^{1}H MRS detected mobile lipids in rodent gliomas: correlation with EPR determined tumour oxygenation

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Introduction

Resonances arising from mobile lipids in brain tumour ^{1}H MR spectra bear multiple potential biomarker functions. Saturated lipid peaks have been associated with high degree of malignancy both in animal [1] and human [2, 3] brain tumours. The MR Spectroscopy (MRS) lipids are correlated with necrotic histopathology, a hallmark of high degree of malignancy [4]. In contrast to saturated lipids, polyunsaturared lipids in brain tumour ^{1}H MR spectrum have been shown to appear in response to successful cytotoxic therapy [1]. Here we have determined oxygen tension in two rat glioma models by electron paramagnetic resonance (EPR) and used single-voxel MRS and MRS imaging (MRSI) to estimate ^{1}H MRS detectable lipids in these tumours.

Methods

9L and F98 cells (50,000 cells in 10 µl) were injected in the left hemisphere of male Fisher rats at the following co-ordinates: AP, -3.0 mm; ML, 1.5 mm and 3.5 mm; and DV, 3.5 mm. Seven days later two aggregates of LiPc crystals (40 µg/each) were injected through the same burr holes at a depth of 2 mm. LiPc high spatial resolution multi-site oximetry for ptO_2 was performed essentially as previously described [5]. Rats (n=5-7 in each tumour type) were anesthetized using 1.5% isofluorane with 30% FiO_2, positioned in the magnet of the 1.2 GHz (L-band) EPR spectrometer. The EPR spectra were recorded at 8 mW to avoid power saturation, with scan times varying from 30-60 seconds. The spectra were averaged over 5 min and the ptO_2 obtained from the two LiPc deposits were pooled together to give an average tumor ptO_2.

For MRI and ^{1}H MRS rats under isofluorane anaesthesia (1-1.5%, in O_2:N_2 50:50) were secured to a custom build head holder using ear bars and nose cone, body temperature was maintained with a thermostated water circulating heating element. Tumour volume and location of LiPc crystals were determined one day prior to EPR, using T_1- weighted MRI after Magnevist, (0.1mmol/kg, ip) injection. T_1-wtd-MRI was acquired with TR=0.7s, TE=9ms, 1mm slice, FOV=4x4cm, matrix size=128x128. Tumor volume was calculated by drawing region of interest on the contrast enhanced regions using software provided by Varian. LASER (Localization by Adiabatic Selective Refocusing) technique was used for single-voxel ^{1}H MRS with TR=2.5 s and TEs of 27 and 136 ms (F98 n=15 for F98 and n=8 for 9L). STEAM –based ^{1}H MRSI (n= 3 for F98, n=4 for 9L) [6] was used with the acquisition parameters: TR=2.5 s, TE=5 ms, TM=40ms, FOV=20mm, 3mm slice, matrix=16x16). jMRUI was used for spectral data analyses. Values shown are means ± SEM. The lipid MRSI images were generated by integration over ±60 Hz in MATLAB.

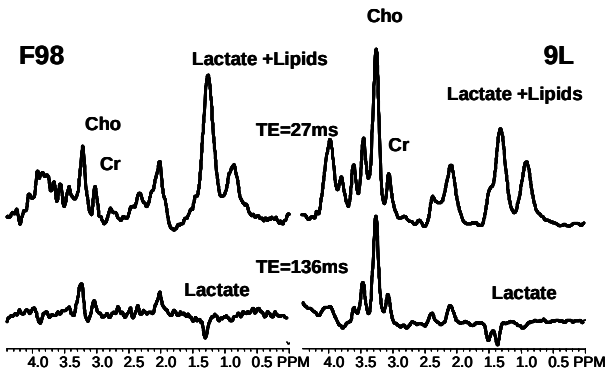

Figure 1. LASER ^{1}H MR spectra of F98 and 9L gliomas at two TE (27 and 136 msec)

Results

Prior to EPR Gd-enhanced tumour volumes were 205±17 and 234±19 mm^3 for 9L and F98 gliomas, respectively. LiPc crystals were localised within enhancing tumour parenchyma in all gliomas. EPR indicated ptO_2 of 34.9±3.3 and 5.9±0.8 mmHg ($p<0.01$) for 9L and F98 gliomas, respectively. Typical LASER spectra from a F98 and 9L acquired with two echo times are shown (Fig. 1). Cho/Cr ratios (LASER with TE=27ms) were 3.0±0.3 and 4.2±0.5 ($p<0.05$) for F98 and 9L gliomas, respectively. The respective 1.3ppm/Cr ratios were 2.9±0.5 and 7.2±0.8 ($p<0.01$). In TE=136ms MRS lactate/Cr ratio was 1.1±0.2 for F98 and 0.9±0.4 for 9L gliomas, thus lactate levels were not different. ^{1}H MRSI 1.3 ppm lipid maps from typical F98 and 9L tumours are shown in Fig 2. It is evident that in F98 the 1.3 ppm peak is localized to the tumour central region, in a 9L more to tumour periphery. In tumour ^{1}H MRSI voxels 1.3ppm/Cr ratios were 19.4±4.6 and 8.6±1.5 ($p<0.05$) for F98 and 9L gliomas, respectively.

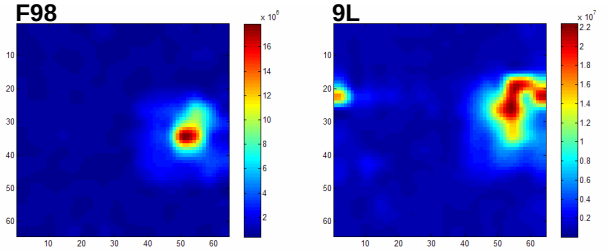

Figure 2. ^{1}H MRSI lipid (1.3 ppm) map of both F98 and 9L gliomas.

Conclusions

These data show that F98 gliomas are very hypoxic, when ptO_2 in 9L is close to that in normal parenchyma [5]. The former glioma type shows elevated 1.3ppm peak compared with the latter, despite similar lactate levels. It is proposed that high ^{1}H MRS-detectable lipids are associated with hypoxic brain tumours.

References [1] Hakumäki JM et al., Nature Med. (1999) 5: 1323. [2] Negendank W & R Sauter, Anticancer Res. (1996) 16: 1533 [3] Murphy PS et al., Br J Radiol (2003) 76: 459. [4] Kuesel AC et al., NMR Biomed. (1994) 7: 149. [5] Williams BB et al., Antioxid Redox Signal (2007) 9: 1691 [6] Liimatainen TJ et al., Magn Reson Med [2008] 59: 1232.

Acknowledgements: We would like to acknowledge Mr J. P. Lariviere for assistance in cell culture preparations.