

# Sodium MRI clocks pre-necrotic alterations in rat glioma model without changes in tumor diffusion

V. D. Schepkin<sup>1</sup>

<sup>1</sup>National High Magnetid Field Laboratory, Florida State University, Tallahassee, Florida, United States

## Introduction

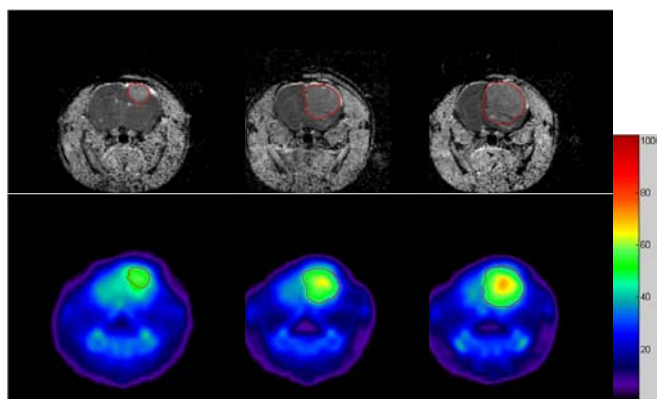
In-vivo MRI biomarkers are crucial for drug development and tailoring individualized therapy. Sodium MRI and proton diffusion (ADC mapping) show strong potentials for evaluating changes inside tumors. In this study, for the first time, high resolution sodium MRI with voxel size less than 1  $\mu$ L was applied for assessment of tumor therapy using a rodent glioma model. The goal was to monitor alteration of intact tumors and their response to chemotherapy. Results from two physiological windows (sodium and diffusion) represent cellular changes inside the tumor and demonstrate the ability of sodium MRI to grade the pre-necrotic development of non-treated tumors. This grading power of sodium MRI is especially important because, during the same time, there were no observable changes in tumor diffusion.

## Materials and Methods

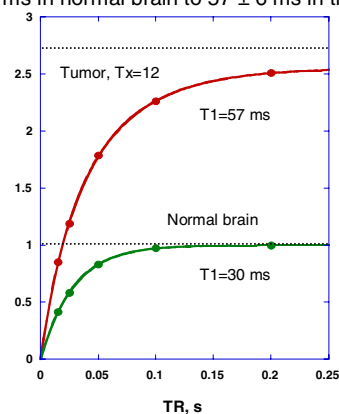
Male Fisher 344 rats with 9L brain tumors were subjects for MRI study when their tumor size reached  $\sim 50 \mu$ l (n=6). Tumor treatments were performed by chemotherapeutic agent 1,3 bis(2-chloroethyl)-1-nitrosurea (BCNU). Single IP injections of BCNU (26.6 mg/kg) were applied  $\sim 14$  days after tumor implantation. Animals in the control group (n=3) remained untreated. Tumor alterations were observed in-vivo by both high resolution sodium MRI and proton diffusion mapping. Imaging experiments were performed on Varian MRI scanner 9.4T. Three-D sodium images were acquired by a back-projection GE pulse sequence with an echo time of 1 ms, TR = 100 ms, matrix 64x128x128, FOV 64 mm and acquisition time of 2 h. For proton diffusion mapping the isotropic DW SE pulse sequence was used with "high-b" (b=1082 s/mm<sup>2</sup>) and "low-b" (117 s/mm<sup>2</sup>), 15 axial slices, FOV 30x30 mm, slice thickness 1.0 mm, TR/TE = 3000/40 ms. All measurements were repeated every 2-3 days. MR images were co-registered in 3 dimensions in order to monitor the same area of the tumor over time. Animal experiments were conducted according to the protocols approved by the University of Michigan LARC.

## Results

During tumor growth, diffusion in untreated gliomas remained unchanged at  $(1.1 \pm 0.05) \cdot 10^{-3}$  mm<sup>2</sup>/s. These values were above diffusion in normal contra-lateral brain ( $0.78 \pm 0.02$ )  $\cdot 10^{-3}$  mm<sup>2</sup>/s (Fig. 1). Sodium MRI, performed simultaneously with diffusion, showed elevated and consistently increasing Na content in tumor relative to a normal brain. The central area of tumors with high Na becomes more noticeable with time (Fig. 1). Furthermore, the rate of tumor sodium increase accelerated with tumor progression. The glioma response to BCNU treatment was dramatic, exhibiting a consistent correlated increase of Na and ADC. The time of the largest values for tumor sodium and diffusion correlated with intensive tumor cell destruction. Sodium T1 relaxation time dramatically increased during this period from  $30 \pm 3$  ms in normal brain to  $57 \pm 6$  ms in treated gliomas (Fig. 2).



**Fig. 1.** Intact 9L rat glioma detected by ADC mapping (upper row) and Na MRI (bottom row). Images were co-registered and represent the same area in the same animal on three different days: D = 14 (left); D = 18 days (center) and D = 20 (right) after tumor implantation.



**Fig. 2.** Sodium T1 relaxation curves for 9L rat glioma (12 days after BCNU) and normal rat brain. Relaxation time in treated tumor was almost 2 times more than in normal brain and, correspondingly, tumor sodium concentration was 2.8 times larger.

## Discussion

Sodium MRI revealed a consistent increase of non-treated tumors' sodium concentration over time. The growth of sodium corresponds to the increasing metabolic stress of tumor cells in gliomas. It is important to note that a persistent increase of tumor sodium preceded any changes in diffusion. Sodium MRI performs tumor grading prior to the development of necrosis. Necrosis, itself, is usually observed in the areas where both Na and diffusion are increasing simultaneously. During tumor therapy, the alteration of tumor sodium over time correlated with proton diffusion, and both were predictive of future tumor shrinking. The near doubling of sodium T1 relaxation time represents a significant decrease in sodium binding and indicates large structural changes in the brain tumor during therapy.

## Conclusion

High resolution Na MRI in non-treated rat gliomas quantitatively reflects pre-necrotic cancer development well in advance of any changes in tumor diffusion. Simultaneous use of both imaging modalities is a valuable tool for assessment of tumor therapy. Our findings, together with the results of other investigators, elucidate the values of sodium and diffusion MRI for oncology and biomedicine [1-7].

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