In vivo and ex vivo Characterization of Extracellular Space (ECS) in mouse GBM using PGSE and OGSE

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TARGET AUDIENCE – Researchers interested in ECS / tumor microstructure characterization

PURPOSE: The extracellular space (ECS) forms a conduit for essential substances to move between blood vessels and cells. In cancer, the ECS volume fraction is closely related to cell density and the interstitial fluid pressure (IFP), making of the ECS fraction a very interesting marker for tumor diagnosis and treatment response evaluation. Information about the ECS volume fraction can also help better understand many of quantitative MRI data, including dynamic contrast enhanced MRI. To date, however, accurate measurement of ECS fraction remains challenging. Diffusion weighted imaging (DWI) is a promising tool to measure cellular structural characteristics non-invasively. Nuclear and cellular restriction of water diffusion in cancer cells is typically expected in 5-10 µm range which cannot be fully assessed by using either pulsed gradient spin echo (PGSE) or oscillating gradient spin echo (OGSE) diffusion experiment alone on conventional preclinical hardware. However, there is a paucity of studies utilizing a combined PGSE and OGSE DWI experiment for the investigation of the ECS. Hence, the purpose of this study was to characterize the tumor environment geometry and extract ECS information using a DWI experiment with a range of diffusion times using PGSE and OGSE, both in vivo and ex vivo. Validation is performed by comparison of ex vivo data with immuno-staining.

METHODS – 10^6 GL261 murine GBM cells (5µL) were injected into the subcortex of 6-8 week old C57BL/6 mice (n=9, female) under anesthesia (air +3% isoflurane). The MR protocol consisted of 10 OGSE and 4 PGSE measurements at 7T (Bruker Biospec Avance2, Etlingen), probing diffusion properties with diffusion times = 6/8/16/31 ms for PGSE and frequencies in the range of 60-225 Hz for the fast ramp cos-OGSE (TR/TE=3000/70ms, BW=300kHz, 1 readout segment, NA=20, NR=2, res. 250x250x1500 µm, FoV 20x20 mm, b=[0,200,400] s/mm^2, dir. (1,1,1), TA=6 min, total time 84 min). The mice were scanned once between Day 14 and 28 after tumor implantation and sacrificed for histology. After fixation, 100µm thick coronal slices were sectioned for direct ex vivo MR examination using a dedicated custom-built histological coil [1] (Fig. 2A). To improve multi-modality co-registration, the neighboring 20µm slices were used for immuno-histochemistry (GLUT1 with hematoxylin counterstaining). Our geometrical model for the tumor environment consists of 3 compartments (cytoplasm/nuclei/ECS) characterized by 6 parameters: f, the intracellular fraction (=1-ECS); R_c and R_n, the radii of cells and nuclei; D_n, D_c and D_{ECS}, free diffusivities inside nucleus, cytoplasm and ECS, respectively. For short diffusion times (1.25-30ms) used in this study, water exchange was assumed negligible such that the total MR signal can be described by a linear combination of MR signals in individual compartments. The attenuation inside a sphere or a shell was calculated as in [2] for PGSE and [3] for OGSE. Diffusion in the ECS was considered Gaussian as a first approximation. The fit stability was improved by setting D_n/D_c=1.6/1.3 µm^2/ms [4-5], and R_n=3.5µm (based on electron microscopy). Parameter estimation was performed in Matlab (The Natick, MA) using non-linear fitting and in-house programs as follows: **1.**voxel-by-voxel to derive parametric maps and **2.**after MR signal average in tumor sub-ROIs based on ADC_{PGSE} (9 bins x 0.1 µm^2/ms window).

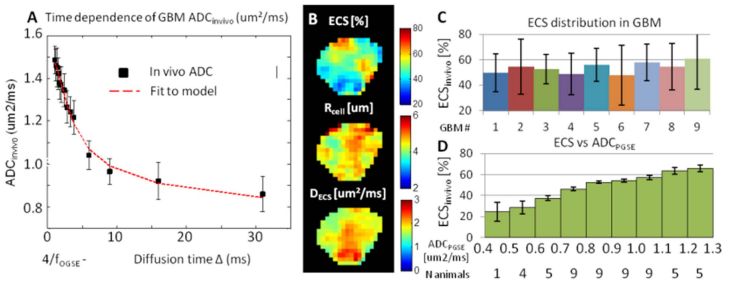

Figure 1.In vivo experiment. A. average ADC_{invivo} in GBM and best fit to model. **B.** ECS, R_{cell} and D_{ECS} parametric maps **C.** Intra-tumoral ECS distribution (9 animals) **D.** ECS estimation after averaging of MR-signal based on ADC_{PGSE}. The errorbars represent the standard deviations (Nanimals<9) weighted for the different number of voxels per animal.

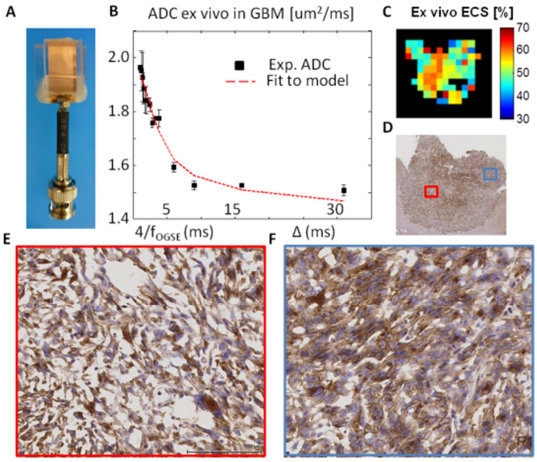

Figure 2. Ex vivo experiment. A. MR histocoil used to observe **B.** time-dependence of ex vivo ADC. **C.** Parametric ex vivo ECS map (thickness 100µm) and **D.** Immuno-staining (GLUT1) of the same tumor (thickness 20µm). **E-F.** 20x magnification in ROIs presenting high (B, red) and low ECS (C, blue) in C.

RESULTS – IN VIVO: Inside the GBM, the ADC_{invivo} was highly dependent on diffusion time / oscillation frequency (Fig. 1A, +80% ADC between Δ=31/1.2 ms). The average tumor ADC_{invivo} (black squares, n=9) could be best described by the following geometry: ECS=45%, R_n/R_c=3.5/4.6µm, $D_n/D_c/D_{ECS}$=1.6/1.3/2.1µm^2/ms. The average GL261 ECS_{invivo} varied between [45-60]% (N=9), with important intra-tumoral ECS variability (>30%, Fig. 2B-C). Both R_c and D_{ECS} followed normal distribution with moderate variability inside GBM (R_c=4.2±1.2µm, D_{ECS}=1.7±0.4µm^2/ms). ADC_{PGSE} correlated mildly with D_{ECS} and ECS_{invivo}, but not with R_c (corr. coefficient $\rho_{SPEARMAN}$ =0.32/-0.25/0.08, N=906 voxels). Fitting after MR signal averaging based on ADC_{PGSE} highlighted a clear relationship between ECS_{invivo} and ADC_{PGSE} (Fig. 1D). EX VIVO: Fits performed with MR histocoil data (Fig. 2A) on ex vivo samples (Fig. 2B) suggest good agreement between ECS (Fig. 2C) and preliminary data obtained with immuno-histochemistry (Fig. 2D), as illustrated by Figs. 2E and 2F in regions of high and low ECS, respectively.

DISCUSSION AND CONCLUSION - Compared to other geometrical models, such as VERDICT [6], this 3-compartment model provides voxel-based values for the ECS (45-60 %), R_c (4-10 µm) and D_{ECS} (1.6-2.5 µm^2/ms), at the expense of the vascular compartment. ECS_{invivo} fit results (average and variability) are in agreement with literature review on ECS in brain tumors prone to necrosis (see Table 1 in [7]). Our preliminary data suggest that DWI can be used to characterize and quantify the ECS in tumors non-invasively and detect the formation of necrotic areas, seen as hot spots on ECS maps (see Fig. 1B & 2C). Despite smaller thicknesses, ex vivo measurements were made possible by using a histological coil, enabling greater B_1 uniformity and unsurpassed increase in sensitivity (> 650%) compared to any room temperature animal RF coil. Further quantitative processing of immuno-staining is being performed in order to validate the MR-based ECS estimation using PGSE+OGSE time-dependent diffusion.

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