Evaluation of the anti-vascular effects of combretastatin in rodent tumours by dynamic contrast enhanced MRI.

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Introduction

Anti-vascular and anti-angiogenic drugs are currently of great interest in the treatment of cancer. Such drugs should have a selective effect in destroying or inhibiting the formation of tumour vasculature, respectively, therefore depriving tumour cells of nutrients and contributing to cell death. Combretastatin A4 Phosphate (CA4P) is a tubulin-binding agent which has been shown to act on vascular endothelial cells *in vitro* and to reduce blood flow in animal tumour models *in vivo*[1]. It may also have anti-angiogenic activity.

Dynamic contrast enhanced MRI (DCE-MRI) has been used in Phase I trials of CA4P to evaluate the extent and time course its anti-vascular effect in human tumours. However, DCE-MRI with Gd-DTPA does not provide an umabiguous measurement of tumour blood flow since the kinetics of this contrast agent in tumours are also sensitive to the permeability-surface area product. This particularly complicates the interpretation of CA4P effects since the drug is expected to increase vascular permeability (to macromolecules, at least) as well as reducing blood flow.

The present study utilises the DCE-MRI protocol used for the Phase I clinical trials in a rat tumour model which has also been studied in terms of uptake of radiolabeled iodoantipyrine (a freely diffusible tracer) to give absolute blood flow measurements. The two modifications used in this animal tumour study are (i) higher spatial resolution and (ii) pre-contrast T_1 mapping. Unsupervised cluster analysis [2] has been incorporated to identify pixels with similar Gd-DTPA kinetics which can be averaged before mathematical fitting of T_1 and DCE-MRI data.

Methods

Rats bearing subcutaneosuly implanted P22 adenosarcomas on the flank were treated with saline or combretastatin-A4-phosphate (10 mg kg^{-1} or 100 mg kg^{-1}) by intraperitoneal injection at one, six or twenty four hours before MRI examination (n = 3-6 animals per treatment group). Anaesthesia was achieved with Hypnorm/Hypnovel and one tail vein cannulated. Animals were placed in a 4.7 Tesla horizontal bore magnet of a Varian MR spectroscopy/imaging system. A 3.0 cm surface coil was placed around the tumour as a transmitter/receiver. Pre-contrast T_1 values were determined from an inversion recovery sequence (TI = 0.15 s, 0.45 s, 1.3 s, 2.9 s). A sequence of 30 gradient echo images were obtained every 11.8 s; three images before administration of contrast agent followed by infusion of a dose of 0.1 mMol kg^{-1} of Gd-DTPA (Magnevist, Schering) in a volume of 1.0 ml kg^{-1} given over 5 s via an infusion pump. The MRI parameters used were: TR 98ms; TE 10 ms; 2 mm slice thickness a field of view 30 x 30 mm with 256 x 148 data points (giving 0.12 x 0.20 mm in-plane resolution).

Images were subsequently processed using Matlab (The Mathworks, Natick, MA, USA). A region of interest was drawn around the tumour. Neural network cluster analysis was used to segment the images into up to eight classes having similar kinetics of contrast-induced signal intensity changes [2]. T_1 values were calculated for each cluster and used to convert image intensity into Gd-DTPA concentration (C_t) on a pixel-by-pixel basis. The mean contrast agent kinetics was determined for each cluster and fit to :

$$C_t = C_a * K^{trans} . \exp(-k_{ep} . t)$$

where C_a is the assumed arterial concentration of Gd-DTPA based on the parameters described by Rozijn et al.[3] for rats

and * indicates convolution

In addition, area-under-the-curve (AUC) for Gd-DTPA concentration was calculated for each tumour by summing the vales from the 27 post-contrast images (0-5.5 min).

Results

The effects of CA4P on T_1, K^{trans}, k_{ep} and AUC are summarised in Table 1. There was no significant change in mean tumour T_1 relaxation time following CA4P treatment except, possibly, for the 100 mg kg^{-1} at 24h group (e.g. t-test $P<0.05$ vs. 10 mg kg^{-1} at 24h group although N.S. vs. control group) The parameter K^{trans} showed the most marked change with a maximal effect 6 hours after CA4P treatment (four-fold reduction at 10 mg kg^{-1}; ten-fold reduction at 100 mg kg^{-1}). K^{trans} recovered back to control values 24 hours after the low dose but not after the high dose treatment. k_{ep} only showed a two-fold reduction 6 hours after treatment (both doses) but estimation of this parameter was more difficult when tumour Gd-DTPA uptake was low. The relative changes in AUC were similar to those in K^{trans}.

Discussion

The time-dependant changes in K^{trans} following 100 mg kg^{-1} CA4P are consistent with (although lower in magnitude than) previous estimates of tumour perfusion changes in this tumour model determined using radiolabelled iodoantipyrine [1]. e.g. approximately 10-fold and 100-fold reductions in tumour perfusion at one and six hours respectively. Although the reduction in K^{trans} was maintained 24 hours after treatment at 100 mg kg^{-1}, the 10 mg kg^{-1} showed a smaller effect and this was completely reversed by 24 hours. Changes in k_{ep} were much less consistent. Relative changes in AUC were similar to those in K^{trans}. Since calculation of this parameter did not use information about arterial Gd-DTPA concentrations, T_1 values, cluster analysis or any kinetic models it is much simpler to calculate and does not, in itself, require any assumptions. However, K^{trans}, k_{ep} and/or other derived parameters may allow a better physiological understanding. The possible increase in tumour T_1 relaxation time 24 hours after 100 mg kg^{-1} CA4P would be consistent with an increase in tumour necrosis.

References

[1] Tozer GM, et al., Cancer Res. 59: 1626-34 (1999)
[2] Maxwell RJ, et al, ' pp. 93-98 in 'Artificial Neural Networks in Medicine and Biology' Eds. H. Malmgren, M. Borga, L. Niklasson, Springer-Verlag, London, 2000.
[3] Rozijn TH, et al., MAGMA 6: 37-43 (1998)

Table 1. Mean +/- S.D. for each treatment group

dose (mg kg-1)	time (h)	T_1 (s)	K^{trans}	k_{ep}	AUC
0	0	1.07+/-0.13	0.294+/-0.134	0.0036+/-0.0011	5.37+/-2.17
10	1	1.04+/-0.08	0.097+/-0.044	0.0003+/-0.0026	2.65+/-1.18
10	6	1.02+/-0.05	0.068+/-0.036	0.0018+/-0.0020	1.49+/-0.82
10	24	1.03+/-0.06	0.298+/-0.101	0.0037+/-0.0015	4.67+/-1.67
100	1	1.08+/-0.11	0.090+/-0.029	0.0028+/-0.002	1.6+/-0.49
100	6	1.04+/-0.09	0.028+/-0.007	0.0018+/-0.0018	0.50+/-0.33
100	24	1.19+/-0.06	0.037+/-0.036	0.0021+/-0.0011	0.63+/-0.76