Investigating ΔR₁ and ΔR₂* as Biomarkers of Tumour Oxygenation

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Introduction: Tumour hypoxia is associated with aggressive, malignant phenotypes and is a major determinant of treatment response and patient outcome. A range of imaging methods are being investigated that may provide a means of assessing tumour hypoxia in vivo [1]. Molecular oxygen is paramagnetic, and therefore in solution shortens the MR longitudinal relaxation time T₁ of surrounding water protons [2]. In vivo, the level of molecular oxygen dissolved in blood plasma has been shown to cause tissue T₁ to change proportionally to the variation in O₂ concentration [3]. Controlled perturbation of blood plasma O₂ through breathing a hyperoxic gas mixture such as carbogen (85% O₂, 5% CO₂) results in a reduction in T₁ (ΔT₁), providing information about changes in tissue oxygenation status. ΔT₁ has therefore been proposed as a novel imaging biomarker of tissue and tumour oxygenation [3, 4]. This approach is distinct to intrinsic susceptibility MRI, which relies on the dependence of the tissue transverse relaxation rate R₂* on the ratio of oxy- to deoxyhaemoglobin in blood. Baseline R₂* and carbogen-induced ΔR₂* are also being investigated for the provision of imaging biomarkers of tumour hypoxia [5, 6]. Given the dependence of both T₁ and R₂* on blood oxygenation, we hypothesised that parallel use of the two biomarkers may provide a more informative index of tumour oxygenation than either does individually. To this end, changes in oxygenation in GH3 prolactinomas during carbogen breathing was investigated using ΔR₁ (1/T₁) and ΔR₂*.

Methods: GH3 prolactinomas were propagated by subcutaneous injection of 2.5 × 10⁶ cells into the flanks of six nude NCr mice. The tumours were imaged at a diameter of approximately 1cm. All images were acquired on a 7T horizontal bore Bruker system using a 3cm birdcage coil. The mice were anaesthetised and restrained using dental paste in order to limit motion artefacts [8]. A nosepiece was positioned for delivery of air or carbogen. TurboRARE images were acquired for tumour delineation, followed by two sets of baseline multi gradient echo (MGE) images (TR=200ms, TE=6-28ms, 4ms echo spacing, 8 averages, 2min 30s AQ), and one set of inversion recovery TrueFISP images (TE=1.2ms, TR=2.4ms, scan TR=10s, α=60º, 20 averages, 8 min AQ) from 3 contiguous 1mm slices acquired from a 3x3cm FOV and 128x128 matrix whilst the host breathed air. The gas supply was then switched to carbogen, and following a two minute transition time, further identical MGE and TrueFISP image sets acquired. Data Analysis: Data were fitted using a Bayesian maximum a posteriori approach. This approach takes into account the data’s Rician noise distribution, which was used in the calculation of a log-likelihood function which incorporated the Rice probability density function [9]. Tumour T₁ and R₂* values were estimated on a pixel-by-pixel basis. The MGE signal magnitude was modelled as a single exponential decay, enabling estimates of ΔR₂* uncertainty (σΔR₂*) to be defined and the probability that a given ΔR₂* estimate was significantly greater than or less than zero.

Results and Discussion: Representative ΔT₁ and ΔR₂* maps from one GH3 prolactinoma are shown in Figure 1. The ΔT₁ and ΔR₂* responses to carbogen were independently spatially heterogeneous. Carbogen breathing significantly increased R₁ (p<0.02) and reduced R₂* (p<0.05) in all six tumours (see Table). The positive median ΔR₁ is consistent with an increase in dissolved plasma O₂ concentration. The reduction in R₂* indicates increased blood oxyhaemoglobin concentration. A weak yet statistically significant correlation (r=0.54, p<0.05) was determined between ΔR₁ and ΔR₂* (Figure 2). This correlation suggests that tumours exhibiting a relatively smaller reduction in R₂* exhibited a greater increase in R₁. This suggests an oxygenated tumour region with saturated haemoglobin, which therefore exhibited greater blood O₂ concentration during carbogen breathing. Conversely, tumour regions which exhibited relatively large reductions in R₂* showed a less pronounced increase in R₁, consistent with a hypoxic yet erythrocyte perfused tumour region in which the deoxyhaemoglobin binds any dissolved molecular O₂ in the blood.

Conclusions: In a controlled preclinical setting, the GH3 tumour model exhibits a significant and positive ΔR₁ and significantly negative ΔR₂* during carbogen breathing. Large negative ΔR₂* and small ΔR₁ may indicate hypoxic tumour tissue, whereas small negative ΔR₂* and large ΔR₁ suggest oxygenated tumour regions. The combined use of ΔR₂* and ΔR₁ may prove more informative for the assessment of tumour hypoxia.

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