Intrinsic Susceptibility MRI of Chemically-Induced Rat Mammary Tumours: Relationship to Histological Assessment of Hypoxia and Fibrosis

L. D. McPhail[1], and S. P. Robinson[1]

[1]The Institute of Cancer Research, Sutton, Surrey, United Kingdom

Introduction

Intrinsic susceptibility MRI is being investigated to provide imaging biomarkers of tumour hypoxia[1]. Deoxyhaemoglobin, which is paramagnetic, creates magnetic susceptibility perturbations, increasing the MRI transverse relaxation rate R_2* of water in blood and in the tissue surrounding blood vessels. Gradient Recalled Echo (GRE) MRI methods are sensitive to R_2* and thus to blood deoxyhaemoglobin levels. Deoxyhaemoglobin therefore acts as an intrinsic, blood oxygenation level dependent (BOLD) contrast agent. Changes in tumour R_2* induced by carbogen (95% O_2/5% CO_2) breathing can be used to assess haemodynamic tumour vasculature[2]. As the oxygenation of haemoglobin is proportional to the arterial blood p_aO_2, and therefore in equilibrium with tissue pO_2, measurements of tumour R_2* should also provide a sensitive index of tissue oxygenation.

A BOLD and DCE MRI study of human breast cancers revealed wide heterogeneity in tumour R_2*, and that relatively slow R_2* correlated with highest relative blood volume and grade, but not hypoxia[3,4]. The presence of fibrosis in human breast cancer has been shown to correlate with high carbonic anhydrase IX expression, an intrinsic marker of hypoxia, and poor prognosis[5]. It remains unclear how necrosis and fibrosis contribute to the overall R_2* of tissue as a function of grade in breast cancer. In this study, we further investigate the relationship of R_2* and carbogen-induced ΔR_2* to histopathological status and hypoxia in a chemically-induced rat tumour model of breast cancer.

Methods

Female Ludwig Wistar rats were injected with either a single or triple dose of methyl nitrosourea (MNU), resulting in tumours (n=16, mean volume 1.9cm^3) which developed at various sites associated with the mammary tissue between 100 and 275 days later. Prior to MRI, tumour-bearing rats were administered with 60mg/kg pimonidazole i.p., for subsequent histological detection of hypoxia. Intrinsic susceptibility MRI was performed using a Varian Unity Inova spectrometer interfaced to a 4.7T horizontal magnet, using a 3 turn 15mm solenoid ^{1}H coil. Multi gradient-echo (MGRE) images were acquired from five 1mm thick transverse slices through the tumour, with TR=80ms, TE=3.5ms, TESPACE=3ms and 8 echoes. Tumour R_2* was quantified whilst the host first inhaled air and subsequently carbogen, administered via a nosepiece. Apparent R_2* maps were calculated on a voxel-by-voxel basis and R_2* determined from an ROI over the whole tumour.

Following MRI, tumours were excised and fixed in formal saline for histological processing. Tumour sections (n=3) were subsequently cut and stained with sirius red, cytokeratin 14 antibody and pimonidazole antibody. Composite images of whole tumour sections were acquired on a microscope with driveable stage, from which the degree of collagen (fibrosis), malignancy and hypoxia were then quantified (%), and any correlates with the MRI data investigated.

Results & Discussion

Figure 1 shows carbogen-induced ΔR_2* maps acquired from two MNU-induced rat mammary carcinoma, and the composite images of sections stained for collagen/fibrosis (sirius red) and hypoxia (pimonidazole) from the same tumours. The mean baseline R_2* for all the tumours was 67.3 ± 4s^{-1} (range 31.3 to 100.1s^{-1}). Carbogen resulted in a highly significant reduction in R_2* (mean ΔR_2* -9.9 ± 4s^{-1}, $p<0.001$, range -1.3 to -21.9s^{-1}). Baseline R_2* positively correlated with subsequent carbogen-induced ΔR_2* ($r=0.5$, $p=0.05$). Inter- and intratumoural heterogeneity in the extent and distribution of sirius red, cytokeratin 14 and pimonidazole staining was evident. Statistically significant negative correlations were found between pimonidazole staining and both baseline tumour R_2* ($r=-0.55$, $p=0.03$, Figure 2) and carbogen-induced ΔR_2* ($r=-0.5$, $p=0.05$), consistent with more hypoxic mammary tumours being more poorly perfused. This supports the concept that R_2* in breast cancer is dominated by blood volume. Pimonidazole staining significantly correlated with sirius red staining ($r=0.75$, $p=0.001$), suggesting that these hypoxic mammary tumours are also more fibrotic. In conclusion, intrinsic susceptibility MRI affords useful imaging biomarkers associated with the underlying pathophysiology of breast cancer.

Fig.1

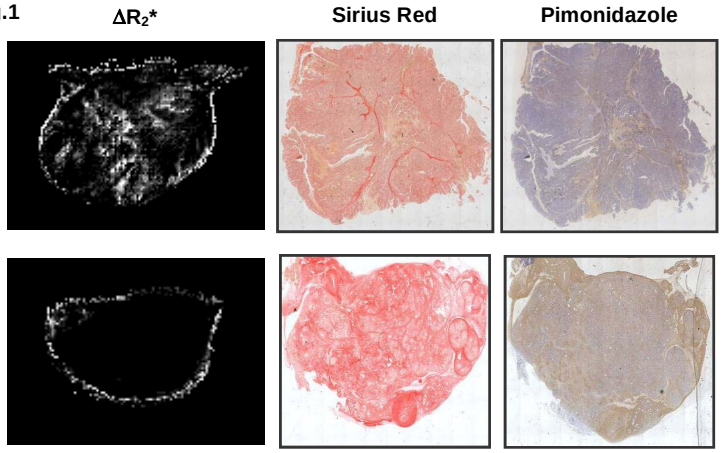
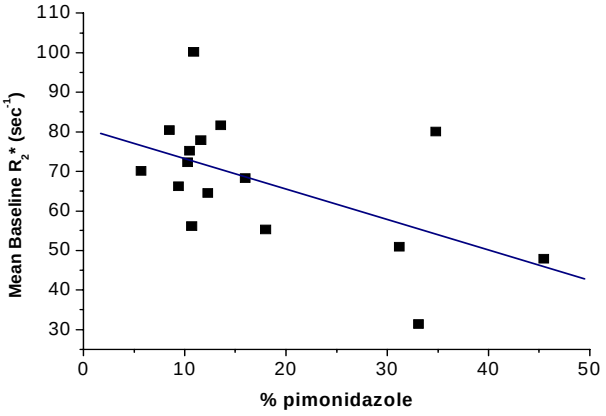


Fig.2



1) Tatum *et al*, Int J Radiat Biol, 82, 699, 2006. 2) Robinson *et al*, JMRI, 17, 445, 2003. 3) Padhani *et al*, Proc ISMRM #90, 2005. 4) Padhani *et al*, Proc ISMRM #1845, 2005. 5) Colpaert *et al*, Breast Cancer Res Treat 81, 137, 2003.

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