DCE-MRI & Fluorescence Microscopy of Microvascular Permeability

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Introduction

Dynamic contrast enhanced-MRI (DCE-MRI) is a non-invasive, functional method that has been used extensively to measure active changes in microvascular hemodynamics (i.e. extravascular extracellular fractional volume (v_e) and the volume transfer constant (K^{trans}) between the blood plasma and the v_e) in tumors [1]. Because anti-angiogenic therapies are designed to affect the abnormal blood vessels recruited by tumors, changes in blood volume, blood flow, or other hemodynamic parameters are promising biomarkers that allow for an *in vivo* assessment of the biological activity of these angiogenic modulators. The ability of DCE-MRI to quantitatively measure response is fundamentally limited by a lack of precise knowledge regarding the relationship between the contrast agent concentration and signal intensity. A major limitation of DCE-MRI is that it does not measure the tracer directly but, rather the effect of tracers on water relaxation. Since water is not exclusively extracellular, exact concentrations must often be inferred [2]. We propose a method by which microvascular hemodynamics as measured by DCE-MRI can be directly compared to the same parameters measured by intravital fluorescence microscopy in a window chamber model [3]. This abstract describes the feasibility of this dual imaging capability to produce K^{trans} and v_e data from both fluorescence and MRI which can be quantitatively compared.

Methods

In order to view the tumor microcirculation with both DCE-MRI and fluorescence microscopy, polyacetal resin window chambers were implanted into the dorsal midline skinfold of male SCID mice (Figure 1a). Shortly following the implantation of the window chamber, green fluorescent protein (GFP)-transfected human prostate cancer cells were transplanted into the chambers. GFP-fluorescence was used to monitor tumor growth and delineate tumor borders (Figure 1b). Fluorescence and MR macromolecular contrast agents were created by coupling Gd-DTPA or Alexa Fluor® 633 (Abs/Em: 621/639 nm) to bovine serum albumin (BSA). Images of dynamic contrast enhanced MR data were obtained using a 1cm surface coil with volume excitation (4.7 Tesla Bruker spectrometer; TR=200ms, TE=5.9ms, Repetitions=30, NEX=2, 5mm slice thickness, Matrix=256x256, 100µm in-plane resolution, scan time≈1h). Following acquisition of the dynamic contrast series, high-resolution images of dynamic fluorophore-labeled contrast agent uptake within the vasculature and subsequent distribution within the interstitial space were acquired using an upright research microscope (Nikon Eclipse E600) at 1X using an on-board Red HeNe laser and 640 bandpass filter for Alexa Fluor® excitation.

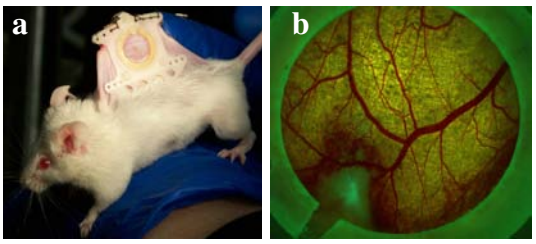

Figure 1. Photograph of SCID mouse bearing plastic chamber (a); simultaneous transillumination & GFP fluorescence (b).

Results

Time-activity curves were analyzed by 2-compartment models for MR [1] and fluorescence [3] to generate maps of permeability and vascular volumes. Figures 2a and b show vascular volume and permeability maps (respectively) derived from the MRI concentration versus time curves. The low permeability associated with the vessels within the chamber is consistent with a normal vessel-selectivity to macromolecules that have long intravascular retention times (e.g. macromolecules with a MW>~20kDa). Figures 2c and d show representative vascular volume and permeability maps (respectively) derived from the concentration versus time curve of the fluorescence microscopy data for a different animal.

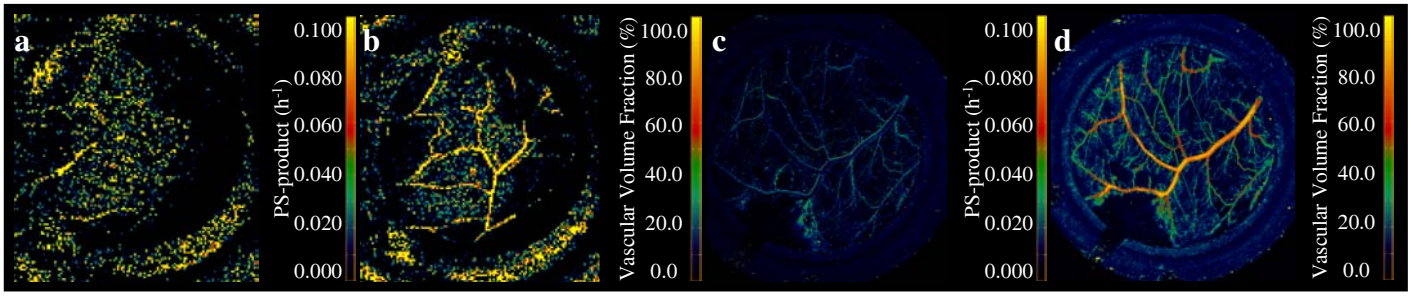

Figure 2. Permeability (a) and vascular volume map (b) derived from MRI concentration vs. time data and permeability and vascular volume maps derived from fluorescence microscope concentration vs. time data.

Conclusions

These data offer a sufficient spatiotemporal resolution to cross-correlate the rate and extent of extravasation of macromolecular contrast agents using both imaging modalities. A near-term goal is to collect data from the same tumors in the same setting by both modalities. Such dual-modality imaging will improve quantitation of MR signal and provide a better understanding of microscopic partial volumes underlying MR signal changes with dynamic contrast. Furthermore, currently available clinical agents have high pre-therapy variance compared to larger molecular weight contrast agents. Once optimized, this multi-modal imaging system will be useful in evaluating contrast agents of different molecular weights to identify sizes that produce the best reproducibility and highest sensitivity/specificity to changes in the tumor microvasculature following antiangiogenic therapy.

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