Multiparametric MRI mapping of oxygen delivery and hypoxia in renal 786-O-R murine xenografts

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Intended Audience: Basic scientists and clinicians with interest in tumor hypoxia, radiobiology, radiotherapy and hypoxia-modifying agents.

Introduction: MRI mapping of tumor oxygen delivery and hypoxia is an unmet clinical need[1]. T_1-weighted oxygen enhanced MRI (OE-MRI) can distinguish well oxygenated voxels from those that are hypoxic[2]; blood oxygenation level dependent (BOLD) imaging can quantify the oxygen binding to haemoglobin after gas challenge[3]; and dynamic contrast enhanced MRI (DCE-MRI) estimates of perfusion have variable relationships to tissue and genetic hypoxia markers[4, 5]. We show that combining these 3 methods enables detailed MRI evaluation of tumor oxygen delivery and hypoxia.

Methods: Cells from a sunitinib resistant 786-O renal carcinoma xenograft (786-O-R) were cultured in RPMI + 10% fetal calf serum treated with antibiotics. Tumors were propagated by injecting 3 x 10^6 cells in 100µl of sterile PBS into the flanks of female SCID mice. Single slice MRI data were acquired on a 7T horizontal bore Bruker system after localization with T_2-w images. Anaesthetized mice were positioned in a 3-cm birdcage coil. Tumors were immobilized using a jig and gas delivery was at 2 l/min via a nosepiece. Warm air maintained the animal core temperature at 37 °C.

One multi-gradient echo image (to map R_2*) and two True-FISP inversion recovery images (to map R_1) were acquired on medical air (21% O_2), using sequences described previously[6]. Voxel size was 0.234 x 0.234 x 1.0 mm.

Four inversion recovery images were acquired during the first 10 minutes of 100% O_2 gas breathing "wash-in" (each taking 2.5 mins). Then one R_1 map and one R_2* map were acquired (identical to initial images on medical air). Finally, 0.1 mmol/kg gadopentetate (GD) was injected and True-FISP DCE-MRI was collected (10 s temporal resolution) (Figure 1).

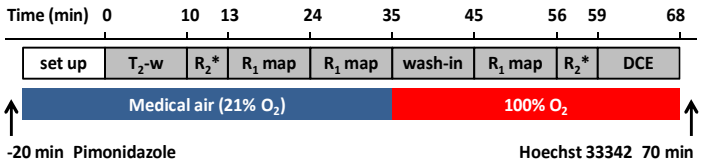


Figure 1: Data collection, gas administration and pathology schedule

Voxel-wise R_2*, R_1 and $IAUC_{60}$ were derived for each map, using a Bayesian maximum *a posteriori* approach, with in-house software[6]. ΔR_2* was calculated by R_2* (O_2) - R_2* (air). ΔR_1 was calculated with R_1 (O_2) data from the final R_1 map and the mean of the two R_1 maps acquired on air breathing.

Intraperitoneal injection of pimonidazole (60mg/kg) occurred 55 minutes before 100% O_2 inhalation began. Hoechst 33342 (15mg/kg) was administered by tail vein injection one minute prior to cull. Hypoxic fraction (HF) and perfused vessel area (PVA) were calculated from 5 µm cryostat sections.

Results and Discussion: Nine mice were imaged. Tumor size (range 178 to 815 mm^3) had no significant relationship to any functional MRI data.

Summary OE-MRI and DCE-MRI data: All tumors showed overall positive R_1 increase, with average ΔR_1 = 0.120 s^{-1} (range 0.012 to 0.201). Within-scan R_1 co-efficient of variation (CoV) was 0.72%. Based on this, individual voxels with R_1 change greater than 2 x (CoV) x (mean baseline tumor R_1) were considered statistically different from baseline. Tumor $IAUC_{60}$ values average = 0.139 mmol.min (range 0.0203 to 0.404). PVA correlated with median ΔR_1 (Spearman's rho 0.905, p=0.002), the % of voxels with positive ΔR_1 (rho 0.857, p=0.007) and median $IAUC_{60}$ (rho 0.893, p=0.007). The HF was correlated to median ΔR_1 (rho -0.783, p=0.013) and the % of voxels with positive ΔR_1 (rho -0.801, p=0.010).

Voxel-wise analysis of heterogeneity: ΔR_1 and $IAUC_{60}$ maps were divided into voxels demonstrating positive enhancement or not. OE-MRI positive ΔR_1 was defined as above. DCE-MRI enhancement was defined as $IAUC_{60}$ > 0. Maps of OE-MRI and DCE-MRI signal mismatch were created to evaluate spatial heterogeneity of oxygen delivery and hypoxia. Analysis revealed four distinct voxel categories: 1) **well perfused and well oxygenated**, 2) **avascular with oxygen build up**, 3) **perfused but hypoxic** and 4) **avascular and no oxygen delivery**. In 6 of the 7 tumors with paired OE-MRI and DCE-MRI data, well perfused, well oxygenated voxels were located in the tumor periphery and central avascular areas were surrounded by a transition zone of hypoxia (example images in Figure 2). Well perfused voxels had greater positive ΔR_1 than the avascular voxels with oxygen build up ($p<0.001$ at all four dynamic time points) and reached plateau within 2.5 mins. Avascular voxels with oxygen build up reached plateau only by 7.5 mins. Perfused but hypoxic voxels became progressively more negative in ΔR_1 values, with significance ($p<0.001$) reached at 7.5 mins (Figure 2).

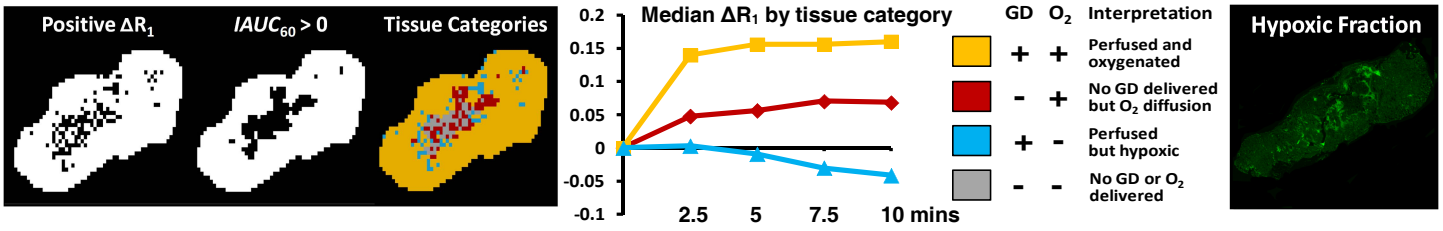
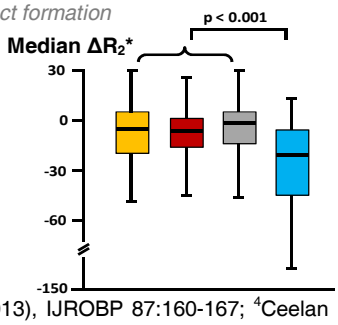


Figure 2 (left): Example maps of O_2 and GD enhancement along with mismatch; (middle): Dynamic ΔR_1 onset and magnitude during 100% O_2 wash in (avascular anoxic category omitted) and interpretation of multi-parametric signal changes; (right): Pimonidazole adduct formation

Relationship of ΔR_2* to other MRI parameters: Average median ΔR_2* was -18.7 ms^{-1}. Median ΔR_2* had no consistent relationship to ΔR_1 or $IAUC_{60}$ and did not relate to PVA or HF on pathology. However, when the voxel-wise ΔR_2* was analyzed in each tumor region parcellated by OE-MRI and DCE-MRI, greatest ΔR_2* were seen in hypoxic tumor regions (median ΔR_2* -19.0 ms^{-1}) and this distribution of voxel values was significantly different from all other tumor voxel categories defined on OE-MRI and DCE-MRI (Figure 3).

Figure 3 (opposite): Box-whisker plots of ΔR_2 for tumor categories defined by OE-MRI and DCE-MRI. Box centre is median ΔR_2*, limits are 25th and 75th centiles and whiskers are 5th and 95th centiles.*



Conclusion: The multi-parametric data presented provide new insight into the spatial and temporal relationships between regional perfusion and hypoxia in tumors. This method has potential for non-invasive delineation of tumor hypoxic volume for radiotherapy and monitoring response to radiotherapy and hypoxia-modifying agents.

References: [1]Tatum (2006), Int J Rad Biol 82:699-757; [2]Linnik (2014), MRM doi: 10.1002/mrm.24826; [3]Baker (2013), IJROBP 87:160-167; [4]Ceelan (2006) IJROBP 64:1188-1196; [5]Halle (2012), Can Res 72:5285-5295; [6]Burrell (2013), JMRI 38:429-434.

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