Histological Verification of Oxygen-Enhanced MRI for Detection of Hypoxia

I. V. Linnik[1,2], M. Scott[3], N. Woodhouse[3], J. C. Waterton[1,3], H. Young[3], C. Liess[3], H. Barjat[3], J. Ulloa[3], C. L. Hodgkinson[4], T. Ward[4], C. Dive[4], D. Roberts[4], J. H. Naish[1,2], and G. J. Parker[1,2]

[1]Imaging Science and Biomedical Engineering, School of Cancer and Enabling Sciences, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom, [2]Biomedical Imaging Institute, University of Manchester, Oxford Road, Manchester M13 9PT, United Kingdom, [3]Imaging, Translational Sciences, AstraZeneca, Alderley Park, Macclesfield, Cheshire, SK10 4TG, United Kingdom, [4]Paterson Institute for Cancer Research, Manchester, United Kingdom

BACKGROUND: Tumour hypoxia is a key regulatory factor in resistance to various therapies. A noninvasive and quantitative method for measuring oxygen levels in tumour would therefore be of great value to help plan chemotherapy, radiotherapy or a range of combination therapies. Dynamic oxygen-enhanced (OE) MRI monitors the tissue change in longitudinal relaxation (T_1) caused by switching from breathing air to 100 % O_2. This effect is completely independent of the BOLD effect, which depends on changes in T_2 and T_2*. It has been shown using dynamic OE-MRI that some tumour regions demonstrate an increase in the longitudinal relaxation rate R_1 consistent with the delivery of paramagnetic molecular oxygen via the blood plasma.[1,2] However, our recent studies [3,4] have demonstrated that tumours also exhibit regions that paradoxically reduce R_1 with the switch to O_2. Here we provide a theoretical explanation of this difference in response between regions and demonstrate that OE-MRI may be used to measure the proportion of a tumour that is hypoxic.

PURPOSE: To characterise the R_1-increasing and R_1-decreasing regions when switching from breathing air to 100% O_2 in OE-MRI by comparison of OE-MRI and immunohistochemistry.

METHODS: MICE: Experiments were carried out in compliance with the UK Animals (Scientific Procedures) Act 1986. Male nude mice (n=6) with human glioblastoma xenografts (U87-MG) implanted on their right flank were anaesthetised throughout imaging with 1.5 – 1.9% isoflurane in either air, or 100% O_2. Temperature and respiration rate were monitored using SA Instruments equipment (Stony Brook, NY).

MRI: Data were acquired at 9.4 T (Varian Inova, USA) with a 38 mm I.D. quadrature volume coil. R_1 was measured prior to both OE-MRI and DCE-MRI using a slice selective IR FLASH sequence[5]: TR/TE= 21 ms/2.2 ms; flip angle = 20°; 1 average, acq. matrix 128 x 64; inversion slice thickness = 20 mm; FOV = 40mm x 40 mm; 4 axial slices, slice thickness 2 mm; inversion times (TI) of 0.01, 0.25, 0.5, 0.75, 1, 2, 5, 7, 9, and 12 s. OE-MRI dynamic data were acquired using a T_1-weighted 2D FLASH sequence with the same parameters as the IR FLASH sequence, but with no inversion preparation. OE-MRI: A dynamic sequence was run continuously over 10 min: 2 min breathing air, 6 min - 100% O_2, then 2 min - air. ANALYSIS: A region of interest encompassing the whole tumour was delineated on each slice. Baseline R_1 on air, R_1 on O_2 and ΔR_1 (O_2 minus air) were measured for each voxel in the tumour. t-statistic maps of ΔR_1 calculated from each voxel's time series allowed identification of sub-regions of R_1-increasing/R_1-decreasing voxels ($p \leq 0.05$). All other voxels were classed as having no change. Area under the curve during 6 min breathing 100% O_2 (AUC_{OE}) was derived. HISTOLOGY: 105 min prior to euthanasia and tumour excision, animals were injected *i.p.* with 0.1mg/g pimonidazole (HPI, Inc., MA, USA) dissolved in saline. Tumours were fixed in 4% formalin for 24 h and transferred to 70% ethanol and then embedded in paraffin. The histological sections of the tumour were cut parallel with the imaging plane and were stained with PM. Histological staining was analyzed using an Ariol SL-50 (Genetix Ltd).

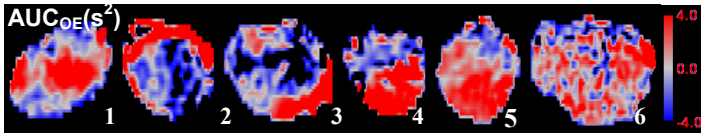

Fig.1 *Example of AUC_{OE} maps in a single slice from 6 tumours.*

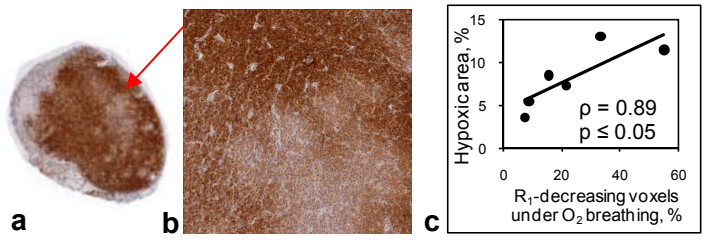

Fig. 2. *PM–stained section (a) of tumor 2. Arrow indicates region presented as high-resolution (magnification x20) histological image (b). Correlation between R_1-decreasing areas under oxygen inhalation and areas stained positively for PM (c).*

RESULTS: Each tumour showed spatially contiguous regions of T_1-weighted signal intensity change in response to O_2 inhalation. Voxelwise analysis indicated that some regions of each tumour demonstrated an increase in R_1 and some a decrease in R_1 in response to O_2 inhalation. A further component of all tumours showed no R_1 change. AUC_{OE} maps demonstrate the spatial distribution of the positive and negative ΔR_1 (Fig. 1). PM staining exhibited regions of hypoxia in all tumours (an example of tumour 2, Fig.2b). A strong positive correlation was observed between R_1-decreasing parcellations under 100% O_2 inhalation and areas stained positively for PM (Fig.2c).

DISCUSSION: Increase in R_1 following the switch to O_2 inhalation is in agreement with increased dissolved paramagnetic molecular oxygen in the blood and tissue plasma, consistent with the known relaxivity of dissolved O_2.[6, 7] However, it is also known that increases in haemoglobin (Hb) saturation by oxygen lead to reductions in R_1[8, 9] consistent with the known relaxivity of deoxyHb. Our observations of changes in R_1 may therefore be interpreted as reflecting two broad regimes. Firstly, regions with high level of deoxyHb on air demonstrate large increases in Hb saturation by oxygen when switching to O_2. All or most of the additional available oxygen is carried by the Hb, leading to no increase in dissolved O_2 in the plasma. The net effect is a reduction in R_1. The extent of these regions correlates strongly with the extent of PM staining and therefore appears to be a good non-invasive marker of hypoxia (Fig 2). Secondly, regions with high Hb saturation on air (normoxia) do not show large changes in Hb oxygen saturation but do demonstrate increases in O_2 carriage in the plasma, leading to a net increase in R_1. A third intermediate regime may be inferred from regions that show neither positive nor negative R_1 change.

CONCLUSIONS: We have demonstrated significant correlations between R_1-decreasing tumour regions under normobaric O_2 inhalation and hypoxic areas stained positively for pimonidazole**.** This finding provides a strong indication that the OE-MRI-based oxygenation changes may provide a powerful new non-invasive method for quantifying hypoxic extent in tumours.

REFERENCES:1: Matsumoto *et al*, *Magn Reson Med***, 56**: 240-246, 2006. 2: O'Connor *et al, Int J Radiat Oncol Biol Phys* **75** (4): 1209-1215, 2009. 3: Linnik *et al*, *Proc. Intl. Soc. Mag. Reson. Med. 17*, 4367, 2009. 4: Linnik *et al*, *Proc. Intl. Soc. Mag. Reson. Med. 1*8, 2010. 5: Haase *et al*, Magn Reson Med, **13**: 77-89, 1990. 6: Chiarotti *et al*, *Nuovo Cimento,* **1**: 863–873, 1955. 7: Zaharchuk *et al*, *Acad Radiol,* **13**:1016–1024, 2006. 8: Blockley *et al*, *Magn Reson Med.*, **60**: 1313-1320, 2008.9: Silvennoinen *et al*, *Magn Reson Med.*, **49**: 568–571, 2003.