Using BOLD MRI with Carbogen to Evaluate Tumour Response to Antiangiogenic Therapy

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Introduction

Tumour growth and survival is dependent on the development of a functional vascular network to provide oxygen and nutrients, and for the removal of metabolic waste products. Antiangiogenic agents exploit the reliance of the tumour on angiogenesis for survival as target for therapy, and are designed to target neovasculature. Vandetanib (ZACTIMA™ (ZD6474), AstraZeneca) is a low molecular weight inhibitor of vascular endothelial growth factor (VEGF) receptors 1 and 2, shown to significantly inhibit tumour angiogenesis and growth in a wide range of models *in vivo*. (Ryan AJ and Wedge SR, Br J Cancer, 92, S6-13, 2005). As antiangiogenic agents do not typically induce tumour regression, non-invasive imaging biomarkers sensitive to disrupted tumour perfusion are continually being developed and applied. The aim of this study was to investigate tumour response to vandetanib in murine PC3 (prostate adenocarcinoma) xenografts using blood oxygen level-dependent (BOLD) MRI and diffusion MRI.

Method & Materials

Animals and tumours: Tumours were propagated by injecting $5x10^6$ PC3 cells subcutaneously in male NCr nude mice (n=12). Tumours were considered suitable for MRI once a volume of $500mm^3$ was reached.

Drug preparation and administration: The antiangiogenic agent vandetanib was dissolved in a solution of deionised water with 1% polysorbate 80 to a concentration of 3.75mg/ml, and milled overnight at room temperature. Mice were given 25mg/kg vandetanib by oral administration on day 0 and day 1 of the study.

Imaging: MRI was performed on day 0 (pre-dosing), and day 2 (48 hours following first dose; 24 hours following second dose) on a 7T Bruker horizontal bore magnet. Anaesthesia was induced by an intraperitoneal injection of a combination of fentanyl citrate (0.315mg/ml) plus fluanisone (10mg/ml). Once anaesthetised the mouse was positioned so that the tumour hung down into a 1cm diameter surface coil. Multi-gradient echo (MGE) images (TR/flip = 200ms/45°, 8 averages and 8 echo times ranging from 6.2 to 28.2ms), and diffusion-weighted spin echo images (TR/flip = 1000ms/45°, 1 average and 5 b-values ranging from 3.1 to 507.8 s/mm^2) were acquired from 3 transverse, 1mm thick slices through the tumour. MGE images were acquired again whilst the mouse breathed carbogen (5% CO_2, 95% O_2). From these data, maps of baseline (i.e. prior to carbogen) R_2*, ΔR_2* (the change in R_2* due to carbogen) and ADC (apparent diffusion coefficient) were estimated on a voxel-by-voxel basis, using analysis software developed in-house (ImageView).

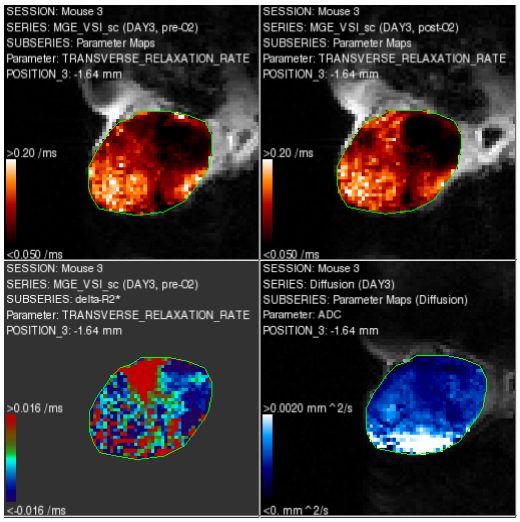

Figure 1: Screenshot from imaging analysis software (ImageView) showing example images of (left-right, top-bottom) baseline R_2*, R_2* during carbogen-breathing, ΔR_2* and ADC.

Results

Example images of each parameter are shown in Fig.1. Median R_2*, ΔR_2* and ADC were estimated for each tumour, pre- and post-therapy (day 1 and day 3, respectively). A paired t-test revealed that the median baseline R_2^* decreased significantly across the group following therapy ($p<0.01$, Fig.2a), consistent with a reduction in paramagnetic deoxyhaemoglobin. Similarly, the median carbogen-induced ΔR_2^* also decreased significantly ($p<0.05$, Fig.2b), implying an increase in the delivery of oxygenated blood to the tumour following therapy. The median ADC, however, did not display a significant change ($p>0.1$, Fig.2c).

Discussion & Conclusion

The results of this study demonstrate that treatment of PC3 tumours with 25mg/kg vandetanib for a period of 2 days significantly decreased baseline tumour R_2* and carbogen-induced ΔR_2*. Similarly acute reductions in perfusion/permeability of PC3 tumours following treatment with vandetanib have been previously reported (Checkley, Br J Cancer, 17, 1889-95, 2003). The data herein are consistent with vandetanib-induced vascular normalisation, whereby antiangiogenic agents can initially improve tumour blood vessel structure and function (Jain RK, Science, 307, 58-62, 2005). Normalisation may prune immature blood vessels and enhance blood flow, and the results of this study support this concept. These data also highlight the potential of BOLD MRI to provide non-invasive biomarkers of tumour response to VEGF inhibition.

Acknowledgements

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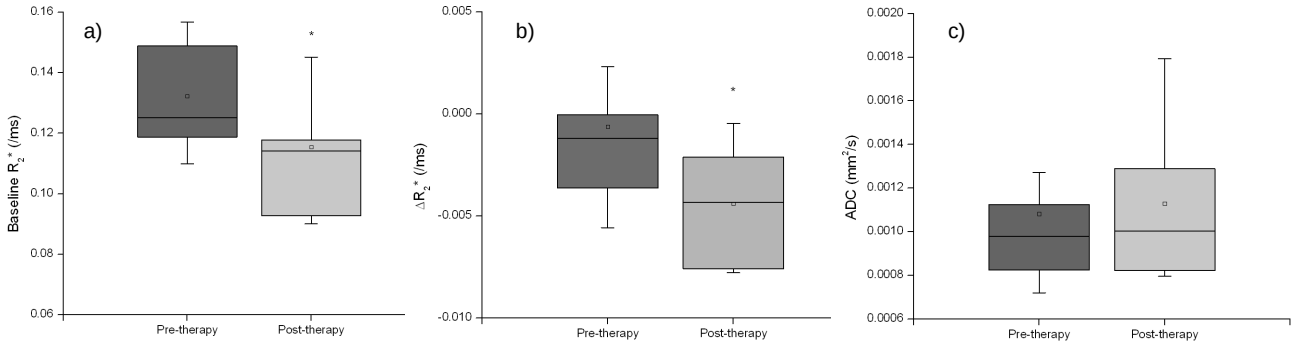

Figure 2: Box plots for the median change in a) baseline R_2*, b) carbogen-induced ΔR_2* and c) ADC, following a dose of vandetanib (square: mean; box: 25th, 50th and 75th percentiles; whiskers: 10th and 90th percentiles). Differences in mean values due to therapy were significant in the median R_2* and ΔR_2* ($p<0.01$ and $p<0.05$, respectively), but not in the median ADC.