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 need1. T1-weighted oxygen enhanced MRI (OE-MRI) can distinguish well oxygenated voxels from those that are hypoxic2, blood oxygenation level dependent (BOLD) imaging can quantify the oxygen binding to haemoglobin after gas challenge3, and dynamic contrast enhanced MRI (DCE-MRI) estimates of perfusion have variable relationships to tissue and genetic hypoxia markers4,5. We show that combining these 3 methods enables detailed MRI evaluation of tumor oxygen delivery and hypoxia.

Methods: Cells from a sunniltubus 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 106 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 T2-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 R1) and two True-FISP inversion recovery images (to map R1) were acquired on medical air (21% O2), using sequences described previously1. 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% O2 gas breathing “wash-in” (each taking 2.5 mins). Then one R1 map and one R1 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 acquired (10 s temporal resolution (Figure 1)).

Voxel-wise R1, R2 and IAUC06 maps were derived for each map, using a Bayesian maximum a posteriori approach, with in-house software6. R1 was calculated by R1 (O2) - R1 (air); R2 was calculated with R1 (O2) data from the final R1 map and the mean of the two R1 maps acquired on air breathing. Intraportal injection of pimonidazole (60mg/kg) occurred 55 minutes before 100% O2 inhalation began. Hoechst 33342 (15mg/kg) was administered by tail vein injection one minute prior to pimonidazole fraction (HF) and perfused vessel area (PVA) were calculated by 5 μm cryostat sections.

Results and Discussion: Nine mice were imaged. Tumor size (range 178 to 815 mm3) had no significant relationship to any functional MRI data. Summary OE-MRI and DCE-MRI data: All tumors showed overall positive R1 increase, with average ΔR1 = 0.120 s-1 (range 0.012 to 0.201). Within-scan R1 co-efficient of variation (CoV) was 0.72%. Based on this, individual voxels with R1 change greater than 2 x (CoV) x (mean baseline tumor R1) were considered statistically different from baseline. Tumor IAUC06 values average was 0.139 mmol.min (range 0.2023 to 0.404). PVA correlated with median ΔR2 (Spearman’s rho 0.857, p=0.007) and median IAUC06 (rho 0.893, p=0.007). The HF was correlated to median ΔR1 (rho -0.783, p=0.013) and the % of voxels with positive ΔR1 (rho -0.801, p=0.010).

Voxel-wise analysis of heterogeneity: ΔR1 and IAUC06 maps were divided into voxels demonstrating positive enhancement or not. OE-MRI positive ΔR1 was defined as above. DCE-MRI enhancement was defined as IAUC06 > 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 1). Well perfused voxels had greater positive ΔR1 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 ΔR1 values, with significance (p<0.001) reached at 7.5 mins (Figure 2).

Relationship of ΔR1* to other MRI parameters: Average median ΔR1* was -18.7 ms-1. Median ΔR1* had no consistent relationship to ΔR1 or IAUC06 and did not relate to PVA or HF on pathology. However, when the voxel-wise ΔR1* was analyzed in each tumor region parcellated by OE-MRI and DCE-MRI, greatest ΔR1* were seen in hypoxic tumor regions (median ΔR1* -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).

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 hypoxia volume for radiotherapy and monitoring response to radiotherapy and hypoxia-modifying agents.


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