The effect of increasing concentrations of intracranial albumin on fluid flow rates within adjacent white matter tracts in rats Carmen Kut¹, Vadappuram Chacko², Betty Tyler³, Arvind Pathak², Zaver M Bhujwalla², Xingde Li¹, Elliot R McVeigh¹, Daniel A Herzka¹, and Stuart A Grossman⁴ Biomedical Engineering, Johns Hopkins, Baltimore, MD, United States, ²Radiology, Johns Hopkins, Baltimore, MD, United States, ³Neurosurgery, Johns Hopkins, Baltimore, MD, United States

TARGET AUDIENCE: This study is intended for clinicians and researchers interested in imaging brain cancer dissemination patterns using MRI.

PURPOSE: In most systemic cancers, tumor cells are passively carried by extracellular fluid flow to regional lymph nodes. Although the brain does not contain lymphatics, the dissemination of gliomas within the brain may be a function of extracellular fluid flow [1-5]. Malignant brain tumors are characterized by a disrupted blood-brain-barrier that results in albumin leaking from blood vessels, which osmotically pulls water into brain parenchyma thereby increasing extracellular fluid flow rates in white matter tracts (WMT). High flow rates may increase tumor dissemination via the WMT. This study sought to determine if increasing intracranial albumin concentrations affect WMT flow rates. If true, restricting albumin extravasation (through steroids or VEGF inhibitors) has the potential to reduce glioma dissemination and determining WMT draining patterns non-invasively via MRI has the potential to identify high-risk areas for recurrence in the brain in patients.

METHODS: 1. Effect of Albumin Concentrations on Extracellular Fluid Flow Rates Along Adjacent White Matter Tracts: Albumin (0.12-6 mg) were stereotactically injected directly into brain parenchyma of 15 adult female SD rats (A1-15) to simulate passage of increasing amounts of albumin across the blood brain barrier. The albumin was labeled with Evans Blue. The distance that the labeled albumin was carried over a fixed period of time was used to estimate WMT fluid flow rates using histologic (Evans Blue-albumin complex) measurements. 2. Comparison of Evans Blue Labeled Albumin (Autopsy) and Gadolinium Labeled Albumin

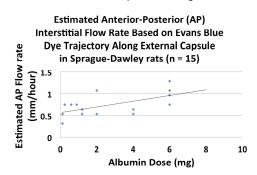


Figure 1 Higher Albumin Concentrations Increase Fluid Flow Rates Along White Matter Tracts Based on Evans Blue Trajectories (R = 0.57)

(MRI) Flow Down White Matter Tracts. A gadolinium-albumin-Evans Blue solution was stereotactically injected directly into brain parenchyma of 4 rats to simulate passage of known amounts of albumin across the blood brain barrier. MRI Scans (2D and 3D FLASH, RARE) were obtained and distribution of the EB-albumin from autopsy) and GD-albumin (from MRI Scans) and respective flow rates were roughly compared. Sequences used include 2D FLASH with TE/TR = 2.3/300 ms, flip angle = 30° and slice thickness = 0.5mm; 3D FLASH sequence with TE/TR = 2.3/20 ms and flip angle = 15°; 2D RARE at TE/TR = 8-120/4000 ms and slice thickness of 0.5mm. A control rat was imaged to ensure that Evans blue alone does not affect image quality.

RESULTS: 1. Albumin Study 15 rats received bovine albumin and 2% Evans blue intracranial. Average anterior-posterior (AP) flow rate for rats receiving 0.12 mg albumin (n = 2) was 0.43 mm/hour; and for 6 mg albumin (n = 4), the average AP flow rate was 1.02 mm/hour (p = 0.029). Positive correlation was observed between albumin dose administered and interstitial fluid flow rates (n = 15, R = 0.57). 2. MRI Study At 6-8 hours post injection, the Evans blue-gadolinium-albumin dye traversed 1.1 mm/hour at 3 mg albumin dose (Figure not shown) and traversed 1.4 mm/hour at 6 mg albumin dose based on 2D T1 FLASH scans (Example shown in Figure 2). This is consistent with our Evans blue WMT flow rate results, which reports AP flow rate of 0.5-1.1mm/hour at 2-4 mg albumin dose (n = 4), and 0.8-1.3 mm/hour at 6 mg albumin dose (n = 4). In addition to flow rates, bright edema signal surrounding the external capsule at the right frontal lobe at 2 hours and 6 hours post injection (Figure 2). This is in contrast to the dark gadolinium-albumin signal along the external capsule (since gadolinium decreases both T1 and T2 values). Water travels faster than albumin, leading to edema presence before enough water is drawn osmotically into the white matter tracts, seen at the bottom of the external capsule in T2 Image (RARE) 2 hours post injection. Evans blue results from autopsy post-imaging are also reported (Figure 2)

DISCUSSION: As expected, when albumin labeled with Evans blue was injected intracranially it was carried by the bulk flow of extracellular fluid along nearby white matter tracts (Figure 1). This suggests that the injected albumin osmotically pulls water into the brain from the capillaries producing more edema around the injection site which increases bulk flow rates down the white matter tracts. This is also supported by the MRI studies which demonstrated markedly increased edema (T2 signal) in the hemisphere where albumin was injected, and T2 abnormality extended further than the signal from gadolinium, suggesting that water travels faster than albumin and fluid flow rates are dependent on particle size (Figure 2). Finally, our results suggest that non-invasive MRI scans can be used to track the flow of labeled albumin down white matter tracts draining a focal site of albumin entry in the brain. Identification of the white matter tracts "draining" extracellular fluids from the tumor could lead to modification of standard radiation fields akin to standard practice in systemic cancers where areas at high risk of recurrence, such as draining lymph nodes, are included in the initial radiation fields. As a result, further studies outlining the role of extracellular bulk flow in patients with high grade gliomas are of critical importance.

CONCLUSION: Our study for intracranial injections suggests higher intracranial albumin concentrations result in accelerated extracellular fluid flow along WMT as demonstrated by MRI and corroborated by staining. These findings suggest that restricting extravasation of albumin through the blood-brain-barrier (e.g. via glucocorticoids and VEGF inhibitors) has the potential to reduce glioma dissemination. In addition, determining specific WMT draining extracellular fluid from a patient's malignant glioma using MRI has the potential to non-invasively identify regions of the brain at highest risk for recurrence.

REFERENCES: [1]: Geer CP et al (1997) J Neurooncol 32(3):193-201; [2]: Cserr HF et al (1971) Physiol Rev 51 (2): 273-311; [3] Cserr HF et al (1981) Am J Physiol 240(4):F319-328; [4] Abott NJ et al (2004) Neurochem Int 45 (4):545-554; [5] Rosenberg GA et al (1980) Am J Physiol 238 (1):F42-49

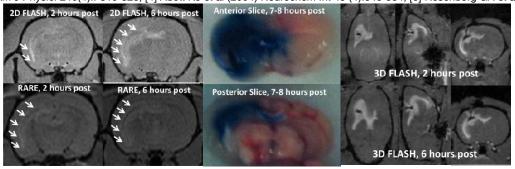


Figure 2 (Left): Gd-Albumin trajectory ofT1 FLASH images demonstrate bright gadolinium signal (arrows) along external capsule ipsilateral to injection site. T2 RARE images demonstrate gadolinium signal (dark) and accompanied by surrounding edema (bright) along the external capsule (arrows). (Middle): Evans blue flow patterns, as shown in coronal slices obtained at autopsy. (Right) 3D Gd-Albumin Trajectory; dark spot indicates injection track.