Validation of different MRI vessel caliber index models with in-vivo 2-photon microscopy measurements of vessel caliber in a U87 mouse brain tumor model

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Introduction: MRI measurements of blood vessel caliber may provide an important tool for assessing tumor angiogenesis and response to anti-angiogenic drug treatment. While magnetic resonance methods for determining vessel caliber index (VCI) have been validated against histology [1,2], no validation studies have been performed *in vivo* where the structural deformations frequently associated with histological tissue preparation are not present. In addition, two models for calculating the VCI have been proposed – a linear dependence on the $\Delta R_2^* / \Delta R_2$ ratio [1] and 3/2 power dependence [3]. While Monte Carlo simulation suggest that there is a sub-linear dependence of ΔR2 on $\Delta\chi$ for sufficiently large $\Delta\chi$ [3,4], it is unclear at what field strength and contrast agent dose this should occur. Here we directly compare the linear and 3/2 power VCI models, generated from data acquired at 9.4T with a MION dose of 20 mg/kg, with the VCI measured from *in vivo* 2-photon microscopy data in a U87 mouse brain tumor model.

Materials and Methods:

In vivo two-photon microscopy: U87GFP tumors were implanted in nude mice with previously implanted cranial windows. When tumors reached a diameter of 1.5 to 2.0 mm, animals were anaesthetized and 3-6 locations per animal were investigated using a multi-photon laser-scanning microscope. To visualize the vessels, 150 µl of tetramethylrhodamine labeled dextran (MW 2 million, 10 mg/ml) was injected intravenously. Stacks of 250 µm depth and 5 µm Z-steps were acquired, and a virtual vascular cast was generated in 3D by custom image analysis software. Length-weighted average vessel diameter was calculated based on the virtual cast.

MRI rCBV and rVCI measurements: U87GFP tumors were implanted in nude mice (n=5) and imaged on a 9.4 Tesla MRI scanner (Bruker Biospin, Billerica, MA). R2 and R2* maps were acquired before and after tail vein injection of 20 mg/kg MION ($\Delta\chi$~$2x10^6$). Multi-echo spin-echo and multi-echo gradient-echo data were used to generate the R2 and R2* maps. A tumor rVCI was calculated from the tumor ΔR2*/ΔR2 ratio, normalized to the contralateral cortex brain tissue. An average rVCI was determined from analysis of 16 image slices acquired from 5 mice with tumors ranging from 2-3 mm in diameter. rVCI models where compared with the *in vivo* 2-photon rVCI using an ANOVA calculation and statistical significance was defined as $p<0.05$.

Results and Discussion: Shown in Figure 1 are 2-photon images of tumor (Fig 1, left) and normal (Fig 1, middle) tissue. Illustrations show maximum intensity Z-projections of 250 µm stacks. Blood vessels are indicated in red and GFP expressing tumor tissue in green. A significant increase in the tumor vessel caliber, with respect to normal brain tissue, of 1.33±0.08 times is observed in the 2-photon images (Fig 1, right). While the *in vivo* optical rVCI is in excellent agreement with the linear rVCI model (rVCI=1.36±0.04), with no statistically significant difference (p=0.772), there is a statistically significant difference (p=0.014) between the optical and the 3/2 power model (rVCI=1.59±0.07).

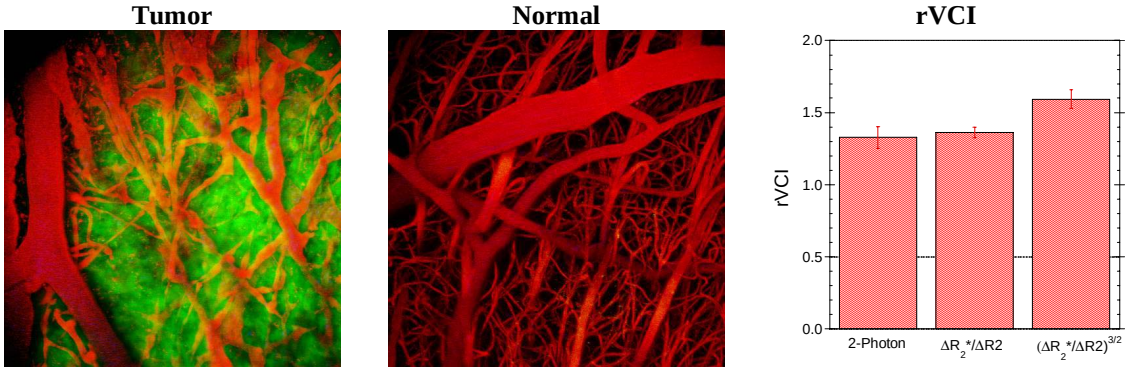

Figure 1: *In vivo* 2-photon microscopy images of a U87GFP mouse brain tumor model. Images of both tumor (left) and normal (middle) tissue are shown. An increase in average vessel caliber of 1.33±0.08 (2-photon), 1.36±0.03 (linear MRI), and 1.59±0.07 (3/2 power MRI) times is observed in the tumor compared to normal tissue (right).

Conclusions: The linear VCI model is in excellent agreement with the *in vivo* 2-photon microscopy measurement of the tumor VCI. In contrast, the 3/2 power model gives a significantly greater increase in VCI than observed in the *in vivo* optical images. These results suggest that the linear VCI model is appropriate for studies at 9.4 Tesla and MION doses of 20 mg/kg.

References:

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