Interstitial fluid pressure correlates with water diffusion coefficient in mouse mammary tumor model

S. Kim[1], L. DeCarlo[2], G. Y. Cho[1], J. H. Jensen[1], D. K. Sodickson[1], L. Moy[1], S. Formenti[3], R. J. Schneider[2], and E. E. Sigmund[1]
[1]Center for Biomedical Imaging, Radiology, New York University, New York, NY, United States, [2]Microbiology, New York University, New York, NY, United States, [3]Radiation Oncology, New York University, New York, NY, United States

Introduction:
Effective delivery of therapeutic drug is often impeded by physiological barriers. Such barriers can be created by abnormal (fast growing, leaky, sometimes dysfunctional) tumor blood vessels, high tumor cellularity, and lack of functional lymphatics, which result in elevated interstitial fluid pressure (IFP) (1). It has been reported earlier that elevated IFP can lead to reduced tumor blood flow (2). However, to date, it has not been reported such reduced flow can be measured using MRI noninvasively. The purpose of this study was to investigate the feasibility of using Intra-Voxel-Incoherent-Motion (IVIM) diffusion weighted imaging (DWI) to measure tumor blood flow and the association of IVIM diffusion coefficients with IFP.

Figure 1. Representative images of subcutaneous 4T1 tumor: (a) T2 weighted RARE image, (b) EPI without b=0, (c) EPI with b=1000 s/mm^2. Region of interest was drawn to select the entire tumor indicated by the arrow.

Materials and Methods: Six- to eight-wk-old BALB/c mice (n = 10) were given a subcutaneous injection in the right flank (n = 7) or in both flanks (n = 3) with 1 x 10^6 4T1 mouse mammary tumor cells suspended in 0.1 ml of PBS on day 0. Five mice were scanned on day 10~13 when the longest diameter of the tumor was about 10 mm. The other five mice were given an intraperitoneal injection of 0.1 ml of 1mg/ml Avastin (Genentech, CA) and were scanned approximately 24 hours later.
MRI was performed using a 7T horizontal bore magnet with a volume transmit and receive coil. General anesthesia was induced by 1.5% isoflurane in air. The animal was mounted on a cradle with respiratory and temperature monitoring probes. The animal body temperature was maintained at 32 ± 2 ºC during the scan. A T2-weighted rapid acquisition with relaxation enhancement (RARE) sequence was used to image the entire tumor (TR = 2s, TE = 35ms, FA=180º, res = 0.18 x 0.18 x 1.5 mm, 10 slices), and to select two slices near the tumor center. A pulsed gradient spin echo (PGSE) diffusion measurement (δ = 7ms, Δ = 14 ms) was performed with 16-shot echo planar imaging sequence (TR = 1.5 s, TE = 32 ms, resolution = 0.36 x 0.36 x 1.5 mm, 2 slices). The diffusion weighting gradient was varied from 0 to 28 G/cm to have diffusion weighting of b = 0, 30, 60, 100, 150, 200, 300, 500, 750, and 1000 s/mm^2. In addition to estimating apparent diffusion coefficient (ADC) by fitting a monoexponential model to the data, the following biexponential model was also used:

$$S/S(b=0) = f_p \cdot \exp\left(-b \cdot D_p\right) + \left(1 - f_p\right) \cdot \exp\left(-b \cdot D_t\right)$$

where S is the MR signal intensity, f_p perfusion fraction, D_p pseudodiffusivity, and D_t tissue diffusivity.

Following MRI, IFP was measured using the wick-in-needle method (3). A 23-gauge needle with a ~5 mm notch located ~5 mm from the tip and filled with nylon sutures was connected to a fluid-filled pressure transducer and pressure monitoring system (Power Lab 8/30, AD Instruments, Inc.). Pressure was recorded continuously as the needle was inserted into a central part of the tumor. Assuming that the pressure reached maximum when both the tip of the needle and the notch were positioned near the center of tumor, the maximum pressure was used as a representative pressure value of the tumor. Milosevic et al (2) reported that blood flow can be inversely related to IFP in the range 7 mmHg < IFP < 20 mmHg, but not for non-elevated IFP (< ~ 7 mmHg). Hence in this study, the association of the maximum pressure with the mono- and biexponential model parameters was evaluated for the data with IFP > 5 mmHg. This study was approved by the institutional animal care and use committee.

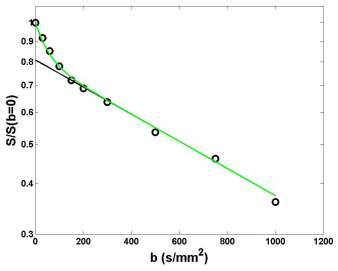

Figure 2. The average diffusion weighted signal from the tumor shown in Fig.1 (circles) shows a non-monoexponential decay. Black line represents a monoexponential model fitted to the values with b ≥ 200 s/mm^2. Green line represents a biexponential model fitted to the data.

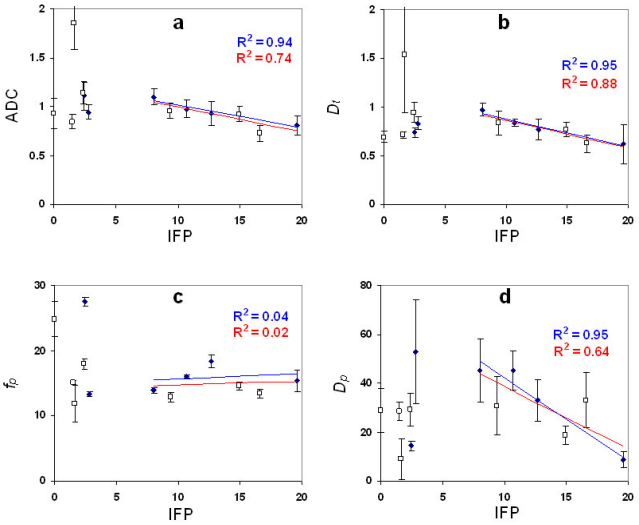

Figure 3. Linear relationship between elevated IFP and diffusion coefficients. Plotted are the estimates for untreated (dark diamonds) and treated (open squares) tumors. Error bars represent the estimation errors from the Levenberg-Marguardt method. Red lines are linear regression for all data points with IFP ≥ 5, and blue lines for untreated tumor only. Units: ADC, D_t, and D_p in μm^2/ms, IFP in mmHg, and f_p in percentage.

Results and Discussion: Fig.1 shows an example of a 4T1 tumor in the right flank. Signal-to-noise ratios of the tumor (arrow) were 38 and 14 for b=0 (Fig.1b) and b=1000 s/mm^2 (Fig.1c), respectively. Fig.2 shows the diffusion weighted signals in the tumor, demonstrating the signals with low b-values (< 200 s/mm^2) substantially deviate from a monoexponential trend (black line). A biexponential model (green line) appears to be adequate to represent the data for all b-values used in this experiment. Fig.3 shows plots between IFP and diffusion parameters. Strong correlations (R^2 > 0.64) were observed between the elevated IFP (> 5 mmHg) and all three types of diffusion coefficients; ADC (total average diffusion), D_t (associated with cellularity-restricted diffusion) and D_p (a marker of microvascular blood velocity). The largest relative parametric change (factor of 4) appeared in D_p. Stronger association between IFP and diffusion coefficients (R^2 > 0.94) was observed when the data from only non-treated mice were considered (blue lines), compared with those from both treated and non-treated (red lines). This may be due to heterogeneous progress of tumor vascular normalization induced by Avastin, an anti-angiogenic drug. The data points with low IFP (< 5 mmHg) did not show any remarkable trend, and these data could also include the cases with measurement error. Although generated with a small sample size, the current preliminary result suggests a high potential of DWI parameters as surrogate markers for IFP.

Reference: **1**. Jain and Baxter., Cancer Res. 1988; 48(24 pt 1):7022-7032. **2**. Milosevic et al., Int. J. Rad. Onc. Biol. Phys. 1999; 43(5):1111-1123. **3**. Boucher et al., Cancer Res. 1991; 51(24):6691-6694.

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