Investigation of the BOLD response to carbogen breathing with tumour blood volume in an intracranial F98 rodent glioma model

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Introduction Blood oxygenation level dependant (BOLD) MRI has been investigated as a marker of tumour vascularity and oxygenation by measuring the signal change induced by carbogen (5% CO_2 / 95% O_2) breathing[1,2]. Tissue R_2* can be modelled as a linear function of tissue deoxyhemoglobin (dHb) concentration, but the BOLD response with carbogen breathing depends on factors including blood volume and blood flow, so is not straightforwardly interpretable[1,2].The BOLD response in brain tumours also has the added complexity of tumour infiltration and co-option of brain tissue blood vessels. In this study in orthotopic F98 rodent gliomas, we have determined the fractional blood volume (fBV) after administration of a USPIO blood pool agent to investigate the impact of blood volume on the brain tumour carbogen breathing induced BOLD MRI response.

Methods Orthotopic tumours (*n=4*) were grown by stereotactic injection of F98 cells ($2.5x10^4$ cells in 10µl PBS) into the left cerebral cortex (2 mm lateral, 5 mm depth from the Bregma) of female Fischer rats. Rats without tumours (*n=3*) were used as controls. MRI was performed between days 14 and 21 post-implantation for tumour sizes similar to that shown in Figure 1 with a 4.7T Varian Unity INOVA using a dedicated rat brain coil (body coil transmit with 15 mm diameter receive coil, Rapid Biomedical, Germany). 11 axial images were acquired for each tumour (1 mm contiguous) with a 4 cm FOV and 128 x 128 matrix with: dual spin echo (MEMS; TR 1000 ms, TE 14 & 40 ms) and multi-gradient echo (MGRE: TR 460 ms, even echoes at TE 6, 14, 22 & 30 ms, flip angle 20°). MGRE images were acquired during air and carbogen breathing and pre- and 5 minutes post-contrast after intravenous administration of a USPIO blood pool contrast agent Molday ION® (BioPAL, Massachusetts, US) at a dose of 44 µmol Fe kg^{-1} body weight via a tail vein cannula. MRI data was analyzed using ImageJ and Matlab to co-register images and calculate R_2*. fBV was determined from the change in R_2* due to the USPIO[3]. Regions of interest (ROIs) for tumour, contralateral normal brain and normal tissue of non-tumour bearing rats were defined from pre-contrast MEMS images. Average measures and voxel by voxel analyses were performed over these ROIs for fBV and the carbogen breathing induced change in R_2* (ΔR_2*). Blood oxygen saturation was measured during air and carbogen breathing with a MouseOx Pulse Oximeter (Braintree Scientific, Massachusetts, US).

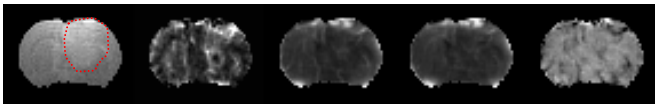
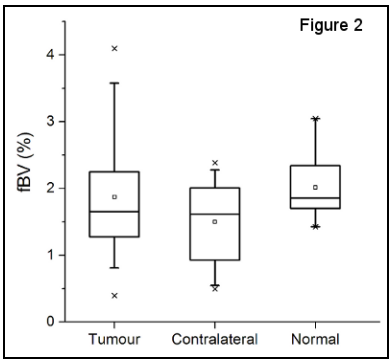
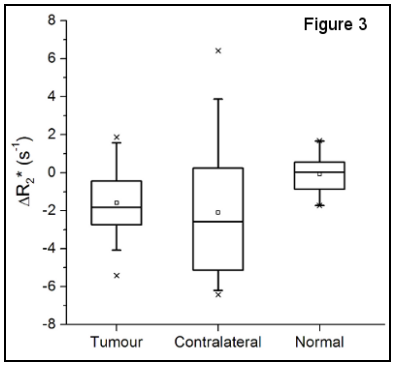


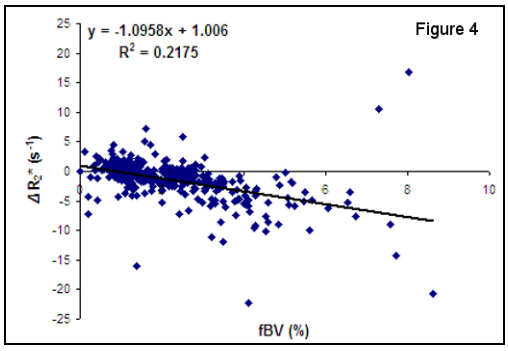
Figure 1

Results Figure 1 shows a tumour ROI on the MEMS image and calculated fBV, R2* air, R2* carbogen, ΔR_2* images. The average fBV and ΔR_2* were determined for each ROI of the individual images for tumour, normal appearing brain contralateral to the tumour and normal brain. The distributions are shown as box plots in Figures 2 and 3. Tumour fBV was more heterogeneous than normal brain, and ranged from 0 to greater than 8% for individual voxels. Contralateral fBV lower compared to normal fBV and ΔR_2* was negative on average for tumour and contralateral brain regions, whereas the BOLD response to carbogen for normal brain was close to zero. One-Way ANOVA statistical analysis showed a significant difference between the contralateral and normal brain fBV (p=0.028) and ΔR_2* (p=0.014). A voxel by voxel analysis of tumour ROIs showed a strong correlation (R > 0.4 for 10 out of 28 slices, average slope -1.43 ± 0.56 (n=10)) between fBV and ΔR_2* as shown in Fig.4 for the tumour slice shown in Fig.1. MouseOx was used to confirm the arterial bold response and the mean ΔY was found to be 4.2 ± 2.2 % (n = 7).



Discussion A simple expression[1] for the change in relaxation rate with carbogen breathing is ΔR_2* = k.[ΔV(1-Y)-V.ΔY] where k is constant that depends on the field strength and vessel size, V is the blood volume and Y the blood hemoglobin (Hb) saturation. R_2* decreases if the blood Hb saturation increases and there is no blood volume change, but R_2* could increase if there was vasodilation and increased dHb but no change in blood saturation. Most likely there is a combination of effects, hence there is not always a simple correlation between ΔR_2*, Y and fBV. However, in some tumour regions there is a large range of fBV and fBV dominates the size of the BOLD response. Tumour blood is generally thought to be more deoxygenated due to their high metabolic rate and poorly structured blood vessels, so there is the potential for a large dHb change, whereas normal brain is well oxygenated so the BOLD response is not so easily detected[4]. The results show that for high fBV there is the largest R_2* decrease, which is consistent with an increase in blood oxygenation due to carbogen breathing. Our data also indicate that normal appearing contralateral brain has lower fBV and a larger R_2* response compared to normal brain. This could be an effect of tumour compression and reduced blood flow leading to a higher baseline dHb level, and hence larger R_2* change with carbogen. Although there was a range of measured ΔY, this did not account for the difference between contralateral and normal brain BOLD response. Whether the contralateral brain is truly normal and is not partially infiltrated by tumour still needs confirmation with histology. However, if there is tumour compression of normal vasculature this has implications for BOLD studies of intracranial tumours and also for fMRI brain tumour studies in general[5].





In conclusion, fBV is a dominant factor that determines the size of the BOLD response in approximately 30% of tumour regions in this study. Detailed histological analysis will next be performed to assess whether the combination of BOLD and fBV can be used to distinguish between well vascularised regions and those with poor vascular development and severe hypoxia. The apparent compression of normal brain blood vessels may be a confounding factor in using BOLD to determined tumour regional boundaries.

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