Assessment of Emerging Chemotherapeutic Tumor Resistance to BCNU

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Introduction

Intrinsic resistance to tumor therapy is often present in malignant gliomas or can be developed during unsuccessful therapeutic treatments. The modest efficiency of BCNU (1,3-bis (2-chloroethyl)-1-nitrosourea) is well known to clinicians. Therefore, it is usually used in combination with other therapies and generally not used for a second time due to a possible change in tumor sensitivity. In this study sodium and apparent diffusion (ADC) tumor imaging, as promising tools for monitoring therapeutic cellularity changes, were tested to reflect emerging BCNU resistance in 9L rat subcutaneous tumor.

Materials and Methods

Male Fisher 344 rats with 9L subcutaneous tumors were included for MRI study when their tumor size reached ~300µl (n=15). Tumor treatments had been performed by chemotherapeutic agent 1,3 bis(2-chloroethyl)-1-nitrosurea (BCNU). IP injections of BCNU with doses 26.6 mg/Kg (2X) were applied twice with an interval of 21 days (n=5). A comparable protocol was reproduced with BCNU dose of 13.3 mg/Kg (1X) with an interval of 14 days (n=5). Animals in the control group (n=5) remained untreated. Response to tumor treatments was monitored in-vivo by proton diffusion mapping and sodium MRI and in-vitro, by using BCNU growth inhibition assay of extracted tumor cells, at the end of experiments. Imaging experiments were performed on Varian MRI scanner 9.4T. For proton MRI the isotropic diffusion weighting SE pulse sequence was used with "high-b" (b=1082 s/mm2) and "low-b" (117 s/mm2), 15 axial slices, FOV 40x40 mm, slice thickness 1.5 mm, TR/TE = 3000/40 ms. Three-D Na imaging was performed by a back-projection pulse sequence with an echo time of 1 ms, TR = 100 ms, matrix 64x32x32, FOV 64 mm and acquisition time 28 min. Amplitude of Na RF pulses were 5 KHz. All measurements were repeated every few days for 38 days after BCNU administration. Sodium 3D images and ADC maps were reconstructed in Matlab and co-registered in 3 dimensions. Tumor volume, sodium concentration and ADC were determined over 3D VOI for each temporal measurement. Animal experiments were conducted according to the protocols approved by the University LARC.

Results

Chemotherapy increased tumor Na concentration and ADC values very heterogeneously (Fig. 1). Time course of the average tumor Na and ADC for two applications of BCNU 2X dose showed dramatic increases of ADC and Na after first treatment and remarkably low changes after second BCNU administration (Fig. 2). Decrease of BCNU dose to 1X produced comparable changes in ADC and Na for the first and the second applications of chemotherapy (Fig. 2). In vitro assay of non-treated tumor cell gave a 50% decrease in survival at 121 ± 6 µM of BCNU in growth media. Treatment with BCNU dose 2X produced an increase in tumor resistance and the same level of tumor cell survival was obtained at 202 ± 16 µM. Tumor treated with BCNU dose of 1X showed in-vitro no noticeable changes in tumor resistance.

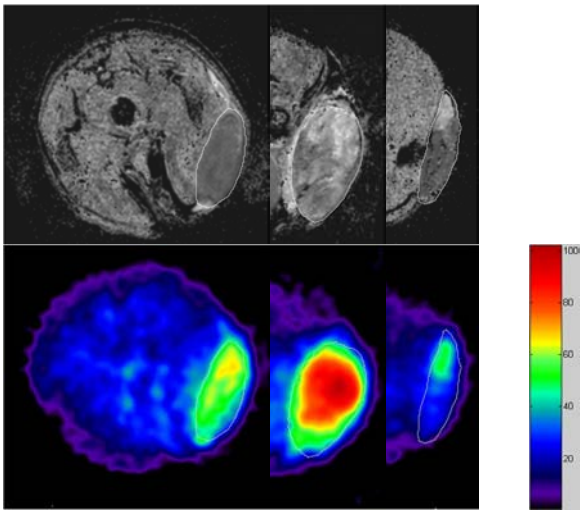

Fig. 1. ADC maps (upper row) and Na MRI (bottom) of 9L rat subcutaneous tumor. Images were taken for the same animal at three different times: before treatment (left); 8 days (center) and 17 days (right) after BCNU administration of 26.6 mg/Kg.

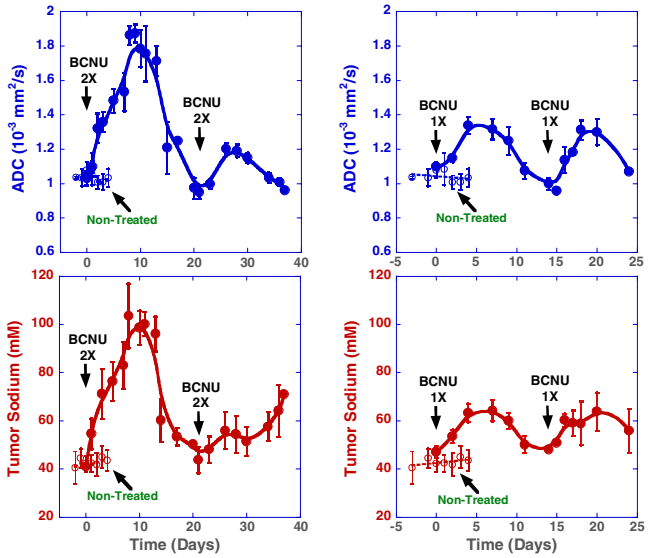

Fig. 2. Time courses of tumor ADC and Na in 9L rat subcutaneous tumor. Responses of ADC and sodium to a second 2X BCNU treatment (26.6 mg/Kg, left) were dramatically reduced in comparison to a first one, reflecting the emerging tumor resistance. Administration of 1X dose of BCNU (13.3 mg/Kg, right) gave no difference in tumor responses between two sessions of treatment, revealing no changes in tumor sensitivity.

Discussion

Changes in efficiency of tumor cell destruction by BCNU therapy were demonstrated here in a dose dependent manner. A large chemotherapeutic dose initiated a strong impact on tumor cells which is reflected by dramatic initial response of diffusion and sodium to therapy (Fig. 2) and by a major suppression of tumor volume changes (data not shown). Both ADC and sodium demonstrate a remarkable correlation, representing the same process of changes in tumor cellularity. A more aggressive treatment also leads to a more advanced change in tumor cell sensitivity to BCNU treatment. A second application of high BCNU yielded dramatically reduced responses in both ADC and sodium. The corresponding in-vitro tumor resistance was ~1.7 times more at this time. Alternatively, a decreased dose of treatment yielded no changes in tumor BCNU sensitivity in-vitro and, correspondingly, only a minor effect on the rate of tumor growth (data not shown). The absolute changes of tumor ADC and sodium were small and almost the same for the first and second low dose BCNU applications.

Conclusion

The results of this study demonstrate that ADC and sodium MRI can reflect the efficacy of tumor therapy as well as the effects of emerging tumor resistance. These experiments illustrate that both imaging modalities can be valuable tools for tumor assessment in oncology.

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