

Assessment of mouse glioma vasculature using SPIO and small molecule contrast agents: sequential implementation of alternate perfusion MRI methodologies

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Prognosis remains extremely poor for glioblastoma multiforme (GBM), the most common of the primary brain tumors, and novel therapeutic strategies are required. The mortality associated with GBM can be attributed largely to the invasiveness of glioma cells and the robust angiogenesis associated with it. By definition, the angiogenic process in brain tumors involves interaction between tumor and brain tissue, and hence angiogenesis and the testing of anti-angiogenic strategies, must utilize *in vivo* models. While potentially of great utility in this regard, the application of perfusion MRI methodologies to the noninvasive monitoring of this angiogenic process in models of mouse glioma is relatively new and uncharacterized. Dynamic contrast enhanced MRI (DCE-MRI) can be implemented to assess the permeability of mouse glioma vasculature, a key angiogenic marker. This vascular permeability however, complicates blood flow measurements with dynamic susceptibility contrast MRI (DSC-MRI) methodology, due to first pass contrast agent extravasation. Depending on the severity, correction algorithms or preloading strategies may not sufficiently address this problem. The use of macromolecular blood pool contrast agents, such as the superparamagnetic iron oxides (SPIO, molecular diameter 150 nm) could be used to circumvent first pass extravasation, but their use in mouse glioma models for this purpose has not been characterized. Our goals were: **1)** To employ an SPIO contrast agent to acquire reliable DSC-MRI blood flow/volume data in an angiogenic mouse glioma model. **2)** To implement DCE-MRI methodology using a small molecule contrast agent, in order to assess vascular permeability. **3)** To enhance the diagnostic utility of these approaches by testing and validating their implementation in the same imaging session.

Brain tumors were induced in B6D2F1 mice (n=10) by intracerebral injection of 4C8 glioma cells. Imaging was performed between 3 and 11 weeks after injection. Prior to imaging, tail vein cannulation was performed for contrast agent injection. MR imaging was performed on a Bruker-Biospin 8.5T vertical wide-bore DRX-360 with an AVANCE console, a Paravision 3.0.2 software platform, and a Mini0.5 imaging system equipped with a 56 mm inner diameter gradient set. Mice were anesthetized and positioned in a 20 mm birdcage resonator. Multislice T₁ and T₂ weighted precontrast images were obtained prior to the perfusion series (slice thickness 0.5 mm, 98 μm in-plane resolution). For DCE-MRI, a series of 200 rapid spoiled gradient echo T₁ weighted images were obtained (Bruker-Paravision FLASH sequence, TR 15.6 ms, TE 2 ms, FA 20°, 128x128 matrix, 1 mm slice thickness, 195 μm in-plane resolution, 2.0s/image). Magnevist (Gd-DTPA, Berlex Inc) was injected (10X diluted, 3.0 μl/g) 30s after initiation of the perfusion image series. DSC-MRI was implemented with 150 rapid T₂* weighted images, (Bruker-Paravision FLASH sequence, TR 12 ms, TE 6 ms, FA 5°, 128x128 matrix, 1 mm slice thickness, 195 μm in-plane resolution, 1.5s/image). Ferridex, an SPIO agent (Berlex Inc, 4X diluted, 2.4 μl/g) was injected 30s after initiation of the DSC-MRI perfusion image series. Perfusion MRI data were processed using the Jim software package (Xinapse Systems). Calculation of DCE-MRI parameters followed the general approach described in Tofts PS *et al.*¹ For DCE-MRI, the jugular veins were used for determination of arterial input functions (AIF). Parametric maps were generated for K^{trans}, the volume transfer

constant for the contrast agent, and v_e, the extra-vascular extra-cellular space volume fraction. Calculation of DSC-MRI perfusion parameters followed the method in Ostergaard L. *et al.*² For DSC-MRI, the AIF was determined from brain pixels identified by an automatic scanning and selection routine. Parametric maps were generated for cerebral blood flow (CBF) and cerebral blood volume (CBV). **Figure 1A** indicates a representative example of a coronal T₁ weighted precontrast image at 11 weeks after cell implantation, indicating a large tumor. Panel **B** and **C** indicate the associated CBF and CBV maps for the masked brain areas obtained from the Ferridex DSC-MRI image protocol, which was not preceded by a DCE-MRI experiment. The CBV and CBF maps correlate well, and reveal the tumor flow heterogeneity with high resolution, indicating local areas with 10 times greater flow than is present in the contralateral area, as indicated by CBF_{rel} in panel **C**. Panels **D-F** indicate parametric maps obtained 24 hours later from the same mouse and geometric position. These data were obtained from the Magnevist DCE-MRI protocol, as well as the Ferridex DSC-MRI image protocol which was implemented 20 min after completion of the DCE-MRI. Panels **D** and **E**, indicate the K^{trans} and v_e parametric maps, which indicate the rate and extent of contrast agent entry into the extracellular space, and provide important complementary information to that of the CBF, shown in panel **F**. The essential agreement of the CBF maps in panels **C** and **F** (plotted with identical intensity scales), indicate that the Ferridex DSC-MRI experiment is unaffected by a preceding Magnevist DCE-MRI experiment. This is consistent with the experimental design, which provided a stabilization period between the bolus experiments, as well as the fundamentally different basis of the two perfusion experiments (T₁ vs T₂*). **In conclusion**, an SPIO contrast agent can be successfully employed with DSC-MRI and a high field micro-MRI system to obtain high resolution CBF/CBV data in models of mouse glioma. Furthermore, this approach can be effectively combined with DCE-MRI assessments of K^{trans} and v_e in the same imaging session, in order to obtain a comprehensive assessment of mouse glioma vascularization. The development and characterization of this approach lays the groundwork for noninvasive, longitudinal and highly diagnostic investigations of anti-angiogenesis strategies in mouse models of malignant glioma.

1. Tofts PS *et al*, *JMRI* **10**: Pages 223-232 (1999).

2. Ostergaard L. *et al*, *Magn. Reson. Med.* **36**:715-728 (1996)

