A ^{1}H MRS Study of Tumour Overexpression of Dimethylarginine Dimethylaminohydrolase (DDAH) and Response to Taxotere

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INTRODUCTION: Dimethylarginine dimethylaminohydrolase (DDAH) is a cytoplasmic enzyme that metabolises endogenous inhibitors of nitric oxide synthase (NOS). Overexpression of DDAH indirectly increases nitric oxide (NO) production[1], and enhances tumour growth and angiogenesis[2,3]. NO has been shown to be both pro- and anti-apoptotic, with the nature and degree of response varying with both cell type and NO concentration[4-6]. The aim of this study was to investigate the utility of *in vivo* ^{1}H magnetic resonance spectroscopy to assess choline concentrations and water diffusion of tumours overexpressing DDAH, and their response to mitotic catastrophe/apoptosis following treatment with taxotere[7].

METHODS: C6 glioma cells transfected to either overexpress DDAH (clones D26 and D27) or containing the empty vector (clone M8) were used. Time lapse microscopy revealed significantly greater apoptosis of D26 and D27 cells, compared to M8 cells, 24 hours after treatment with taxotere. Cells were inoculated subcutaneously in the flanks of female MFI nude mice, and an enhanced tumour growth rate was found for both D26 and D27 tumours as before[2]. Size-matched tumours were positioned into a 15mm diameter two turn RF coil, and ^{1}H MRS was performed using a 4.7T Varian Unity Inova MR spectrometer. Localised voxels within the tumour in the range of 50–150 mm^3 were selected from scout gradient echo images. PRESS localisation with water suppression (TR=2s, 64 transients and varying echo times from 20 - 408ms) was used. Choline concentration (t-choline = PC + GPC + choline) was calculated using unsuppressed tumour water as a reference[8]. Subsequently, diffusion weighted (DW) ^{1}H MRS was performed using a localized STEAM sequence with diffusion sensitising gradients in echo-time periods. The acquisition parameters for DW-MRS were TE=24ms, TM=100ms, TR=3sec, δ=6ms, Δ=112ms, with increasing diffusion gradients from 0 to 13G/cm with 1G/cm interval. The DW water signal was quantified by MRUI software. Plots of normalised water signal (M/Mo) against b-values were fitted with a stretched exponential model[8], giving an estimation of heterogeneity index (α) and distributed diffusion coefficient (DDC). Following pre-treatment ^{1}H MRS measurements, mice bearing D26,D27 and M8 tumours were treated with 15mg/kg Taxotere i.p., and ^{1}H MRS was repeated 24 hours later.

RESULTS: The *in vivo* ^{1}H MRS data are summarised in Table 1. There was no significant difference in the baseline choline concentration of M8, D26 and D27 tumours. Water T_2 and DDC were significantly higher in M8 tumours compared to D26 and D27 tumours. The choline concentration of the M8 tumours significantly increased 24 hours after treatment with taxotere, whereas the D26 and D27 tumours showed no change. Water T_2 significantly decreased and water DDC significantly increased in the D26 tumours following treatment with taxotere. Tumour apoptosis was confirmed with elevated caspase-3 activity in taxotere treated D26 and D27 tumours, detected by western blot.

Table 1	M8 (n=6)		D26 (n=6)		D27 (n=5)	
	Pre	Post	Pre	Post	Pre	Post
t-Choline (mM)	3.72 ± 0.8	4.47 ± 0.7$^{\#}$	2.85 ± 0.5	2.69 ± 0.5	3.31 ± 0.6	2.54 ± 0.5
Water T_2 (ms)	78.3 ± 2**	75.7 ± 2	70.9 ± 2	66.6 ± 2$^{\#}$	63.9 ± 4	62.8 ± 1.4
t-Choline T_2 (ms)	131 ± 15	116 ± 14	182 ± 9*	217 ± 50.2	147 ± 13.4	202 ± 19
Heterogeneity	0.69 ± 0.02	0.71 ± 0.04	0.66 ± 0.01	0.68 ± 0.01	0.70 ± 0.02	0.75 ± 0.03
DDC (10^{-5} cm^2/sec)	1.76 ± 0.3**	1.95 ± 0.3	0.85 ± 0.03	1.08 ± 0.1$^{\#}$	0.82 ± 0.04	1 ± 0.12

(mean ± s.e.m., **p<0.05 pre-treatment M8 compared to both D26 and D27, *p<0.05 pre-treatment D26 compared to both D27 and M8, Student's t-test, $^{\#}$p<0.05, Student's paired t-test).

DISCUSSION: Despite the increased growth rates of the D26 and D27 tumours, there was no significant difference in baseline choline concentration, implying no apparent link between choline metabolism and DDAH-enhanced vascularisation. The significantly longer baseline water T_2 and faster DDC in the M8 tumours is consistent with a greater extravascular space and lower degree of vascularisation, compared to the D26 and D27 tumours overexpressing DDAH which results in an enhanced angiogenic phenotype[2,3]. The choline concentration of the M8, but not D26 or D27, tumours increased following treatment. This, coupled with the caspase-3 activity data, suggests that overexpression of DDAH enhances apoptotic tumour response to taxotere. The data also suggest that increased NO production through overexpression of DDAH confers pro-apoptotic activity *in vivo*.

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