Measuring Non-invasively Tumor Perfusion and Diffusion as A Function of Tumor Progression using Proton Magnetic Resonance Imaging (1H MRI)

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Introduction: We hypothesized that the tumor microenvironment can be evaluated non-invasively by measuring the changes in tumor water diffusion and tumor perfusion. Our hypothesis is that necrotic tumor tissue areas have defective cell membranes and loss of cellularity leading to higher ADC (Apparent Diffusion Coefficient) values. Also, necrotic tumors have poor vascularity which results in reduced or non-existent blood flow. Our hypothesis for viable tumor tissue is that the cell membranes and cellularity are intact leading to limited diffusion and "normal" blood flow. Our goal is to map the tumor water diffusion by diffusion-weighted ^{1}H MRI (DW MRI) and to map tumor vasculature by dynamic contrast-enhanced ^{1}H MRI (DCE MRI) and compare the diffusion and vascular maps obtained from MRI with the *ex vivo* histology images of viable and necrotic tumor regions obtained from Hematoxylin & Eosin (H&E) stained tumor sections.

Materials and Methods: The Dunning Rat Prostate Cancer Cell Line, R3327-AT, was cultured in Dulbecco's Modified Essential Medium supplemented with 10% Fetal Calf Serum, 100 U/ml Penicillin and 100 µg/ml Streptomycin in a humidified CO^2 incubator at 37ºC. Nine Copenhagen rats were injected with 3-4 million R3327-AT cells suspended in 200 µl phosphate-buffered saline on their right hind legs. Tumor volumes were determined using Vernier caliper and corrected for skin thickness. Tumor diffusion and perfusion experiments were performed on 9 rats with tumors of volumes ranging from 149 mm^3 to 2806 mm^3 using a Bruker 7T horizontal-bore magnet (Fig. 1). For DW MRI, the T_2-weighted signal intensity of each individual voxel in the DW MR images of the tumor slices was acquired for different b-values with a constant diffusion time. From the slope of b vs bADC, ADC values were calculated for each pixel in a slice using the polyfit function of Matlab and the corresponding ADC map was generated for each slice (Ross, 2003, Bammer, 2003). For DCE MRI, the experimental dynamic build up curves from T_1-weighted water signal after Gd-DTPA administration for each individual voxel in the MR images were processed, the build up curves fitted using the Hoffman model (Hoffmann, 1995), and normalized with respect to the baseline signal before contrast agent administration. The kinetic modeling is based on the linear relationship between measured MR signal and the concentration of contrast agents in the tissue. An amplitude (A), reflecting the relative signal enhancement and an exchange rate (kep), reflecting the velocity of signal increase, can be analyzed via a two compartment model (Ulf Hoffmann, 1995). Consequently the magnitude of Akep represents the slope of MR signal enhancement and it represents vascular flow or perfusion of the tumor tissue (Ulf Hoffmann, 1995). **Akep** values of individual voxels from the dynamic build up curves were estimated for each tumor slice (4-5 per tumor) and **Akep** maps were generated for the related tumor slices (field of view: 37.5 mm x 37.5 mm (128 pixel x 128 pixel), slice thickness: 1 mm). Hematoxylin and Eosin (H&E) staining was performed on tissue sections (8 µm slice thickness) to distinguish viable from non-viable tissue and were set in relation to ADC and Akep obtained from non invasive MRI.

Figure 1: Photograph illustrate animal positioning and molding processes on the home- built MR animal holder with stereotactic marker system

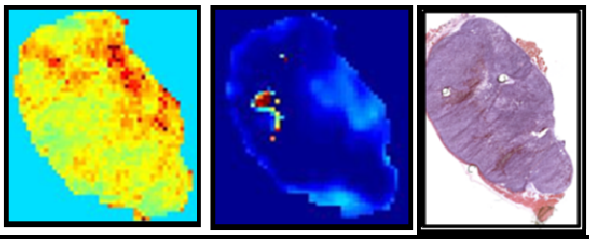

Figure 2: Representative images (from left to right) of ADC map, Akep map and H&E histology of a tumor slice.

Results: We mapped for each animal the **ADC** values and **Akep** values across the tumor (4 to 5 slices per tumor, Fig. 2). We found that with increasing tumor size the maps become more heterogenous as tumor necrosis develops with the tumor rim still being well perfused and a drop in tumor perfusion in the cent ral regions of the tumors. The median **ADC** across the tumors increased slightly but not significantly with tumor size (Fig. 3, left panel). However, median Akep values across the tumor decreased significantly with tumor volume ($p<0.05$) (Fig. 3, right panel). Additionally, the drop in median tumor Akep with tumor size was significantly related to the slight increase of median tumor ADC with increasing tumor size ($p=0.0339$).

Conclusion: Our preliminary data indicate that obtaining ADC values by DW-MRI in addition to DCE-MRI may increase our understanding of the tumor microenvironment which in turn could influence the choice of tumor treatment and the evaluation of treatment response. Currently, we are in the process of evaluating the relationship between viable tumor tissue and tumor necrosis obtained from H&E staining of tumor tissue sections, tumor perfusion, and diffusion characteristics of the tumor (fig-3, fig-4).

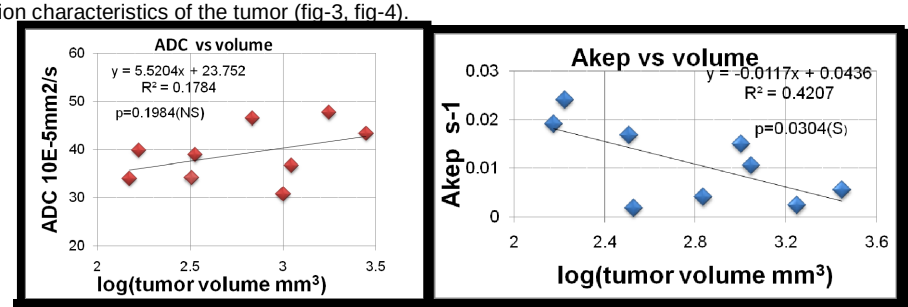

Figure 3: The median ADC across the tumors increased slightly but not significantly with tumor size (left panel). However, median Akep across the tumor decreased significantly with tumor volume ($p<0.05$) (right panel). Field of view: 37.5 mm x 37.5 mm (128 pixels x 128 pixels), Slice thickness: 1 mm. (4 to 5 slices per tumor).

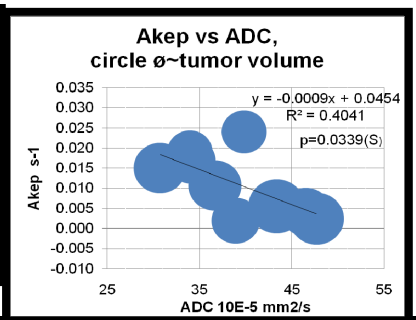

Figure 4: ADC and Akep with increasing tumor volume (circle diameter proportional to tumor volume).

References: [1] B Ross, B. M. (2003). Evaluation of Cancer Therapy Using Diffusion Magnetic Resonance Imaging. Molecular Cancer Therapeutics , 2, 581-587. **[2]** Bammer, R. (2003). Basic principles of diffusion-weighted imaging. European Journal of Radiology , 45 (3), 169-184. **[3]** Ulf Hoffmann, G. B. (1995). Pharmacokinetic Mapping of the Breast: A New Method for Dynamic MR Mammography. Magnetic Resonance in Medicine , 33, 506-514.

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