Effect of Cyclophosphamide on the Apparent Diffusion Coefficient of Water and 23Na MRI in the Subcutaneously Implanted RIF-1 Tumor

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Introduction

The diffusion of tissue water *in vivo* is sensitive to tumor chemotherapy and can be accurately and noninvasively estimated as water ADC by using diffusion-weighed ^{1}H NMR (1,2). Water ADC may be correlated with ^{23}Na signal intensity in tumor tissue because both are sensitive to changes in extracellular space (3). Monitoring and imaging tissue Na^+ by MR techniques may be useful for assessing response to therapy because of the biological importance of sodium. In this study, we used ^{1}H and ^{23}Na MRI to examine and correlate the changes in water ADC and tumor tissue $[Na^+]$ ($[Na^+]_{tumor}$) in response to the chemotherapeutic drug Cp using the RIF-1 tumor model. We also investigated the mechanism of the observed changes in $[Na^+]_{tumor}$ and water ADC through histology and destructive chemical analysis.

Methods

^{23}Na MRI and water ADC imaging were performed on Cp-treated (300 mg/kg, i.p.; n = 6) and untreated control (n = 5) C3H mice. MRI images were acquired with a Varian 9.4 Tesla 31-cm horizontal bore system. Each animal was examined before treatment and daily for three days following treatment. 3D transaxial ^{23}Na MR images of the tumor were obtained using a gradient-echo imaging sequence. The following imaging parameters were used: 100 μs non-selective excitation RF pulse, 50 ms repetition time (TR), 10 ms echo time (TE), and 64 x 32 x 8 data points over a 40 x 40 x 36 mm field of view (FOV). Water ADC in the tumor was measured using a multi-slice diffusion-weighted imaging (DWI) sequence. The following imaging parameters were used: 1,100 ms TR, 10 ms TE, 256 x 128 data points over a 40 x 40 FOV, 2.0 mm slice thickness, and 0.6 mm slice gap. Four interleaved b-factors (b= 0, 236, 945 and 1,679 s/mm^2) were used. ^{23}Na T_1 was measured using a pulse-burst saturation recovery pulse sequence consisting of 50 saturation pulses followed by an incremental delay and a 90° observe pulse and acquisition with Cyclops phase-cycling. ^{23}Na T_{2f} and T_{2s} were measured using a Hahn spin-echo sequence consisting of a composite 180^{o} pulse. H&E histology and destructive chemical analysis were performed after the last MR measurement.

Results and Discussions

Tumor volumes were significantly lower in Cp-treated animals two and three days post-treatment. At the same time points, *in vivo* MRI experiments showed an increase in both water ADC and ^{23}Na signal intensity in the treated tumors, while the control tumors did not show any significant changes (**Figure**). Water ADC increased from 4.87 ± 0.23 x 10^{-4} mm^2/sec (before treatment) to 7.29 ± 0.40 x 10^{-4} mm^2/sec (day 3 after treatment) ($p \leq 0.05$); $[Na^+]_{tumor}$ increased from 34.2 ± 1.9 mM (before treatment) to 43.5 ± 2.7 mM (day 3 after treatment) ($p \leq 0.05$). The correlation between the water ADC and $[Na^+]_{tumor}$ changes was dramatically increased in the Cp-treated group (R^2=0.97) compared to the untreated group (R^2=0.29), suggesting that the observed increases in both water ADC and $[Na^+]_{tumor}$ were caused by the same mechanism. The increase in ^{23}Na MRI signal intensity after Cp treatment was due to increases in $[Na^+]_{tumor}$, but not due to changes in ^{23}Na relaxation characteristics because T_1, T_{2s}, and T_{2f} values did not change after treatment or during untreated growth. Histological sections showed decreased cell density in the regions of increased water ADC and $[Na^+]_{tumor}$. Destructive chemical analysis showed that Cp treatment increased the relative extracellular space (**Table**) and confirmed an increase of 27-29% in $[Na^+]_{tumor}$ after chemotherapy. We conclude that the changes in water ADC and $[Na^+]_{tumor}$ were largely due to this increase in extracellular space in this tumor model. ^{1}H water ADC measurements and ^{23}Na MRI may provide valuable noninvasive techniques for monitoring responses to chemotherapy.

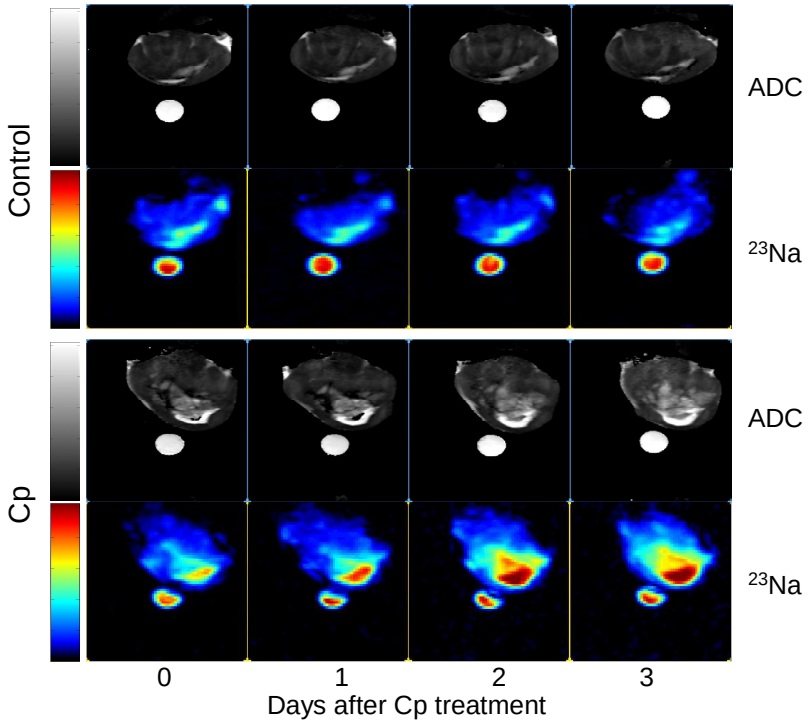

Figure. Water ADC maps and ^{23}Na MR images of representative control and Cp-treated RIF-1 tumors. Water ADC and ^{23}Na signal intensity increased with time after Cp treatment. A vial filled with a 154 mM NaCl solution was placed near the tumor as a reference.

Table. Tissue compartmentalization of control and Cp-treated RIF-1 tumors three days after therapy as measured by destructive chemical analysis

	rDW	rECS	rICS
Control	0.21 ± 0.01	0.26 ± 0.04	0.53 ± 0.04
Cp-treated	0.16 ± 0.01*	0.46 ± 0.08*	0.43 ± 0.08

Note. Values are reported as mean ± SE. rDW – relative dry weight, rESC – relative extracellular space, rICS – relative intracellular space. Significance: * - $p \leq 0.05$ (control vs. Cp-treated).

References

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