Perfusion MRI-based quantification of mouse glioma vascularization

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INTRODUCTION

Despite therapeutic advances prognosis remains extremely poor for glioblastoma multiforme (GBM), the most common of the primary brain tumors, and novel therapeutic strategies are required. The morbidity and mortality surrounding the diagnosis of GBM can be attributed to the invasiveness of glioma cells and robust angiogenesis associated with this tumor. By definition, the angiogenic process in brain tumors involves interaction between tumor and brain tissue, and hence such a pathologic condition must be evaluated in *in vivo* models. The testing of anti-angiogenic strategies requires noninvasive and longitudinal measurements of tumor perfusion in such models. However, the application of noninvasive perfusion MRI to studies with mouse glioma models is relatively new and has not been well characterized. Our goal was to develop and characterize noninvasive perfusion MRI methodology capable of providing longitudinal and quantitative *in vivo* noninvasive measurements of cerebral blood volume (CBV) and cerebral blood flow (CBF) in mouse gliomas. To accomplish this, we utilized dynamic susceptibility contrast MRI (DSC-MRI).

MATERIALS AND METHODS

Brain tumors were induced in B6D2F1 mice (n=12) by intracerebral injection of 4C8 glioma cells. Imaging was performed between 3 and 9 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 3.0.1 software platform, and a Mini0.5 imaging system equipped with a 56 mm inner diameter gradient set (Billirica MA). Mice were positioned in a 30 mm birdcage resonator, and heads were fixed in place with a specially designed head holder. A layer of aqueous gel and a gel filled latex balloon were positioned above the mouse head to distance the air interface from the brain area and improve magnetic field homogeneity. Mouse tail vein cannulation was performed for contrast agent injection. Multislice T_1 and T_2 weighted images were obtained prior to the perfusion series (slice thickness 0.5 mm, 98 µm in-plane resolution). For perfusion imaging, a series of 100 rapid spoiled gradient echo T_2* weighted images was employed on a single slice traversing the tumor (TR 18 ms, TE 6 ms, FA 5º, 128x128 matrix, FOV = 25 mm, 1 mm slice thickness, 195 µm in-plane resolution, 2.3s/image). Magnevist (Gd-DTPA) was injected (3X diluted,2.9 µl/g) 30s after initiation of the perfusion image series. Multislice T_1 and T_2 weighted post-contrast images were then acquired. The images were masked using an interactive MATLAB routine, in order to remove areas outside of the brain and to ensure the accurate determination of the arterial input function. Perfusion analysis was performed with MedX software (Sensor Systems, Sterling, VA). The relative CBV maps were determined though gamma variate fitting of the concentration curves and integration, while the relative CBF maps were generated from the amplitude of the residue curve with results from deconvolution of the tissue curve via singular value decomposition.

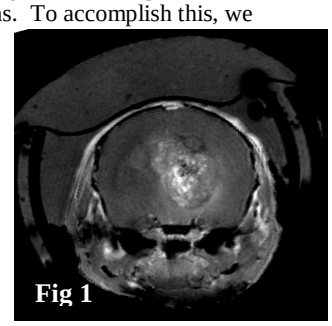
Fig 1

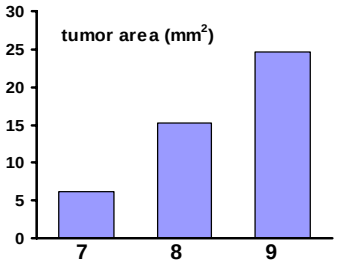

Fig 2

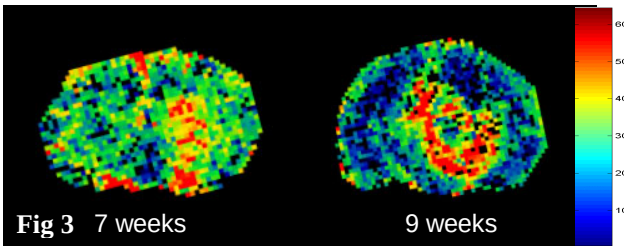

Fig 3 7 weeks 9 weeks

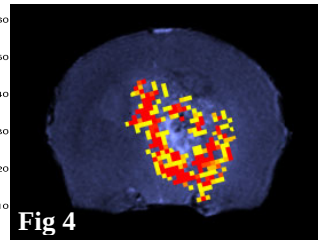
Fig 4

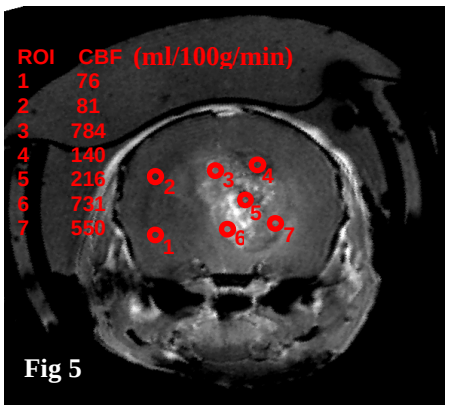

Fig 5

RESULTS

Figure 1 indicates a post-contrast T_1 weighted image of a typical mouse brain at 9 weeks post-injection, and indicates a large well delineated tumor. **Figure 2** indicates the cross sectional area of the tumor during weeks 7-9, as determined from region of interest (ROI) analysis on the post-contrast T_1 images, and reveals substantial tumor growth over that period. **Figure 3** indicates parametric cerebral blood flow parametric maps obtained on the same mouse, for 7 and 9 weeks respectively, from the same slice position as in **Figure 1.** The data indicate that at 7 weeks, greatly increased CBF is observed in the central area of the tumor. At 9 weeks, substantial additional tumor growth had occurred and high flow areas were prominent on the tumor periphery. The patterns in the CBV maps corresponded well with those of CBF (data not shown) **Figure 4** indicates thresholded CBF values at 9 weeks superimposed on a (masked) anatomical T_1 weighted image at the same slice position. **Figure 5** indicates the mean CBF (ml/100g/min) in ROIs superimposed over the post-contrast T_1 weighted image. These data reveal that flow had increased up to 10-fold in the peripheral areas of the tumor in comparison to contralateral brain areas. In other areas of the tumor, the increase in flow is much less prominent. The tumor heterogeneity is also reflected in the anatomical images. Hence, while a very robust angiogenic process is evident at the tumor periphery, the tumor flow becomes heterogeneous at the later stages of growth, suggesting that central necrotic areas may be forming.

In conclusion, using DSC-MRI, a high field microMRI system, and a tail vein contrast injection approach, we have obtained high quality *in vivo* CBF data from the mouse brain in a manner useful for longitudinal studies of tumor angiogenesis. Combination of perfusion MRI with an anatomical MRI assessment of tumor morphology enabled precise localization of tumor neovascularization. Our study documented and quantified a robust angiogenic process occurring in the periphery of 4C8 mouse gliomas. 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.