects model-based analyses were performed with tumor volume being a covariate so that the treatment effect was established after being adjusted for by the volume effect. First, the effect of volume on baseline (1st measurement) parameters of all tumors was determined. Second, the effect of treatment on tumor vascular parameters was studied with tumor volume being used as a covariate and the 1st measurement serving as an internal control (ratio). Third, post hoc analyses to observe volume effects were conducted on differences in those vasomodulator treatment parameters that were found to be statistically significant compared to NS treatment. Analyses were implemented in SAS PROC Mixed (3). A significance level of 0.05 was used for all statistical tests.

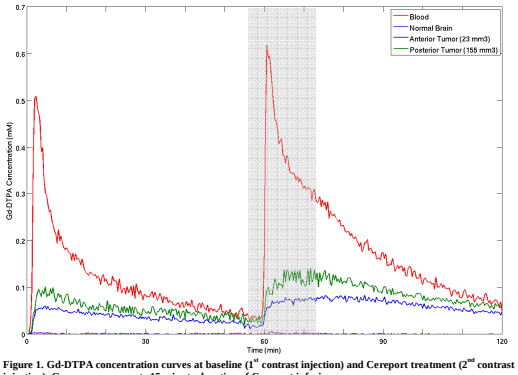

Figure 1. Gd-DTPA concentration curves at baseline (1st contrast injection) and Cereport treatment (2nd contrast injection). Grey area represents 15 minute duration of Cereport infusion.

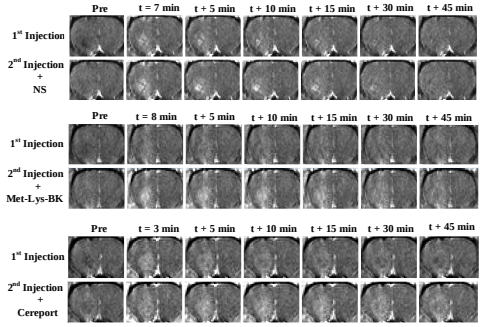

Figure 2. Right frontal RG-2 glioma contrast enhancement at selected time points after initial (1st) and repeat (2nd) Gd-DTPA injection. Second Gd-DTPA injection occurs during infusion of either Normal Saline (NS), Met-Lys-BK, or Cereport. The images of dynamic scans at time points t = 7, 8, and 3 represent maximum contrast enhancement following first Gd-DTPA injection.

RESULTS: There was a visually apparent broadening of the VIF shape in the setting of vasomodulation but not NS treatment (**Figure 1**). There was a statistically significant effect of volume on baseline tumor (N=40) vascular parameters with an increase in mean maximum concentration, AUC, K^{trans}, K_{ep}, V_e and decrease in contrast wash-out with an increase in tumor volume (range 16-882 mm^3). Meanwhile effect of volume on wash-in, time duration at mean maximum concentration, and V_p failed to show significance. These findings indicate that baseline RG-2 permeability increases with increasing tumor size. With volume as a covariate, the effect of Met-Lys-BK (N=14) treatment on tumor parameters compared to that of NS (N=11) was an increase in mean maximum Gd-DTPA concentration and AUC, and decrease in contrast wash-out. The effect of Cereport (N=15) as compared to that of NS on tumor vascular parameters was an increase in time duration of Gd-DTPA at mean maximum concentration, slower contrast wash-in, and a decrease in wash-out, K^{trans}, and K_{ep}. This data shows that Met-Lys-BK increases tumor contrast concentration to a greater extent than Cereport, while Cereport treatment results in a longer persistence of Gd-DTPA within glioma tissue than with Met-Lys-BK (**Figure 2**). Permeability-related parameters (wash-in, K^{trans}, and K_{ep}) were not statistically different with Met-Lys-BK treatment, while there was a slower wash-in and decreased K^{trans} and K_{ep} during Cereport infusion. Post hoc analyses revealed positive volume effect on wash-in (faster) and wash-out (slower) with Cereport but not Met-Lys-BK.

DISCUSSION AND CONCLUSIONS: The broadening of VIF reflects systemic hemodynamic changes that lead to prolonged intra-vascular retention of Gd-DTPA. Due to the longer residence time of Gd-DTPA within the poorly auto-regulated tumor microvasculature, the permeable fraction of the contrast agent would persist within the extra-vascular extra-cellular tumor space. The lack of measurable tumor permeability-related vascular parameter changes with vasomodulation may be attributed to Gd-DTPA being a low molecular weight extra-cellular contrast agent that is blood flow dependent. Taken altogether our Gd-DTPA DCE MRI findings suggest that enhanced delivery of Gd-DTPA across the RG-2 glioma BTB during bradykinin analogue-mediated vasomodulation is largely due to the systemic actions of these peptides and not *per se* selective for the brain tumor micro-vasculature.

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