The Response of RIF-1 Fibrosarcomas and LS174T Colon Carcinomas to Chemotherapy Assessed by *In vivo* ^{1}H MRS Methods

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INTRODUCTION: ^{1}H MRS methods of quantifying tumour choline content[1,2] and diffusion weighted water[3,4] signal are being exploited to provide early *in vivo* markers of tumour response to chemotherapy. Chemotherapeutic agents predominantly induce cell death either by necrosis or apoptosis. Previous studies have shown that the antimetabolite 5-fluorouracil (5FU) induces both necrosis and apoptosis in RIF-1 fibrosarcomas[5], whereas treatment with the mitotic poison taxotere results in cell cycle arrest and the induction of apoptosis in colon carcinomas[6]. In this study, localised ^{1}H MRS techniques[7] have been applied to quantify tumour choline concentration and water diffusion coefficients (DDC) prior to and following treatment with either 5FU or taxotere, to assess whether the different modes of action of the two drugs can be determined by these ^{1}H MRS characteristics.

METHODS: RIF-1 fibrosarcomas were grown subcutaneously in female C3H mice and LS174T colon carcinomas were grown subcutaneously in female MFI nude mice. Tumours were placed into a 15mm diameter two turn RF coil, and ^{1}H MRS was performed using a 4.7T Varian Unity Inova MR spectrometer. Voxels in the range of 50–150 mm^3 were selected from scout gradient echo images.

Acquisition protocols: 1) PRESS localisation with water suppression (TR=2s, 64 transients and TE=20, 68, 136, 272 and 408ms), which was used to detect total choline (t-choline) compounds[7] (PC + GPC + choline). The choline concentration was calculated using unsuppressed tumour water as a reference[7]. 2) Diffusion weighted (DW) ^{1}H MRS 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 = 112 ms with diffusion gradients from increased from 0 to 13 G/cm with 1 G/cm interval. The DW water signal was quantified by MRUI software.

The plots of normalised water signal (M/Mo) against b-values were fitted with a stretched exponential model[8,9], which resulted in estimation of a heterogeneity index (α) and distributed diffusion coefficient (DDC). Following pre-treatment ^{1}H MRS measurements, RIF-1 tumours were treated with 130mg/kg 5FU i.p. whereas the LS174 tumours were treated with 15mg/kg taxotere i.p., and ^{1}H MRS was repeated 24 hours later.

RESULTS:

The data are summarised in Table 1 (mean ± s.e.m., **p<0.001, *p<0.01, Student's paired t-test). 5FU-treated RIF-1 tumours showed a significant reduction in volume, consistent with previous observations[10] whereas there was no volume change in Taxotere-treated LS174T tumours. Both 5FU and taxotere significantly reduced the t-choline signal at 3.23ppm in both tumour models. There were no changes in either choline T_2 or water T_2 in either case. All RIF-1 tumours showed a negligible change in water DDC and heterogeneity index 24 hours after 5FU treatment. In contrast, all taxotere-treated LS174T tumours showed a decrease in the heterogeneity index (α) but no systematic change in DDC.

Table 1	RIF-1 (n=5)		LS174T (n=5)	
	Pre	Post	Pre	Post
Volume	555.1 ± 73.2	443.4 ±71.3*	258.1 ± 38.1	292.6 ± 32.1
t-Choline (mM)	5.99 ± 0.54	4.95 ± 0.38*	5.83 ± 0.52	4.02± 0.66**
Water T_2 (ms)	52.2 ± 0.9	53.5 ± 0.5	71.4 ± 1.6	68.9 ± 3.1
t-Choline T_2 (ms)	158.0 ± 11.8	174.7 ± 9.6	251.5 ± 17.4	250.0 ± 26.8
Heterogeneity index (α)	0.71 ± 0.03	0.74 ± 0.01	0.72 ± 0.05	0.57 ± 0.04
DDC (10^{-6} cm^2/sec)	2.46 ± 0.41	2.83 ± 0.37	4.22 ± 0.82	4.20 ± 0.84

DISCUSSION: The results with these two tumour models showed that t-choline is a reliable and early metabolic (biochemical) marker to follow the response to chemotherapy. Since tumour tissue is heterogeneous we have used a stretched-exponential model for DW-MRS data analysis. The heterogeneity index (α) gives biophysical information about the distribution of diffusion coefficients due to the heterogeneity of the tumour tissue; for homogeneous tissue the value of α is 1 and as the heterogeneity increases the value of α decreases (α<1). DDC gives an indication of the average diffusion coefficient in the tissue. As tumour tissue contains many small compartments, each with its own diffusion regime, estimation of this parameter along with α is more appropriate for tumour DW-MRS studies. We found that the stretched-exponential model fits to the tumour DW-MRS data were more robust than mono- or bi-exponential models. The decrease in heterogeneity index (α) of the LS174T suggests an early increase in heterogeneity of tissue diffusion coefficients due to the taxotere treatment. This would be consistent with the apoptotic mechanism of cell death previously observed with the taxotere action on colon carcinomas[6]. In conclusion, these data indicate that in tumours undergoing chemotherapy, MRS can monitor biochemical changes related to early events that precede the biophysical changes monitored by MRI techniques such as water diffusion.

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