Vessel size index MRI to monitor the effect of antivascular treatment in a rodent tumour model

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INTRODUCTION:

Vascular disrupting agents (VDA) cause significant reduction in tumour blood flow (TBF) with no apparent tumour regression. Dynamic contrast enhanced MRI using Gd-DTPA has shown a significant reduction of K^{trans} of rat GH3 prolactinomas at 24 hours after treatment with 350mg kg^{-1} of 5,6 dimethylxanthenone 4-acetic acid (DMXAA) (AS1404, Antisoma, UK), but no effect at lower doses[1]. K^{trans} is a function of TBF and permeability surface area product, hence the anticipated effects of DMXAA, increased blood vessel permeability and reduced blood volume due to vascular collapse, may produce little change in K^{trans}. In this current study we have used the macromolecular USPIO blood pool contrast agent Ferumoxtran-10 (Sinerem®, Guerbet, France / Combidex®, Advanced Magnetics, USA) to determine fractional blood volume (fBV) and vessel size (Rv), two parameters we hypothesised might decrease after treatment with DMXAA.

METHODS:

GH3 prolactinomas were grown subcutaneously in the flanks of Wistar Furth rats. MRI was performed with a 4.7T Varian Unity INOVA with tumours suspended into a 3-turn solenoid. Three axial images were acquired for each tumour with a 128 by 64 matrix and 6cm FOV with: MGRE – TR 80 ms, TE 5 – 40 ms with 8 echoes, flip angle 20°; SE – TR 1000 ms, TE 15 & 40 ms; DWI – TR 1000 ms, TE 40 ms, b-factors 0 & 639 s mm^{-2}. DWI was performed pre-contrast only, MGRE and SE images were acquired pre-contrast and at 5 minutes after administration of contrast agent at a dose of 200 μmol Fe kg^{-1} body weight via tail vein cannulation. Immediately after MRI on Day 1 rats were given DMXAA (*n=6*) at 350 mg kg^{-1} body weight intra-peritoneally or vehicle as control (*n=6*). MRI was repeated on Day 2 at 24 hours post treatment.

MRI data was analysed using ImageBrowser (Varian Inc.) to calculate R_2*, R_2 and ADC pixel by pixel and create fBV and Rv maps[2]. To prevent excessively large Rv values arising from pixels with low SNR or very low fBV, a threshold of $\Delta R_2 > 1$ms was imposed; an estimation of the lower limit accuracy of calculating ΔR_2. Regions of interest encompassing tumour, but excluding muscle, skin and highly cystic regions were defined from ADC maps, and the mean and standard deviation of MRI and VSI parameters for each tumour group pre- and post-treatment calculated. Histogram analyses were also performed for fBV and Rv. Parameter F was defined as the fraction of each tumour with calculated blood volume < 1%.

RESULTS:

All quantified MR parameters are given in Tables 1 and 2 below. Tumour size was not significantly different between the groups and increased by +13% ±29% in controls and +5% ±19% in DMXAA treated tumours. DMXAA produced a significant decrease in tumour fBV after 24 hours, of on average –33% (range –10% to -61%), with 4 tumours showing greater decrease in fBV than the controls (range +5 to -29%, average –13%) over the same 24-hour period. Over all tumours there was a significant inverse correlation between pre-contrast R_2* and tumour size on day 1 ($R = -0.77$, $P < 0.005$) and day 2 ($R = -0.79$, $P < 0.005$).

Table 1 Quantified MRI parameters pre-treatment and at 24 hours post-treatment (mean ± st. dev.)

	R_2 (s^{-1})		R_2*(s^{-1})		ADC (10^{-6} cm^2 s^{-1})	
	pre	*post*	*pre*	*post*	*pre*	*post*
Control (*n=6*)	32 ±4	35 ±5	108 ±19	122 ±24^{+}	0.67 ±0.09	0.65 ±0.6
DMXAA (*n=6*)	30 ±6	34 ±7^{++}	96 ±36	113 ±44^{++}	0.65 ±0.04	0.64 ±0.1

Table 2 Calculated VSI parameters pre-treatment and at 24 hours post-treatment (mean ± st. dev.)

	fBV(%)		F ≤ 1% fBV		Rv (μm)	
	pre	*post*	*pre*	*post*	*pre*	*post*
Control (*n=6*)	2.8 ±0.9	2.4 ±1.0	0.13 ±0.08	0.18 ±0.12	16 ±5	15 ±4
DMXAA (*n=6*)	3.0 ±0.8	2.0 ±0.6* $^{+++}$	0.07 ±0.06	0.30 ±0.17*$^{++}$	15 ±5	13 ±3

Paired t-test post- versus pre-treatment: + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.005$. Student's t-test DMXAA treated versus control: * $P < 0.05$

DISCUSSION:

Reduced fBV and increased F in treated tumours is consistent with the expected action of DMXAA to cause vascular collapse. Assuming that R_2* (and to a lesser extent R_2) is dominated by deoxyhemoglobin (Hb) levels in perfused vasculature, an R_2* increase over 24 hr would be consistent with tumour growth whose increased oxygen needs are not matched by an increased vascular supply for both controls and treated tumours, hence an increase in blood Hb. The inverse correlation of R_2* with tumour size over all tumours however suggests that the dominant effect with growth is of decreasing blood vessel density and necrosis. The significant differences in F and fBV between DMXAA treated and control tumours highlights the sensitivity of using an exogenous contrast agent as compared to endogenous Hb to characterise and differentiate between treatment induced vascular disruption and tumour progression with an increasingly undersupplied vasculature. Rv did not decrease with treatment as hypothesised. The measurement of Rv requires mathematical combination of 5 MR images so has greater measurement error than fBV, which requires just pre- and post-contrast R_2* measurement. Furthermore Rv can only be determined where fBV is sufficient for an accurate measurement of R_2 change with contrast agent, so is biased towards the higher fBV regions. In conclusion, fBV measured using a blood pool contrast agent appears to be a straightforward biomarker for quantifying tumour response to VDAs.

REFERENCES:

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