# The Loss of Sodium Homeostasis and Apoptosis during Rodent Glioma Chemotherapy

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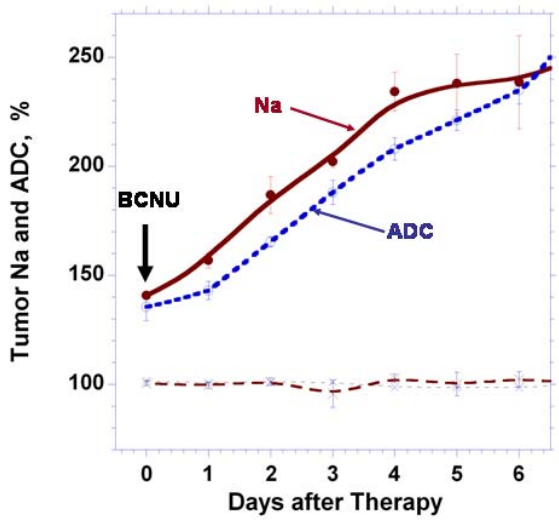
## Introduction

There is growing evidence that the disruption of sodium homeostasis is the one of the first and an important component of cellular response during cancer therapy, indicating a possible goal for drug development (1-9). Multiple tumor cell lines demonstrate this trend in many experiments performed *in vitro* (1-9). The goal of the present study was to assess the hypothesis that an *in vivo* increase of tumor intracellular sodium is a crucial stage for cancer therapy that occurs early during chemotherapy. While proton diffusion tumor mapping, as a biomarker for tumor cell destruction and a sign of positive tumor therapy response, is currently in clinical trials, the role of sodium has not yet been fully established. 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.

## Materials and Methods

Rat 9L gliosarcoma cells were implanted intra-cranially in male Fisher 344 rats (weight ~ 120 g). At ~10 days after tumor implantation, animals (n = 12) were subjected to a single dose of carmustin (BCNU) chemotherapy (IP, 26.6 or 13.3 mg/kg). In the control group (n=4) tumors remained untreated. Tumor sodium, diffusion map and tumor volume were detected daily, during a course of 6 days. The experiments were performed in a 21.1T MRI scanner using proton (900 MHz) and sodium (237 MHz) signals (Bruker Avance III console equipped with Micro 0.75 gradient set, GREAT60 amplifiers and operated by PV5.0 and TopSpin 2.0 software). Sodium 3D back-projection MRI scans had a duration of 27 min, TE =1 ms, TR=100 ms. The high sensitivity at 21.1T allowed sodium MRI in a rat brain with a resolution of 1 μL overall. 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. During processing, additional efforts were applied to minimize any contribution of the partial volume effects in sodium as well in diffusion MRI results. All animal experiments were conducted according to the protocols approved by The Florida State University ACUC.




## Results and Discussion

All tumor measurements were performed relative to a normal contra-lateral part of the brain, where ADC was ~$0.78*10^{-3}$ $mm^2$/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). The mostly static sodium plateau phase (days 4 - 6) suggests that almost all tumor cells have lost their sodium homeostasis because 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 sodium and correspondingly did not lead to tumor shrinking, as it is usually observed for high dose chemotherapy.

**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 loss of sodium homeostasis, as diffusion in tumor is still increasing.

## Conclusion

The changes of sodium homeostasis take place during the first days following chemotherapy. During efficient therapy, sodium reaches a plateau indicating a complete loss of Na homeostasis before the final tumor cell destruction. This dose dependent tumor sodium response can serve as a very early biomarker indicating the onset of apoptosis and forecasting tumor elimination. Both the tumor sodium and ADC mapping are independent windows and represent a unique combination to monitor the *in vivo* biomedical cell processes during cancer interventions.

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