Blood volume fraction (BVF) and characteristics of vascular growth in VX2 tumor measured by MRI, Ultrasound and Micro-CT

X-L. Qi[1], X-W. Yu[2], J. Hong[2], P. Burns[2], M. Henkelman[2], G. Wright[2]

[1]Imaging Research, Sunnybrook &Women's College HSC, Toronto, ON, Canada, [2]Sunnybrook & Women's College HSC, Toronto, ON, Canada

Introduction: It is well known that blood supply is very important for rapid growth of a malignant tumor. MRI and Ultrasound can non-invasively evaluate the tissue microcirculation by calculating relative blood volume (rBV) in the tumor (1), which is valuable and helpful for clinical diagnosis and treatment planning. Many medical imaging studies have been conducted to detect and assess the shape and structures of a tumor and to follow up clinical treatments (2). However, the density of the blood vessels and blood volume in the tumor may be changed at different stages of tumor growth; the relationships between rBV, density of blood vessels and the size of the tumor have not yet been reported. The purpose of this study was to evaluate the rBV characteristics in the rim of a rabbit VX2 tumor model and the relationship of rBV calculated by MRI and Ultrasound to the size of the VX2 tumor (expressed in 3-D volume: cm^3). Moreover, the blood vessel density in the tumor demonstrated by Micro-CT and 3-D MRI was also compared with rBV in the tumor rim for different sizes of tumor.

Materials and Methods: Five New Zealand white rabbits (4 to 4.5 kg) were each injected intramuscularly with about $1x10^6$ VX2 tumor cells in a hind leg. The tumor was detectable in 7 to 10 days and when the tumor reached a size of up to 15 cm^3 around 20 days after injection, the rabbits were anaesthetized with Ketamine (50mg/kg) and Rompun (5mg/kg). Each tumor was scanned both by MRI and Ultrasound. When the imaging studies were finished, tumors were perfused with saline followed by microfil (Flow Tech inc., Carver, MA) for the micro-CT study. All MRI, ultrasound and micro-CT studies were performed within 24 hours. Tumor size was measured in three dimensions by ultrasound.

MRI studies were performed on a GE Signa 1.5 T CV/i equipped with 40 mT/m gradients with a 150 mT/m/s slew rate using a 3-inch receive-only surface coil for signal reception. Quantitative T1 measurements were made using a Look-Looker sequence with spiral readouts (225 ms acquisition spacing, TR=3000 ms, RBW=125 kHz, 20° flip angle, 7 mm slice thickness, FOV=20 cm, 4096x8 spiral readouts, 1.1 mm resolution. T1 measurements were made both prior to, and approximately 4 min following an intravenous bolus injection of Clariscan (0.05 ml/kg), and then at 15 minute intervals thereafter. Dynamic T1-weighted imaging for perfusion and a 3-D spoiled gradient echo acquisition for angiograms were also performed immediately after and 10 min following the Clariscan injection respectively. Small arterial blood samples were drawn both before and after the Clariscan injection and their T1 decay times measured independently.

Under the assumption that Clariscan remains intravascular in tumor, the blood volume fraction (expressed in percentage) was calculated using the equation:

$$BVF = \frac{(1/T1tumor)\,postCA - (1/T1tumor)\,preCA}{(1/T1blood)\,postCA - (1/T1blood)\,preCA} x100\%$$

where preCA and postCA refers to measures before and after injection of Clariscan. We used postCA measures at the latest time point following injection to get the best concordance with the fast exchange assumption used for BVF measurements (3).

Ultrasound: Ultrasound studies were performed using an HDI 5000 scanner (Philips Ultrasound, Bothell, WA) with a 6MHz linear array operating in pulse inversion mode. The intravascular microbubble contrast agent Definity (Bristol Myers-Squibb, Boston MA) was infused intravenously at 3.5 ml/min. The acoustic power, measured by the Mechanical Index (MI), was 0.6-1.0, so that microbubble disruption was induced. Relative blood volume measures were obtained with a destruction-reperfusion method (4), with the reperfusion curves analyzed offline. The contrast signal corresponding to the tumor blood volume was measured after a perfusion interval of 30 seconds. This signal was then compared to a signals in adjacent muscle and, if available, a major blood vessel.

Micro-CT: The tumor was excised about 90 minutes after microfil perfusion and stored in 10% formalin for 24 hours. The specimen was mounted in 10% gelatin and three-dimentional CT data sets were acquired using a microCT scanner (MS-8, GE Medical Systems, London, Ontario). A cone beam CT acquired projections of 1000 x 1000 pixels at 600 angular increments.

Regions of interest (ROI) were placed around the edge of the tumor determined from dynamic images in MRI (the dark rim area which filled quickly with ClariScan) and from images at a 30 second delay in Ultrasound (bright rim area filled with contrast agent, Difinity). We have chosen an ROI in the rim of tumor tissue to assess the microcirculation and to avoid bigger vessels just outside of the tumor.

Results: The blood volume fraction (BVF) of the tumor calculated from the MRI using Clariscan and rBV measured by Ultrasound using Definity shows good correlation (R=0.84). The rBV from ultrasound is a relative measure only, normalized with respect to muscle. Our results also indicate for the first time that relative blood volume in the tumor rim seems to decrease with increasing size of VX2 tumor (Fig.1). Interestingly, rBV in the tumor rim measured by MRI and ultrasound was qualitatively consistent with the density of blood vessels demonstrated by micro-CT and 3-D MRI. Micro-CT and 3-D MRI show that smaller tumors (4 cm^3 in volume) have a higher density of blood vessels and bigger tumors (12cm^3 in volume) have a lower density of blood vessels (Fig 2&3).

Discussion and Conclusion: Relative blood volume in the microcirculation of tissue measured by MRI and Ultrasound may be of value in the evaluation of the vascular characteristics of a tumor, and changes with growth or intervention. The observation on MRI, ultrasound and micro-CT that small tumors have a higher density of blood vessels may be related to their rapid growth and the relative absence of necrosis. If carried over to clinical studies, these results may help elucidate optimal timing in the administration of anti-vascular cancer therapies.

References:

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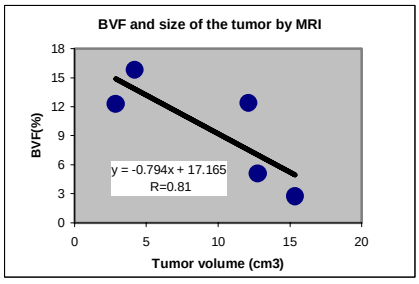

Fig.1: BVF measure by MRI in VX2 tumor is inversely related with size of tumor measured in 3-D by ultrasound.

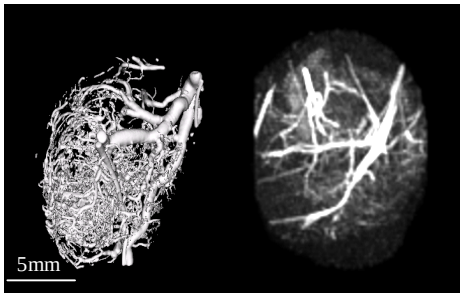

Fig.2: small tumor (4 cm^3), left: micro-CT, right: 3-D MRI, higher density of blood vessels in peripheral area.

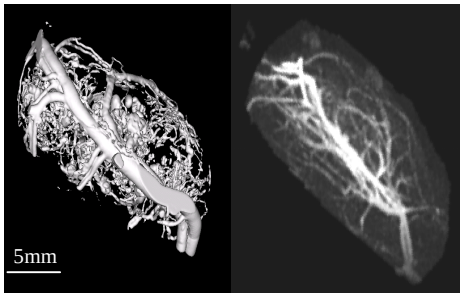

Fig.3: Bigger tumor (12 cm^3), left: micro-CT, right: 3-D MRI, lower density of blood vessels in peripheral area.