Increased Diffusion Coefficient Correlates with Decreased Choline in Response to Cyclophosphamide Therapy of RIF-1 Tumors

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Introduction:

Tumor response to therapy is generally associated with changes in physiological and biochemical parameters that can be observed with MR. Untreated tumors exhibit high choline and low ADC values which correlate well with cell density (1). In response to therapy, decreases in lactate (2) and transient increases in NMR-detectable lipid resonances (3) have been observed in rodent tumor models. The decrease in lactate correlates with increased tumor perfusion/permeability as measured by MRI (2). In this study we used a combined imaging and spectroscopy approach to evaluate the relationship between ADC and metabolite changes in response to cyclophosphamide (Cp) treatment of RIF-1 tumors.

Methods

Subcutaneously grown RIF-1 tumors were treated with Cp (300 mg/kg, i.p., n = 9) or with saline (control tumors, n = 6). NMR measurements were made prior to treatment (time 0), 24 hr and 72 hrs after treatment using a 9.4T vertical bore spectrometer and a slotted tube resonator. T_2 measurements were performed using a multi-slice spin echo sequence (TR = 2.5s, TE = 15 - 75 ms, slice thickness = 1 mm, FOV = 1.5 x 1.5 cm). Multi slice trace of diffusion tensor images (ADC_{av}) were computed using a diffusion-weighted spin echo sequence (4) with diffusion gradients applied in the three orthogonal planes (TR = 2 s, TE = 60 ms, 4 b values: 0-112506 s/cm^2). Pixel by pixel T_2 and ADC_{av} maps were computed from all imaging slices and average T_2 and ADC_{av} value for the entire tumor was calculated. Single voxel STEAM (2.5 x 2.5 x 2.5 mm^3) spectra were acquired using a TR = 3s, TE = 8 ms and 128 acquisitions.

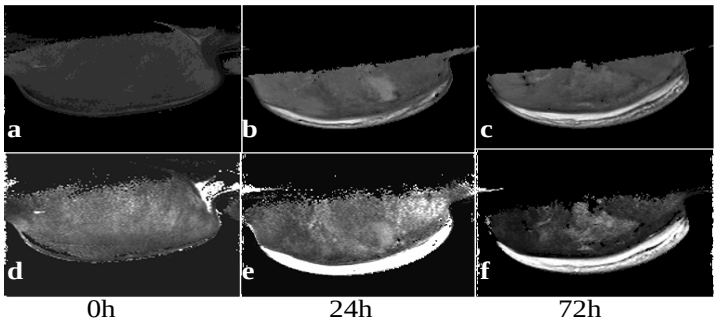

Figure 1: T_2 (a-c) and ADC_{av} (d-f) maps from a RIF-1 tumor before (0h) and after Cp treatment.

Results and Discussion

Fig 1 shows the T_2 (a-c) and ADC_{av} (d-f) maps of a RIF-1 tumor before (0h), 24 and 72h after Cp treatment. Regions of increased ADC_{av} are seen in the tumor as early as 24h after treatment which further increased at 72h indicating focal areas of increased cell death and necrosis. The ADC_{av} for the entire tumor increased significantly after 72h of Cp treatment from 0.85±0.02 to 1.08±0.01 x10^{-9} m^2/s ($p < 0.001$, Figure 2) while T_2 decreased from 32.2±0.3 ms to 29.6±0.1 ms. During the same time frame, a significant decrease in total choline (TCho)/CH_3 ratio was observed in Cp treated animals (Figure 2, 3) that was not observed in controls (Figure 2). The inverse correlation between changes in ADCav and TCho after Cp treatment indicates an increase in the extracellular volume fraction and a decrease in viable tumor cell density accompanying a positive response to therapy (1).

Tumor CH_2/CH_3 ratio decreased from 2.09±0.23 to 1.32±0.19 ($p < 0.005$) 72h after Cp treatment. We have earlier reported a significant decrease in tumor lactate after 24h of Cp treatment using the Sel-MQC pulse sequence (2). Since no significant differences in the CH_2/CH_3 peak were observed at 24h, it is possible that the increase in fatty acyl chains was cancelled by the decrease in lactate. Transient increases in lipid resonances have in fact, earlier been reported accompanying apoptotic cell death (3). We have shown that Cp induces apoptosis in RIF-1 tumors (5), thus a decrease in CH_2/CH_3 ratio after 72h of Cp treatment may indicate apoptotic cell death. In conclusion, a combined imaging and spectroscopy approach provides an insight into the tumor microenvironment and metabolism which may assist in monitoring treatment response to therapy.

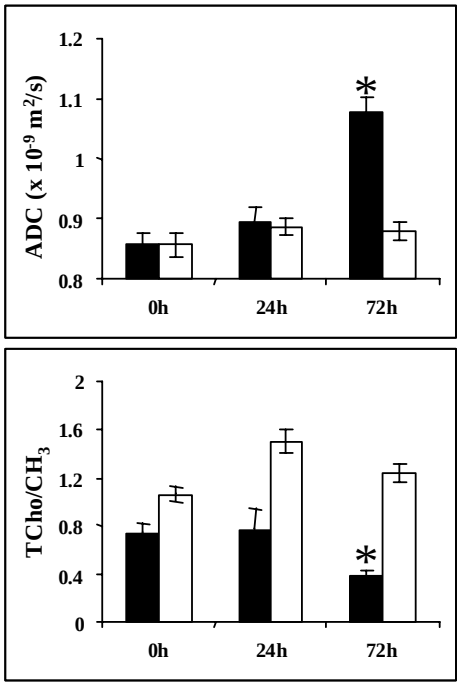

Figure 2: ADCav (x10^{-9} m^2/s, top) and TCho/CH3 ratios (bottom) from RIF-1 tumors. Cp treatment (filled bars) and sham controls (empty bars). * indicates $p < 0.001$ compared to 0h. value.

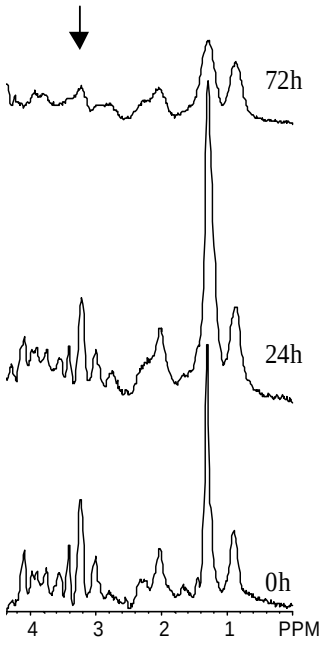

Figure 3: Single voxel STEAM spectra from a RIF-1 tumor showing a significant decrease in TCho (arrow) ratio after Cp treatment

References

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Acknowledgements

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