

Blood volume fraction mapping for angiogenesis assessment in a novel human glioblastoma stem cell model

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Introduction: It is important in clinics as well as in preclinical studies to identify parameters for tumor angiogenesis that can predict response to antiangiogenic therapies. One such parameter related to angiogenesis is the tumor blood volume. In this study, we quantified the blood volume fraction (BVf) in a novel orthotopic mouse model derived from human glioblastoma stem cells [1] using the Rapid Steady State T_1 (RSST $_1$) magnetic resonance MRI technique [2] with Gd-DOTA, a contrast agent approved for clinical use.

Material and methods: Human glioblastoma stem cells (5×10^5 in 5 μ l phosphate buffered saline) were injected into the right caudate nucleus (Bregma level, 2 mm lateral, 2.5 mm depth) of $n = 6$ nude mice. One to three months after tumor implantation, they were imaged in a 47/40 Bruker Biospec USR AV III scanner using a homogenous body coil for transmission and a Bruker mouse head surface coil for signal reception with a field of view of $15 \times 15 \text{ mm}^2$ and 8 slices of 0.7 mm for all acquisitions. The mice were equipped with an intraperitoneal catheter and injected with 6 mmol/kg (12 ml/kg) Gd-DOTA inside the scanner 5 minutes after the start of the dynamic RSST $_1$ acquisition (Inversion Recovery prepared Modified-Driven-Equilibrium-Fourier-Transform (MDEFT), TR/TR $_{\text{echo}}$ /T $_{\text{inversion}}$ /TE = 750/6.5/303/1.2 ms, flip angle = 10°, matrix 32×32, duration 6 s per repetition) of 60 minutes duration, followed by T_1 weighted images (Multi-Slice-Multi-Echo, TR/TE = 300/6.3 ms, NA = 8, matrix 128×128) acquired 60 minutes after Gd-DOTA injection. Prior to contrast agent injection, T_2 weighted (Rapid-Acquisition-Relaxation-Enhanced imaging, TR/TE $_{\text{effective}}$ = 3500/33 ms, NA = 6, matrix 128×128), T_1 weighted and proton density weighted MDEFT images TR/TR $_{\text{echo}}$ /T $_{\text{inversion}}$ /TE = 10 s/6.5 ms/9 s/1.2 ms, matrix 32×32, duration 1 min 20 s) were acquired. Contrast enhancement was quantified as $(S_{\text{post_T1w}} - S_{\text{pre_T1w}})/S_{\text{pre_T1w}}$, $S_{\text{post_T1w}}$ and $S_{\text{pre_T1w}}$ being the T_1 weighted (T1w) pre and post contrast signal, respectively. The BVf maps were obtained according to $S_{\text{norm}} = (S_{\text{post}} - S_{\text{pre}})/S_0$ [2], where S_{pre} and S_{post} are the average pre and post contrast RSST $_1$ images during the steady state phase, while S_0 is the proton density weighted signal. When the contrast agent is confined to the vascular compartment the normalized signal equals the BVf: $S_{\text{norm}} = \text{BVf}$ [2].

Results and discussion: During the 1st month, tumor growth was not detectable with either MR technique. During the 2nd month, the tumor occurrence was hardly visible on T_2 weighted acquisitions, but contrast enhancement in the order of 50 to 400% could be observed (Figure 1) on T_1 weighted acquisitions. Figure 2 displays typical plots of $S_{\text{norm}}(t)$ from one mouse. The dynamic RSST $_1$ acquisition showed no contrast agent leakage in the tumor region, similar to the RSST $_1$ signal from a major vessel. For comparison, a typical contrast agent leakage profile such as observed in muscle tissue is also shown. Fifteen to at least 35 minutes after contrast agent injection the signal is in a steady state reflecting the thermodynamic equilibrium signal from the vascular compartment. Under the assumption that the contrast agent is confined to the vascular compartment after intraperitoneal injection [3], the BVf in the tumor region is 0.034 ± 0.010 (range 0.020 to 0.043) while it is 0.023 ± 0.004 (range 0.018 to 0.029) contralateral to the tumor. In most mice, clinical signs appeared 3 months after tumor implantation.

Conclusion: Similar to clinically encountered tumors, this novel tumor model develops slowly, has an infiltrating growth pattern and it mimics the cellular heterogeneity encountered in clinical tumors. During the 2nd month of tumor development, the tumor vasculature is impermeable to Gd-DOTA since dynamic RSST $_1$ acquisitions revealed no signal time course typical for contrast agent leakage in the tumor tissue. This study demonstrates that quantitative micro- and macrovascular BVf maps for angiogenesis assessment can be acquired noninvasively using the RSST $_1$ technique with intraperitoneal administration of Gd-DOTA. This novel glioblastoma model is useful for the evaluation of new treatment strategies such as antiangiogenic agents.

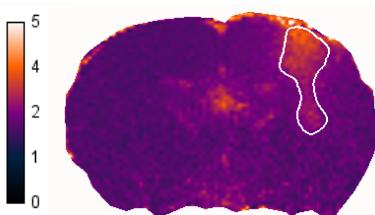


Figure 1:
representative
coronal mouse
brain map of
contrast
enhancement

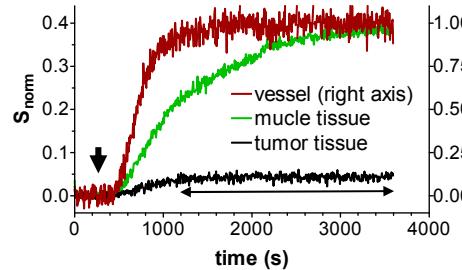


Figure 2:
typical signal time course ($n = 1$) after intraperitoneal injection of 6 mmol/kg Gd-DOTA (vertical arrow), and resulting steady state interval (horizontal arrow)

[1] Platet et al, Cancer Lett. 2007; [2] Perles-Barbacaru and Lahrech, JCBFM 2007; [3] Moreno, NMR Biomed 2006