Vessel Size Index MRI with Viable Tumor Analysis for Monitoring Anti-Angiogenic Therapeutics

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Introduction. *In vivo* monitoring of anti-angiogenic therapies can reveal a host of information about their effect on tumor vasculature. Vessel size index (VSI) MRI has recently been used to detect the effect of an anti-angiogenic agent [1]; VSI MRI determines the fractional blood volume and mean vessel size on a voxel-by-voxel basis using changes in T_2 and T_2* caused by the introduction of an iron oxide contrast agent [2]. This enables longitudinal studies of vasculature that provide additional information to traditional *ex vivo* measures of vascular density. In this study, we use VSI MRI to examine the effect of G6-31, an anti-VEGF therapeutic agent [3]. To account for tumor heterogeneity, we use viable tumor VSI analysis (VT-VSI), restricting the VSI parameter analysis to the viable tumor tissue as determined with multispectral k-means clustering [4]; VT-VSI has been shown to correlate well with *ex vivo* micro-CT angiography as a measure of vessel size and blood volume [5]. The VT-VSI technique requires no additional scans beyond those used for VSI MRI and can improve accuracy of tumor parameter measurement by eliminating necrotic regions from the analysis.

Methods. The institutional AAALAC-accredited review board approved all animal procedures. MRI scans were performed on a 4.7 T Varian Unity Inova MRI system with a 20 mm two-loop surface coil (Varian Inc, Palo Alto, CA), for 8 mice with HM7 colorectal cancer tumors grown subcutaneously on the leg. A multi-slice FSE sequence with diffusion weighting was used to calculate an apparent diffusion coefficient (ADC) map (6 b-values ranging 300-1100 s/mm^2, TR = 3 s, ETL = 4, NEX = 2). A multi-slice spin echo sequence was used to calculate T_2 maps (TR = 3s, TE = 5,26,47,68 ms, NEX = 1), and a multi-slice gradient echo sequence was used to calculate T_2* maps (TR = 300 ms, TE = 4,10,16,22,28,34 ms, NEX = 4). ADC and T_2 data were collected as 8 coronal 1-mm-thick slices (FOV = 25.6×25.6mm, 64×64 matrix); T_2* maps were collected with a 128×128 matrix. A USPIO contrast agent (Molday ION, BioPAL) was then injected via a tail-vein catheter (200 µmol/kg), and the SE and GE sequences were repeated to calculate the post-contrast T_2 and T_2* maps. Mice were randomized into two groups based on tumor volume estimates (caliper measurements) and imaged at day 0, then treated with either ragweed (control antibody) or G6-31 (an anti-VEGF-A monoclonal antibody) based on their assigned groups. Imaging was repeated at 48 hours after treatment.

Vessel size index (VSI) maps and fractional blood volume (BV) maps were calculated voxel-by-voxel using the ADC map and the pre- and post-contrast T_2 and T_2* maps [2]. Multispectral analysis for tissue segmentation was performed using the ADC and T_2 maps using a k-means clustering algorithm [4]. The tumor was segmented into four classes: viable tumor tissue, subcutaneous fat, and two necrosis classes. The viable tissue class was then used as a mask to calculate VSI and BV parameters within the viable tissue, excluding regions of fat and necrosis. Representative images are shown in Fig. 1.

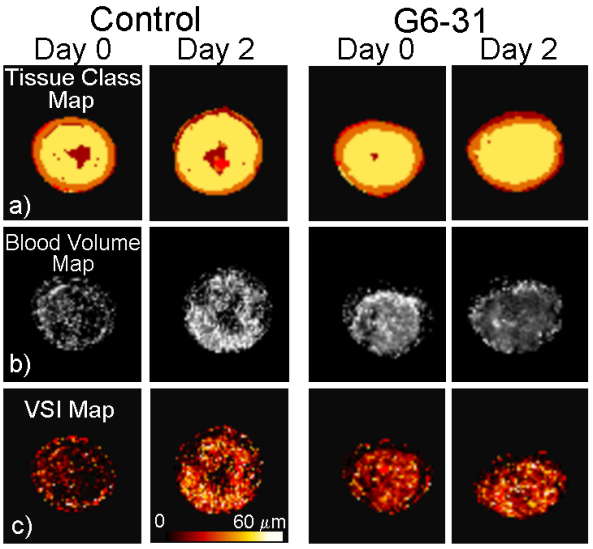

Figure 1. Representative images from one control and one treated tumor, pre- and post-treatment. (a) Tissue class maps from multispectral analysis: yellow = viable tumor tissue, orange = subcutaneous fat, maroon = necrosis + hemmorhage, red = cyst-like necrosis. (b) BV maps show increase for control tumor, but decrease for treated tumor. (Scale = 0-17%) (c) VSI maps show increase in vessel sizes from 2 days of tumor growth in both tumors.

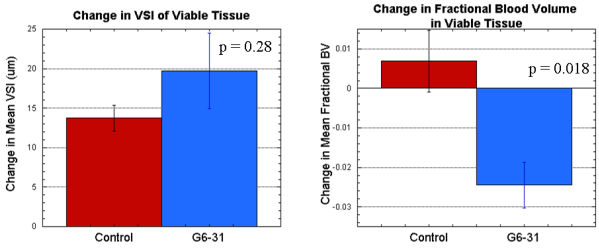

Figure 2. Pre- to post-treatment change in mean VSI and mean BV (with std error) for two treatment groups.

Results. The mean VSI and BV for each tumor were calculated within the viable tumor tissue. Both treatment groups had a significant increase in mean VSI at 48 hours (control group p = 0.001, G6-31 group p = 0.02), with the G6-31 group trending toward a larger increase in VSI than the control (see Fig. 2). The G6-31 group had a significantly reduced blood volume relative to the control group. This may indicate that smaller vessels are more strongly dependent on VEGF than larger vessels, with anti-VEGF treatment resulting in a shift toward larger vessels and reduced overall BV.

Conclusions. We have demonstrated that VT-VSI MRI can detect microvascular changes associated with tumor growth and anti-VEGF therapy. We detected a significant increase in mean VSI at 48 hours for both groups; the mean BV of the G6-31 group was significantly suppressed, indicating a strong therapeutic effect. We are in the process of expanding this study to include more animals.

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