Pre-Clinical Assessment of Anti-Vascular Effects of Novel Combretastatin A-4 Analogues by Dynamic Contrast Enhanced MRI

I. V. Linnik[1], S. R. Williams[1], K. E. Davies[1], A. T. McGown[2], J. A. Hadfield[2], and D. L. Buckley[1]

[1]Imaging Science and Biomedical Engineering, University of Manchester, Manchester, United Kingdom, [2]Centre for Molecular Drug Design, University of Salford, Manchester, United Kingdom

PURPOSE The aim of the study was, firstly, to develop a robust quantitative DCE-MRI protocol for studying tumour vasculature in a rodent model and, secondly, to deploy it for evaluation of the vascular effects of several novel anti-vascular agents. Five newly synthesized combretastatin analogues (labelled agent **3** – **7**), combretastain A-4 (CA-4) and oil as a control were tested for their effects on RIF-1 tumours grown in mice 24 hours after administration.

METHODS ANIMAL PROTOCOL: The tumour model used was a radiation induced fibrosarcoma (RIF-1) grown in the flanks of 10-16 week-old female C3H mice. All animals were scanned once and then assigned to one of five treatment or two control groups (n=4 in each group). Treatment groups were dosed with the five novel combretastatin analogues agent **3** - **7**. Drugs were dissolved in 5% dimethylacetamide (DMA) and hazelnut oil and injected *i.p*. at the following doses: 100 mg/kg for agents **3**, **4**, **5**, **7** and 10 mg/kg for agent **6**. Two control groups were used: one was injected with an equivalent volume of 5% DMA and hazelnut oil as a negative control and another one with CA-4 (100 mg/kg) as a positive control. Each animal was scanned a second time at 24 hours after treatment.

MR PROTOCOL: Data were obtained using a SMIS console (Surrey Medical Imaging Systems, Guildford UK) interfaced to a 16 cm horizontal bore 7 T magnet and shielded gradient coils from Magnex Scientific (Abingdon, UK). A 38 mm I.D. quadrature volume coil (Rapid Biomedical, Germany) was used for excitation and detection. Prior to Gd-DTPA injection, data were acquired for a T1 measurement using a spin echo (SE) and inversion recovery (IR) sequence pair with the following parameters: TR/TE= 2000 ms/25 ms; FOV 33 x 33 mm; 16 contiguous 1 mm slices; 2 averages; pixel resolution =0.26 x 0.52 x 1 mm. An inversion time of 1500 ms was used. A T1-weighted 3D GE sequence was used to obtain dynamic quantitative data in the coronal plane (FOV= 33 x 33 x 19.2 mm; acq. matrix 128 x 64 x 17; recon. matrix 128 x 128 x 32; TR/TE=17 ms/5 ms; flip angle = 20^o; 1 average; acq. time 19 s). The dynamic imaging sequence was run continuously until 50 volumes were acquired, with Gd-DTPA injection into the tail vein (0.25 mmol/kg).

DATA ANALYSIS: A whole tumour region of interest was selected in the slice with the largest cross-section of the tumour. Baseline T1 was estimated from the ratio of IR/SE signal intensities. The average pixel intensity was measured over time then converted to a change in 1/T1 (R1) over time as a measure of Gd-DTPA concentration.[1] An AIF determined in a previous mouse study was used.[2] The data resulting from the dynamic contrast enhanced study were analysed with a compartmental model which allows us to estimate K^{trans} (transfer constant) and v_e (interstitial volume) by fitting the model to the imaging data as suggested by Tofts and Kermode.[1]

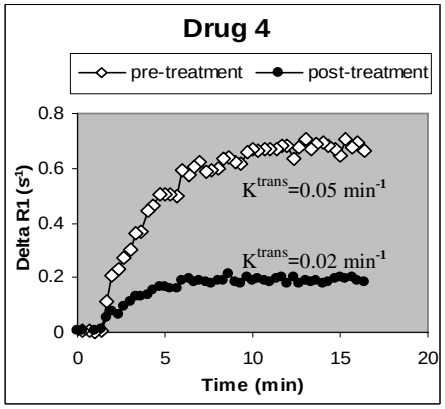
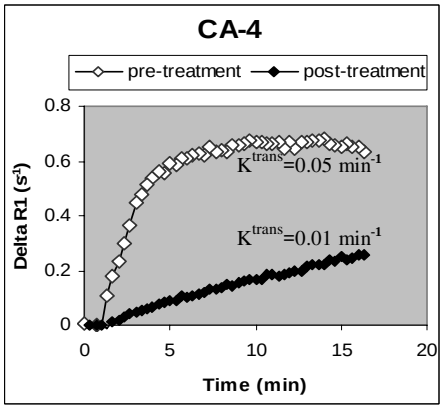


*Fig.1 .Gd-DTPA uptake curves before treatment (white diamonds) and 24 hours after i.p. drug injection (black diamonds) from regions drawn around tumours treated with agent **4** (left hand side) and CA4 (right hand side). Delta R1(s^{-1}) - change in 1/T1 over time.*



RESULTS Estimates of baseline T1, K^{trans} and v_e were obtained for each tumour pre- and post-treatment. The mean pre-treatment tumour K^{trans} value (n=28) was 0.057 ± 0.028 min^{-1} and mean v_e (n=27) was 0.39 ± 0.25. The mean baseline T1 (n=28) was 1972 ± 695 ms. Three drugs produced a significant reduction in K^{trans}: CA-4 - 72% ($p < 0.012$); agent **3** - 75% ($p < 0.041$); and agent **4** - 68% ($p < 0.013$). v_e was significantly reduced by two drugs: agent **4** - 75% ($p < 0.010$) and agent **6** - 40% ($p < 0.019$). No significant effects were seen in the tumours treated with oil-DMA.

CONCLUSIONS In this study, we applied quantitative DCE-MRI to evaluate the effect of new anti-cancer drugs on the vasculature of RIF-1 tumours in mice. By undertaking measurements of the AIF and baseline T1 we have maximized the accuracy of the estimates of tumour vascular parameters[3]. We have shown that three combretastatin analogues, as well as the positive control CA-4, have a significant effect on tumour vascular parameters using only 4 animals per group. The results suggest that the applied DCE-MRI method may be a valuable screening tool to monitor, non-invasively, the effects of anti-vascular drugs *in vivo*.

ACKNOWLEDGMENTS Funded by Cancer Research Technology.

REFERENCES: 1. Tofts and Kermode. *Magn Reson Med*, **17**:357-367 (1991); 2. Linnik *et al*, *ISMRM 14th Scientific Meeting*, Seattle, WA, USA, (2006). 3. Buckley. *Magn Reson Med,* **47**:601-606 (2002).