Evaluation and Immunohistochemical Qualification of Carbogen-Induced ΔR_2* as a Non-Invasive Imaging Biomarker of Improved Tumour Oxygenation

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Introduction

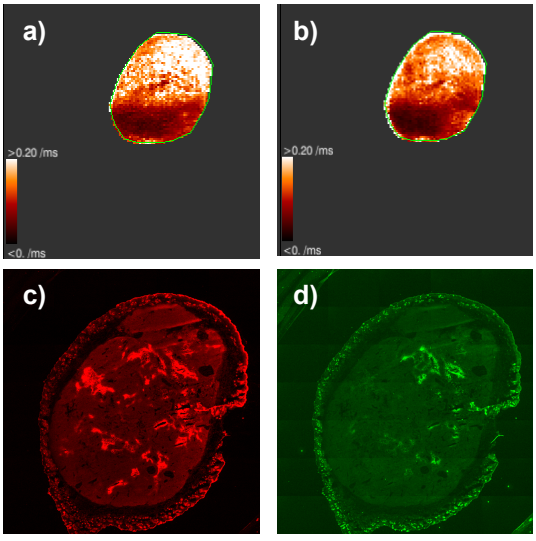
Intrinsic susceptibility MRI is being investigated to provide imaging biomarkers of tumour hypoxia[1]. Paramagnetic deoxyhaemoglobin creates magnetic susceptibility perturbations that increase the MRI transverse relaxation rate R_2* of water in blood and in the tissue surrounding blood vessels. Gradient Recalled Echo (GRE) MRI methods are sensitive to R_2* and thus to blood deoxyhaemoglobin levels. Deoxyhaemoglobin is therefore 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 related to tissue oxygenation. Correlations of carbogen-induced decrease in R_2* and changes in tumour pO_2, determined using invasive electrodes at single locations have shown that a carbogen-induced decrease in R_2* is temporally indicative of increased tumour oxygenation *in vivo*[3]. In this study, we have investigated the relationship of tumour ΔR_2* to changes in hypoxia, the latter quantified immunohistochemically in the same tumour using a double 2-nitroimidazole hypoxic marker approach[4]. Data were acquired from GH3 prolactinomas, in which a carbogen-induced ΔR_2* has been well-described[2].

Methods

Female NCr nude mice were injected with $2.5x10^6$ 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 ^{1}H surface coil. An intraperitoneal line primed with a second 2-nitroimidazole, pimonidazole, was inserted into the abdomen, and a nosepiece positioned for gas delivery. Intrinsic susceptibility MRI was performed using a Bruker 7T microimaging system. To quantify R_2*, multi gradient-echo (MGRE) images were acquired from three 1mm thick coronal slices through the tumour, with T_R=200ms, T_E=6ms, T_ESPACE=4ms and 8 echoes. Carbogen was then continuously delivered at 1l/min, and five minutes later 60mg/kg pimonidazole was administered i.p. At least 45 minutes later, during which the mouse continued to inhale carbogen, a second set of MGRE images were acquired. Finally, the mouse was removed from the magnet, the tumour rapidly excised and frozen over liquid nitrogen. Apparent R_2* maps were calculated on a voxel-by-voxel basis using in-house software (ImageView), and median R_2* for each slice determined from an ROI over the whole tumour. Composite images of whole 10μm thick tumour sections, cut approximately in the same plane as for MRI, were immunohistochemically processed for the immunorecognizable reduced adducts of CCI-103F and pimonidazole. The degree of CCI-103F and pimonidazole adduct formation was quantified as a percentage of the whole tumour section area but excluding the skin (3 sections per tumour).

Results and Discussion

The figure shows calculated R_2* maps acquired from one GH3 prolactinoma during a) air and b) carbogen breathing. Intense (white) regions (relatively fast R_2*) in the initial air-breathing R_2* map are consistent with the presence of deoxyhaemoglobin, whilst dark areas (relatively slow R_2*) are consistent with the presence of oxyhaemoglobin. Carbogen challenge resulted in a clear decrease in R_2*, indicating a decrease in deoxyhaemoglobin. The average baseline R_2* for all the tumours was 113.4 ± 14s^{-1} (n=6). Carbogen breathing resulted in a significant reduction in R_2* (mean ΔR_2* -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 pO_2<10mmHg[5]. 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 R_2* with carbogen breathing was associated with a significantly lower pimonidazole staining than CCI-103F positivity, providing further validation of carbogen-induced ΔR_2* 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 R_2* and ΔR_2*, and ii) whether these relationships hold in other tumour models.

References

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