Changes in choline level, T1, and contrast agent uptake in response to 5FU therapy of RIF-1 tumors in mice

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Introduction

^{1}H MRS and MRI readouts like choline signal, relaxation times and contrast agent uptake (dynamic contrast enhanced MRI=DCE-MRI) can be used to monitor the effect of treatment in tumors. In earlier reports, choline level assessed by ^{1}H MRS decreased in murine tumors after cytotoxic treatment (1), whereas contrast agent (CA) uptake assessed be DCE-MRI increased (2). The aim of this study was to evaluate the time course of choline, T_1, and CA uptake after cytotoxic drug therapy with 5FU in the RIF-1 tumor model in mice to determine the most sensitive and practical method.

Methods

Murine RIF-1 tumors were grown sc in the flank of syngeneic C3H/He mice. Animals were treated once with 160 mg/kg 5-fluorouracil (5FU) (n=6) or saline (n=8).

MR measurements were performed under anesthesia in a 4.7 T Bruker Biospec MR system before treatment and on days 1-2, 5-6, and 8-9 (only 5FU group) post-treatment. Tumor volume was measured with a multi-slice RARE sequence. T_1 was measured with an IR FLASH sequence with incremented IR delays before contrast agent (CA) infusion. Regional CA uptake was assessed by a series of IR FLASH images (IR delay 1.3 s, time resolution 6 s, duration 12 min) after a bolus injection of 0.2 mmol/kg of GdDOTA. After local shimming with FASTMAP, water-suppressed ^{1}H MR spectra were acquired with the PRESS method (TE = 20 ms, TR = 1500 ms, acquisition time 10 min) of two 8 mm^3 voxels placed in the tumor rim and center. Additionally, non-water-suppressed ^{1}H MR spectra were acquired in the same voxels.

Spectra were fitted in the frequency domain. Choline signals were measured relative to water signal of the non-water-suppressed spectrum. CA concentration curves in the voxels chosen for MRS were calculated from the signal change of the DCE-MRI. The initial area under the curve (iAUC) was calculated for the time range 0-90 s after CA injection. T_1 was derived from fitting of the relaxation curve which was averaged over the whole tumor area. Statistical analysis was done with one way and two way repeated measure ANOVA.

Tumors were excised for histology on day 2 (3 5FU-treated, 2 controls) and on day 5 (2 5FU-treated, 3 controls). Slices were H&E stained. Additionally, immunohistochemical analysis was performed for PCNA (proliferation), and with a TUNEL assay for apoptosis and necrosis.

Results and Discussion

In 5FU treated mice, tumors shrank until day 5 and then re-grew. Choline content decreased significantly on day 5, T_1 decreased significantly on day 2, iAUC increased significantly on day 2. On day 8-9, T_1 and iAUC returned to normal levels and choline levels showed this trend, too. In control animals, T_1 did not change and tumors grew during the observation period. DCE-MRI showed well a perfused rim and an ill-perfused, necrotic core in large vehicle treated tumors (confirmed by histology). Choline level was stable in the rim, but decreased in the necrotic core. In some animals, the spectral quality in the core was not sufficient for fitting.

Decreased choline level is marker for reduced proliferation. Histology showed a decrease in proliferation on day 2, and a further decrease on day 5. Increased iAUC, a measure for vascular permeability, may reflect damages to the vasculature and/or increased perfusion. The decreased T_1 was probably due to damaged tissue architecture. In the literature increases of T_1 after treatment (3) as well as decreases (4) have been reported in experimental tumors.

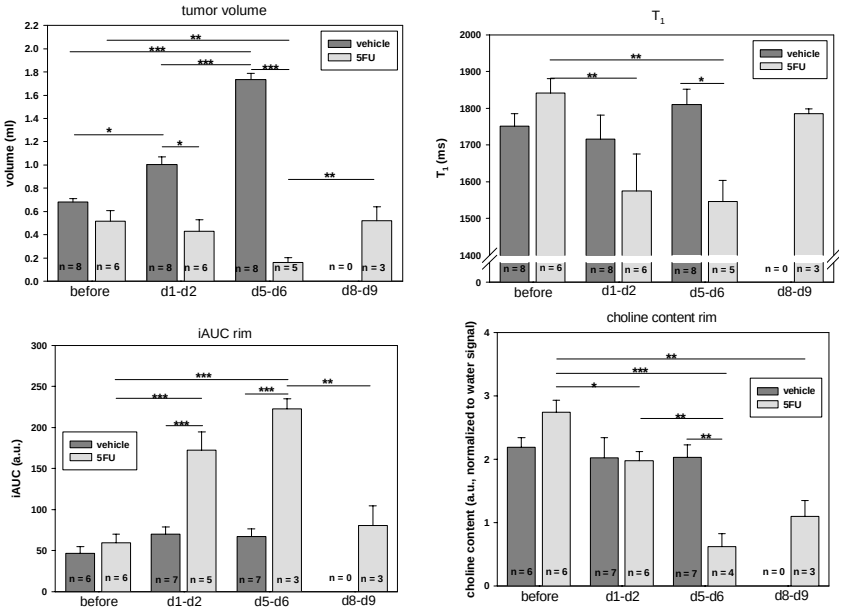

Fig. 1: Tumor volume, T_1, iAUC, and choline content (mean ± SEM) in 5FU treated (light gray bars) and saline treated (dark gray bars) RIF-1 tumors

Conclusion

Choline, T_1 and iAUC changed significantly after treatment with 5FU. These alterations paralleled the change in tumor volume and thus indicated their suitability as biomarkers for treatment response. The most sensitive parameter was iAUC and the easiest parameter to measure was T_1, suggesting these could be very useful clinical biomarkers for cytotoxic activity.

References

(1) Aboagye EO, et al., Cancer Res. 1998;58(5):1063-7.
(2) Poptani H, et al., NMR Biomed. 2003;16:102-11.
(3) Chenevert TL, et al., Clinical Cancer Res. 1997;3:1457-66.
(4) Braunschweiger PE, et al., Magn. Reson. Imaging 1986;4(4):335-42.