Comparison of response to OXi4503 therapy in subcutaneous and orthotopic liver metastasis models using susceptibility and diffusion MRI

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Introduction
The development of novel treatments in pre-clinical studies are primarily carried out in subcutaneous xenograft tumour models. However, it is argued that orthotopic models (in which tumours develop in the tissue or organ from which the cells were derived) would allow for a broader spectrum of responses to treatment. The combination of intrinsic susceptibility and diffusion MRI can provide sensitive measures of the acute response to vascular disrupting agents (1, 2), although this has not been extensively applied to orthotopic tumour models. The aim of this study was to assess and compare the response of an orthotopic liver metastasis model with an equivalent subcutaneous tumour model, following treatment with OXi4503 (a prodrug of combretastatin A1), which acts as a vascular disrupting agent. This inhibits tumour blood flow leading to central necrosis, but leaves a viable rim of tumour cells after treatment (3).

Methods

Tumour models: 1×10⁶ luciferase-transduced SW1222 colorectal carcinoma cells were injected intrasplenically into MFl nu/nu mice (week 0). Development of metastatic disease was monitored by bioluminescence on an IVIS system following 150mg/kg luciferin i.p., injection on weeks 1, 3 and 4. A 9.4T Varian VNMRS 20 cm horizontal-bore system (Varian Inc. Palo Alto, CA, USA), with a 39 mm birdcage coil was used to acquire multi-slice, fast spin echo images revealing liver tumour burden at weeks 2, 3, 4 and 5. All scans were triggered with respiratory gating (SA instruments, New York, US). For the subcutaneous tumour model, 5×10⁶ SW1222 cells were injected subcutaneously into the flank of 6 mice at week 3 and their growth monitored using caliper measurements.

Therapy response: To assess response to treatment at week 5, an axial, diffusion-weighted spin-echo (SE) EPI sequence was used to estimate tumour ADC (see sequence details below). A multi-gradient echo (MGE) sequence was also used to estimate tumour R₂*. Both scans were triggered with respiratory gating. Dosing with 40mg/kg OXi4503 was performed using an i.p. line within the scanner bore. Following a pre-treatment measurement, MGE and SE sequences were interleaved and acquired dynamically, enabling the acute response to the drug to be monitored for up to 3 hours post-dosing. Imaging was also performed, in separate sessions, at 24 hours, 3, 5 and 15 days following dosing. At 3 days (for orthotopic tumours) or 15 days (for subcutaneous tumours), the hypoxia and perfusion markers pimonidazole and Hoechst33342, respectively, were administered. Animals were then sacrificed and tumour samples excised for histology.

SEₐₐ parameters: b=2.5, 214, 413, 609, 805 & 1000 s/mm², TR=1500ms, TE=22ms, 20 slices, 128x128 matrix, 40x40mm FOV, 1mm slice thickness, diffusion gradients in the slice direction. MGE parameters: 8 echoes, TE=2ms, echo spacing=2ms, TR=280ms; all geometric parameters matched to SEₐₐ.

Post-processing: Regions of interest were drawn corresponding to either multiple individual tumour metastasis in the liver or the entire subcutaneous tumour. Median R₂* and ADC values were estimated by model fitting MGE and SE data on a pixel by pixel basis. Hyper-acute measurements of R₂* with time were fitted with the following phenomenological model to assess the rate of drug uptake and action: ΔR₂* = -S[-1(1-e⁻[-r₁t])(e⁻[r₂t] – A)] [Eq.1].

Results
Orthotopic liver model: Fig. 1 shows gated, fast spin echo images from week 3, 4 and 5, from which the total volume was calculated as 6mm³, 178mm³ and 1532mm³, and in which individual, focal lesions can be identified. IVIS bioluminescence images also confirmed the presence of liver metastases, and could detect tumour formation from an earlier time point (week 1).

Acute response: Following treatment at week 5, R₂* measurements during the acute phase (0 to 100 minutes) initially decreased, followed by a slow increase past the baseline level (Fig. 1a). This characteristic vascular response has been reported previously [1]. The effect was greater and more rapid in the orthotopic model than the subcutaneous model, but also showed greater variability between animals and also between lesions within individual animals. From model fitting Eq. 1 to ΔR₂* data, r₁=15.5/6 min was estimated in orthotopics and 2.3±1.1 min in subcutaneous tumours (p<0.01, Wilcoxon rank sum), suggesting a more rapid delivery and/or action of the drug in the orthotopic model. ADC decreased significantly by significantly in the subcutaneous model. Baseline ADC was significantly greater in orthotopic tumours than in subcutaneous tumours.

Chronic response: The chronic ADC response pattern differed between models. In orthotopics, ADC had recovered to baseline by 24 hours, then increased at a linear rate of (0.5±0.2)×10⁻³ mm²/s/day. Subcutaneous tumours recovered to above baseline by 24 hours, then plateaued at (1.2±0.5)×10⁻³ mm²/s. The increase in ADC following therapy is proposed to reflect the onset of necrosis. Histological analysis confirmed the induction of hypoxia and necrosis at 24 hours following treatment and a significant reduction in blood volume.

Discussion & Conclusion
The results of this study have identified differences in the response to OXi4503 in subcutaneous and orthotopic tumour xenograft models. The orthotopic models appeared to respond more rapidly and to a greater magnitude than the subcutaneous model, both acutely (as evaluated by susceptibility MRI) and chronically (as evaluated by diffusion MRI). This is likely to be due to differences in the vascular supply to tumour cells prior to therapy and access to nutrients from surrounding tissues following vascular collapse within the tumour. Ongoing histological assessments are being undertaken to determine the physiological effects that might underpin these observations, however, these results highlight that careful attention should be paid to the choice of tumour model used in the evaluation of anti-cancer therapies and their dependence on tumour sitting.