

THE LOSS OF SODIUM HOMEOSTASIS AND APOPTOSIS DURING RODENT GLIOMA THERAPY

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INTRODUCTION

There is growing evidence that the disruption of sodium homeostasis is one of the first and most essential components of cellular response during cancer therapy. The disruption of sodium balance is also desirable as a possible goal for cancer drug development. Multiple tumor cell lines demonstrate this trend in many experiments performed *in vitro*. The goal of the present study was to assess *in vivo* that an increase of tumor intracellular sodium is an essential step in cancer therapy, and it occurs early during chemotherapy.

METHODS

In the present study, the initial response to chemotherapy was detected each day using a rodent glioma model. The high resolution sodium and diffusion mapping were performed using MRI at 21.1 T (bore 105 mm) capable of accommodating large rodents. Rat 9L gliosarcoma cells were implanted intra-cranially in male Fisher 344 rats (weight ~ 120 g). At ~10 days after tumor implantation, animals were subjected to a single dose of carmustin (BCNU) chemotherapy (IP). Tumor sodium, diffusion map and tumor volume were detected daily. The experiments were performed detecting proton (900 MHz) and sodium (237 MHz) signals using Bruker Avance III console equipped with Micro 0.75 gradient set, GREAT60 amplifiers and operated by PV5.0. Sodium 3D back-projection MRI scans had a duration of 27 min, TE =1 ms, TR=100 ms and a resolution of 1 μ L. Diffusion SE pulse sequence had flow/motion compensated diffusion gradients, two b values of 100 and 1000 (sec/mm²), TE=34 ms and 15 back-projection 2D slices, thk = 0.7 mm. All animal experiments were conducted according to the protocols approved by the Florida State University ACUC.

RESULTS

All tumor measurements were performed relative to a normal contralateral part of the brain, where ADC was $\sim 0.78 \cdot 10^{-3}$ mm²/sec and sodium content ~ 50 mM. During the first day after therapy a noticeably higher rate of sodium increase was observed (~16%/day) in comparison to the diffusion rate (~7%/day). Later sodium and diffusion were increasing at comparable rates of 24%/day and 21%/day, respectively. By day 4, after the initiation of therapy, tumor sodium reached a plateau value and remained unchanged at the level of ~240% (~120 mM) (Fig. 1, 2, 3). The static sodium plateau phase (days 4 - 6) suggests that by this time almost all tumor cells have lost their sodium homeostasis. This conclusion is in agreement with the fact that the continuing tumor cell destruction, detected by the growth of ADC, no longer affects total sodium. It is important to note that a low dose of BCNU chemotherapy (13.3 mg/kg) did not yield this level of tumor sodium and correspondingly did not lead to tumor shrinking, as it is usually observed for high dose chemotherapy.

CONCLUSION

The changes of sodium homeostasis takes place *in vivo* during the first days following chemotherapy and it occurs before therapeutic changes in tumor diffusion. During efficient therapy, sodium reaches a plateau indicating a complete loss of sodium homeostasis before final tumor cell destruction. This dose-dependent tumor sodium response can serve as a very early biomarker for the onset of apoptosis, forecasting tumor shrinking. Both the tumor sodium and ADC mapping represent unique MRI windows to monitor *in vivo* the first steps of cellular changes during cancer interventions.

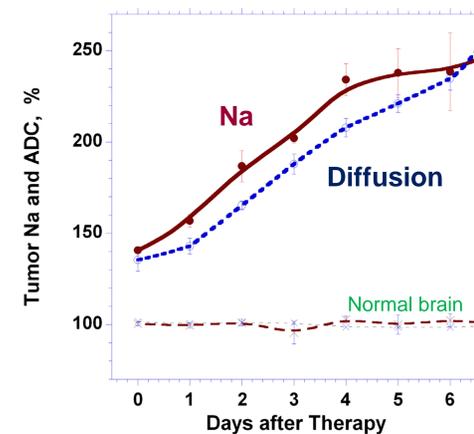


Fig. 1. Sodium and ADC in rodent glioma after BCNU chemotherapy of 26.6 mg/kg. Tumor sodium plateau (~120 mM) at days 4 - 6 corresponds to a loss of sodium homeostasis. Diffusion in tumor is still increasing at this time indicating further cell destruction.

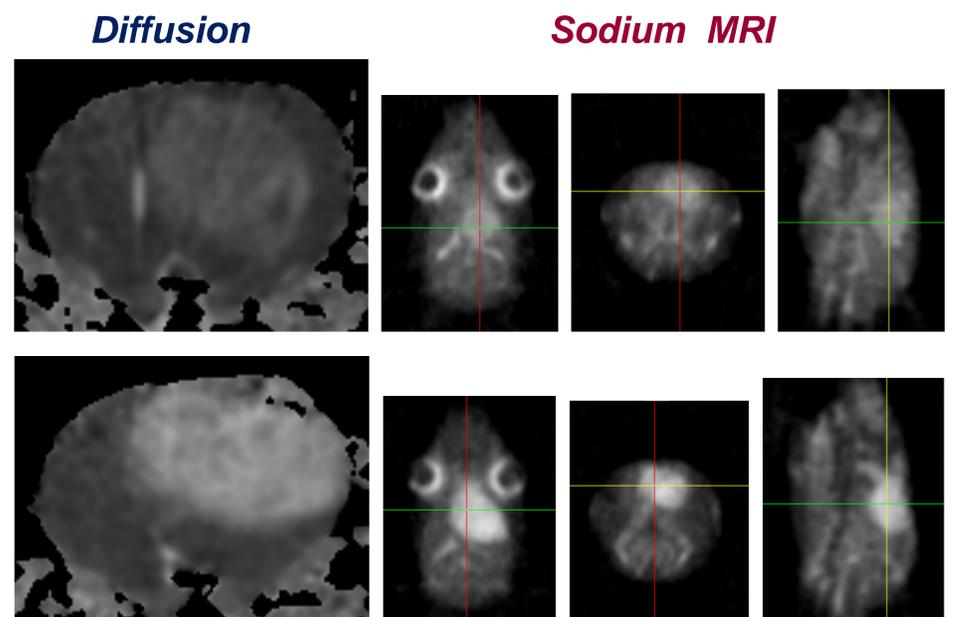


Fig. 2. Diffusion map (left) and sodium MRI (right) in rodent glioma before (upper row) and 6 days after BCNU chemotherapy of 26.6 mg/kg (low row). Tumor diffusion and sodium are dramatically increased as a result of therapy.

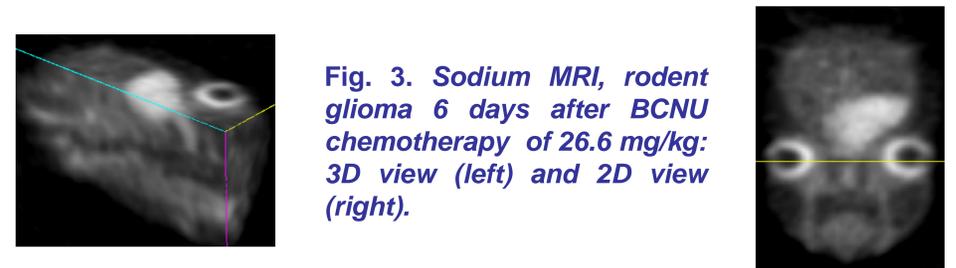


Fig. 3. Sodium MRI, rodent glioma 6 days after BCNU chemotherapy of 26.6 mg/kg: 3D view (left) and 2D view (right).

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