Correlation between Single Quantum and Triple-Quantum-Filtered ^{23}Na MRI Signal Intensities, Water ADC and Histology Changes in Control and 5-Fluorouracil Treated RIF-1 Tumors

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Introduction

It has been assumed that the chemotherapy-induced increase in water ADC and total tissue ^{23}Na signal intensity (SI) in subcutaneously-implanted (sc) RIF-1 tumor (1) and 9L glioma (2) results from the increase in extracellular space due to cell death. Destructive chemical analysis supports this hypothesis for RIF-1 tumor treated with cyclophosphamide (1), but not for 9L glioma treated with BCNU (3). The analysis of histological images of RIF-1 tumors and their correlation with MRI has not been performed. Thus, it remains unclear whether the changes in water ADC or ^{23}Na SI after chemotherapy are general phenomena related only to structural changes, or do they depend on metabolic changes, such as intracellular [Na^+]? The possible contribution of changes in intracellular [Na^+] to ^{23}Na SI after chemotherapy has not been evaluated. In this study, the correlation between single quantum (SQ) and triple-quantum-filtered (TQF) ^{23}Na MRI SI, water ADC and histology in control and 5-fluorouracil (5FU) treated sc-RIF-1 tumors was examined.

Methods

SQ and TQF ^{23}Na MRI and ^{1}H diffusion imaging were performed on untreated control and 5FU-treated C3H mice with sc-implanted RIF-1 tumors. MR images were acquired with a Varian 9.4 Tesla, 31-cm horizontal bore system. Each animal (n = 6 control, 7 treated) was examined before 5FU injection (150 mg/kg, ip) 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^3 field of view (FOV). TQF ^{23}Na pulse sequence parameters were: 90-100 ms 90° excitation RF pulse, 120 ms TR, 10 ms TE, and 64 x 32 x 8 data points over a 40 x 40 x 36 mm^3 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, 60 ms TE, 256 x 128 data points over a 40 x 40 mm^2 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.

Histology was performed following the last ^{1}H and ^{23}Na MRI measurement three days after 5FU injection. Histological sections of the tumor were cut perpendicular to the body wall along the same plane as the MR images. Tissue sections were obtained at 5-µm thickness and stained with Hematoxylin and Eosin (H&E) to identify regions with different cell densities. Three histology slices, one each from the anterior, middle, and posterior regions of each tumor, were compared with water ADC maps and ^{23}Na MRI. Digital micrographs were obtained with a Nicon Coolpix 4500 (Nicon Inc., Torrance, CA), and histological slices were analyzed with Microstar IV (IMEB Inc., Columbus, OH). Necrotic regions and the entire tumor boundary were manually traced, and their areas were measured using Analyze 6.1 (Mayo Clinic, Rochester, MI) image processing software. The necrotic tumor fraction was calculated from the necrotic area normalized by the entire tumor area of the histological slice.

Results and Discussions

The 5FU treatment of RIF-1 tumor significantly decreased the tumor volume from 0.72 cm^3 to 0.49 cm^3 two days after chemotherapy and 0.34 cm^3 three days after chemotherapy. Total tumor Na^+ level monitored by SQ ^{23}Na SI increased by 26% on day 2 and by 49% on day 3 relative to its pre-treatment baseline value ($p \leq 0.05$) and was significantly higher compared to control group at the same time points ($p \leq 0.05$) (Fig.1). Similar to the SQ ^{23}Na SI data, the water ADC measured by ^{1}H MRI increased in treated tumors by 18% and 25% two and three days after chemotherapy, respectively (data not presented). The intracellular Na^+ level measured by TQF ^{23}Na SI increased in control tumors but not in treated tumors (Fig.1). In control tumors, $^{23}\mathrm{Na}_{\mathrm{tumor}}^{\mathrm{TQF}} / {}^{23}\mathrm{Na}_{\mathrm{ref}}^{\mathrm{SQ}}$ increased compared to the pre-treatment level by 31 % ($p \leq 0.05$) on post-treatment day 3. Correlation analysis between all data points of SQ and TQF ^{23}Na SI and water ADC showed that the highest level of correlation existed between water ADC and SQ ^{23}Na SI in the 5FU-treated group ($R^2 = 0.99$). The control group also showed slight correlation ($R^2 = 0.75$) between water ADC and SQ ^{23}Na SI. There was no significant correlation between SQ and TQF ^{23}Na SI ($R^2 = 0.49$ for 5FU-treated group and 0.03 for control group) or between water ADC and TQF ^{23}Na SI ($R^2 = 0.50$ for 5FU-treated group and 0.01 for control group).

H&E histology shows that the necrotic areas were larger (A) and cellular density in necrotic regions (B) was lower in the 5FU-treated group compared to control tumors three days after treatment (Fig. 2). The histological sections of 5FU-treated tumor at day 3 show cellular abnormalities in the necrotic regions, such as nuclear dusting, neutrophil invasion, pyknosis, and karyorrxesis. The increase in necrotic area and decrease the cellularity after 5FU treatment suggest that the increase in SQ ^{23}Na SI was caused primarily by the increase in extracellular space due to cell death following effective chemotherapy. A comparison of the H&E-stained tumor section with water ADC maps, SQ ^{23}Na MRI and TQF ^{23}Na MRI demonstrated that necrotic regions with low cellular density (b) showed high water ADC and SQ ^{23}Na SI (Fig. 3). However, in some regions, when SQ ^{23}Na SI was high, water ADC did not dramatically increase. Histology showed that although these regions had a large extracellular space, it was filled with collagen material that limited water motion and decreased water ADC.

Conclusion

There was a good correlation between water ADC and SQ ^{23}Na MRI SI 2-3 days after effective chemotherapy, which was accompanied with an increase in necrotic area and a decrease in cellularity in necrotic regions. However, correlation between changes in TQF ^{23}Na SI and both water ADC and SQ ^{23}Na SI was not found. The lower TQF ^{23}Na SI in treated tumors compared with controls may result from increased oxidative metabolism and/or a decrease in cell density.

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References: (1) Babsky A et al. Neoplasia 2005; 7: 658-66 (2) Schepkin VD et al. MRI 2006; 24: 273-8 (3) Winter P et al. Cancer Res 2001; 61:2002-7

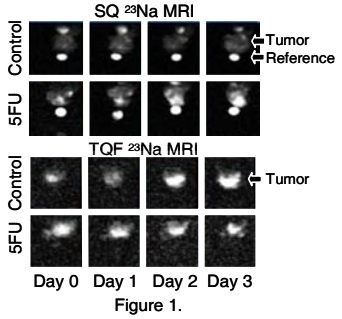

Figure 1.

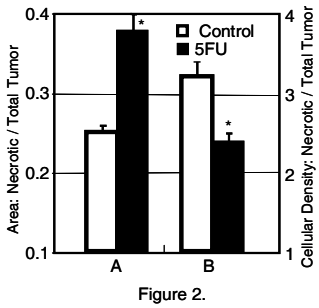

Figure 2.

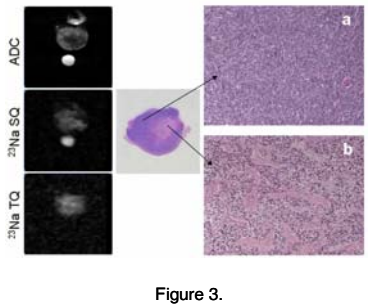

Figure 3.