BOLD signal and T_2 responses in rat F98 and 9L gliomas to hypoxic hypoxia

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Introduction

Malignant gliomas are the most angiogenic tumours; angiogenic vessels are abnormal with increased non-selective permeability of endothelium leading to elevated interstitial pressure and poor perfusion, hence gliomas are inherently deprived of metabolisable substrates and removal of waste products [1]. Level of communication in terms of oxygen delivery to brain tumours has been recently assessed, using BOLD signal changes and showing that in 9L gliomas tissue O_2 tension (ptO_2) increases under hypercapnia [2]. BOLD contrast is known to correlate with pO_2 although in a qualitative manner [3], with underlying physiology making full analysis of hypercapnic challenge difficult [4]. Here we have examined BOLD and T_2 responses in F98 and 9L rat gliomas to hypoxic hypoxia in order to indirectly assess vascular responsiveness in these tumours.

Methods

Male Fisher rats were inoculated with $5x10^4$ tumour cells in 10 µl [F98 (n=7) or 9L (n=6)] into the left hemisphere. Rats were anaesthetised with isofluorane (1-1.5% in 70:30 O_2:N_2) and secured using tooth and ear restraints for MRI; heart rate and oxygen saturation (Y) were monitored with Nonin V8600 pulse-oximeter and core temperature controlled via a heated blanket throughout. MRI experiments were performed on a horizontal-bore 7T magnet using a Varian Inova console with a surface coil. Gd-enhanced (Omniscan, 0.2 mmol/kg ip) T_1-weighted images were used to assess blood-brain-barrier leakage. After tumours reached a Gd-volume greater than 12 mm^3, spontaneously breathing rats were exposed to 3 min of either hypoxic hypoxia (12% O_2 in N_2) or carbogen (5% CO_2 in O_2) through a nose cone. A single slice through the tumour volume showing Gd-leakage in T_1-weighted MRI a day or two earlier was chosen for BOLD-sensitive SE-EPI time-course imaging (TR=1s, TE=60ms, 64x64 pixels, FOV 3x3 cm, 1mm slice, 1 shot) and T_2 mapping (TR=2s, six TEs range 25 – 90 ms, 64x64 pixels, 3x3 cm, 1 mm slice, 2-shots), the latter performed both before and during (with a mean time of 2 min 30 sec) the respiratory challenges. Analysis of ROI time courses and T_2 map generation was performed using Matlab platform. Relative CBV was computed from the 1st passage curve acquired using FLASH MRI (TE=1.8 ms, flip angle 7°, 3.3 frames/sec) after bolus injection of Omniscan (0.2 mmol/kg). A satellite group of glioma bearing rats (n=3) received i.v. injection of AMI-227 (6 mg/kg Fe) about 30 min before respiratory challenges were performed and MRI data acquired using EPI as above.

Results

Baseline T_2 values for F98 and 9L tumours were 48.7 ± 1.9 and 45.1 ± 2.6 ms ($p<0.01$) respectively; contralateral cortex was 47.2 ± 2.2 ms. Both heart rate and Y responded to hypoxia as expected (Table 1). Signal intensities for tumour parenchyma during hypoxia challenges decreased across all rats, with a more pronounced effect in 9L than F98 (Fig. 1a). Changes under carbogen were less pronounced, with negligible change in Y and pulse rate, and signals increasing at a level lower than that of the contralateral cortex. Corresponding changes in T_2 are shown in Table 1. It is evident that hypoxia produced consistent changes in both BOLD signal and T_2, 9L gliomas being greater than those in F98. Tumour responses to carbogen were less evident. Baseline CBV values for F98 and 9L were computed as 3.3 and 2.2 relative to contralateral cortex. Signal decrease in 9L glioma to hypoxia in AMI-227 injected rats was much larger than in F98 gliomas (Table 1), indicating large increase in CBV of the former tumour type under these conditions. AMI-227 signal responses to carbogen were of opposite sign in the two gliomas (Table 1).

Table 1: Changes in BOLD signal and T_2 in contralateral normal brain cortex ('contra') and glioma parenchyma ('tumour') measured under respiratory challenges for F98 and 9L tumour models (mean ± SD; * indicates $p<0.05$ between the two gliomas; bpm = beats per minute).

	ΔS (%)		ΔT_2 (ms)		AMI–227 ΔS (%)			
	tumour	contra	tumour	contra	tumour	contra	Δsat. O_2	Δ pulse (bpm)
F98 hypoxia	-5.3±1.7	-7.2±1.1	-1.8±2.8	-2.4±1.7	-4.0	-3.8	-20.8±2.6	22±12.6
9L hypoxia	-10.9±4.6	-8.6±3.6	-5.0±2.6	-3.8±1.7	-11.2	-0.14	-17.5±3.0	8.9±4.3
F98 carbogen	2.8±2.5	6.8±2.9	-0.20±0.6	1.2±1.9	-1.0	-3.8	-0.7±1.4	0.4±9
9L carbogen	5.3±4.7	6.7±5.0	0.43±1.6	1.2±2.6	8.7	-2.8	0.4±1.5	0±4.4

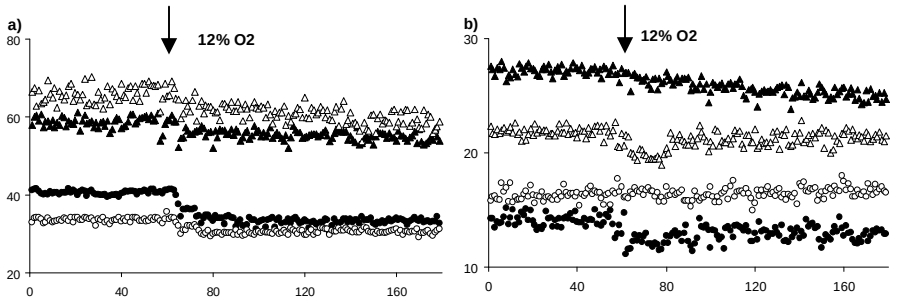

Figure 1: Signal time courses for hypoxia challenge a) without and b) with AMI-227 i.v. (▲ = F98 tumour, Δ = F98 contra, ● = 9L tumour, ○ = 9L contra).

Conclusions

The present data show that the MRI responses to hypoxia are very pronounced and consistent in both gliomas and, interestingly, they are much more reproducible than those to carbogen. This level of hypoxic hypoxia does not influence baseline CBF in normal brain under isofluorane anaesthesia [5]. Mild hypoxia is metabolically well tolerated [5] and it may provide a means to evaluate responsiveness of tumour vasculature to increased need for energy substrates as well as metabolic adaptation under these conditions. Interestingly, our data show that 9L glioma is more sensitive to hypoxia than F98 possibly reflecting greater haemodynamic change and increased CBV.

References:
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