Evaluation and Immunohistochemical Qualification of Carbogen-Induced ΔR₂* as a Non-Invasive Imaging Biomarker of Improved Tumour Oxygenation

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Introduction

Intrinsic susceptibility MRI is being investigated to provide imaging biomarkers of tumour hypoxia¹. Paramagnetic deoxyhaemoglobin creates magnetic susceptibility perturbations that increase the MRI transverse relaxation rate R₂* of water in blood and in the tissue surrounding blood vessels. Gradient Recalled Echo (GRE) MRI methods are sensitive to R₂* and thus to blood deoxyhaemoglobin levels. Deoxyhaemoglobin is therefore an intrinsic, blood oxygenation level dependent (BOLD) contrast agent. Changes in tumour R₂* induced by carbogen (95% O₂/5% CO₂) breathing can be used to assess haemodynamic tumour vasculature². As the oxygenation of haemoglobin is proportional to the arterial blood pO₂, and therefore in equilibrium with tissue pO₂, measurements of tumour R₂* should also provide a sensitive index related to tissue oxygenation. Correlations of carbogen-induced decrease in R₂* and changes in tumour pO₂ determined using invasive electrodes at single locations have shown that a carbogen-induced decrease in R₂* is temporally indicative of increased tumour oxygenation in vivo³. In this study, we have investigated the relationship of tumour ΔR₂* to changes in hypoxia, the latter quantified immunohistochemically in the same tumour using a double 2-nitroimidazole hypoxic marker approach⁴.

Methods

Female NCr nude mice were injected with 2.5x10⁶ GH3 prolactinoma cells subcutaneously in the flank. Tumour-bearing mice were administered with 80mg/kg i.p. of the 2-nitroimidazole CCI-103F to provide a baseline measurement of hypoxia. At least 2 hours later, mice were anaesthetised and positioned such that the tumour hung into a 2cm ¹H surface coil. An intraperitoneal line primed with a carbogen (95% O₂/5% CO₂) breathing can be used to assess haemodynamic tumour vasculature. Gradient Recalled Echo (GRE) MRI methods are sensitive to R₂* and thus to blood deoxyhaemoglobin levels. Deoxyhaemoglobin is therefore an intrinsic, blood oxygenation level dependent (BOLD) contrast agent. Changes in tumour R₂* induced by carbogen (95% O₂/5% CO₂) breathing can be used to assess haemodynamic tumour vasculature. As the oxygenation of haemoglobin is proportional to the arterial blood pO₂, and therefore in equilibrium with tissue pO₂, measurements of tumour R₂* should also provide a sensitive index related to tissue oxygenation. Correlations of carbogen-induced decrease in R₂* and changes in tumour pO₂ determined using invasive electrodes at single locations have shown that a carbogen-induced decrease in R₂* is temporally indicative of increased tumour oxygenation in vivo. In this study, we have investigated the relationship of tumour ΔR₂* to changes in hypoxia, the latter quantified immunohistochemically in the same tumour using a double 2-nitroimidazole hypoxic marker approach. Data were acquired from GH3 prolactinomas, in which a carbogen-induced ΔR₂* has been well-described.

Results and Discussion

The figure shows calculated R₂* maps acquired from one GH3 prolactinoma during a) air and b) carbogen breathing. Intense (white) regions (relatively fast R₂*) in the initial air-breathing R₂* map are consistent with the presence of deoxyhaemoglobin, whilst dark areas (relatively slow R₂*) are consistent with the presence of oxyhaemoglobin. Carbogen challenge resulted in a clear decrease in R₂*, indicating a decrease in deoxyhaemoglobin. The average baseline R₂* for all the tumours was 113.4 ± 14s⁻¹ (n=6). Carbogen breathing resulted in a significant reduction in R₂* (mean ΔR₂* -8.9 ± 4s⁻¹, p<0.05, Student’s paired t-test). Composite fluorescence images showing the distribution of c) CCI-103F (red) and d) pimonidazole (green) adduct formation obtained from the same GH3 tumour are also shown. Spatially, both CCI-103F and pimonidazole adduct formation were co-localised, but the extent of pimonidazole staining was typically lower. Bioreduction of 2-nitroimidazoles and strong adduct formation typically occurs at pO₂<10mmHg. The tumour area of CCI-103F adduct formation (7.1 ± 2%) was significantly greater than for pimonidazole (5.2 ± 2%, p<0.05, Student’s paired t-test), consistent with a carbogen-induced improvement in oxygenation within the hypoxic tumour regions.

Conclusions

The significant reduction in R₂* with carbogen breathing was associated with a significantly lower pimonidazole staining than CCI-103F positivity, providing further validation of carbogen-induced ΔR₂* as a non-invasive imaging biomarker of increased tumour oxygenation. Ongoing studies are i) interrogating the distribution and contribution of tumour perfusion, assessed by Hoechst 33342 uptake, to R₂* and ΔR₂*, and ii) whether these relationships hold in other tumour models.

References


We acknowledge the support received for the CRUK and EPSRC Cancer Imaging Centre, in association with the MRC and Department of Health (England) (grants C1060/A10334 and C16412/A6269), the Biotechnology and Biological Sciences Research Council (grant S20430), NHS funding to the NIHR Biomedical Research Centre and The Royal Society.