

Non-invasive measurement of cellular membrane pH gradient in brain tumors using hyperpolarized ^{13}C -bicarbonate MSRI and CEST imaging

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Target audience: Scientists with interests in magnetic resonance spectroscopic imaging of hyperpolarized ^{13}C compounds, brain tumors, pH imaging, and molecular imaging.

Purpose: Hyperpolarized ^{13}C bicarbonate chemical shift imaging (CSI) can be used to measure extracellular pH (1). Intracellular pH can be measured by chemical exchange saturation transfer (CEST)(2) of magnetization from endogenous amine and amide protons to bulk water. The purpose of this study is to measure the intracellular/extracellular pH gradient in brain tissue and tumors using hyperpolarized ^{13}C bicarbonate CSI and CEST MRI.

Methods: The caudate nucleus of a Wistar rat brain was surgically implanted with one million C6 glioma cells. Ten days after implantation, T_2 -weighted proton images of the rat brain were acquired using a 3T GE Discovery MR750 to localize the extent of the tumor. Extracellular pH was measured by injecting 100-mM hyperpolarized ^{13}C cesium bicarbonate solution in the rat tail vein. ^{13}C spectra of the rat brain were acquired using 2D FID-CSI (TR = 80ms, matrix 8x8, FOV = 60x60mm, slice thickness = 5.5mm and BW = 5000Hz) to measure the regional distributions of ^{13}C -bicarbonate and $^{13}\text{CO}_2$. Extracellular pH maps of the healthy brain and tumor were calculated from the regional ^{13}C data using the Henderson-Hasselbalch equation (1). On the same day, the same rat was imaged on a 9.4T Agilent (Palo Alto, CA) small animal MRI scanner. T_2 weighted proton images were acquired to localize the tumor, followed by single slice CEST MRI (3,4). CEST spectra were acquired using a standard fast spin echo (FSE) pulse sequence (TR/TE = 7000/7 ms, ETL=32, ETE=7 ms, matrix= 64x64, FOV=40x40 mm², 2 prescans, slice thickness=4 mm, 4 s and 1.5 μT pre-image saturation pulse). An intracellular pH map was calculated from the CEST data using a previously described amine and amide concentration-independent detection (AACID) technique (4).

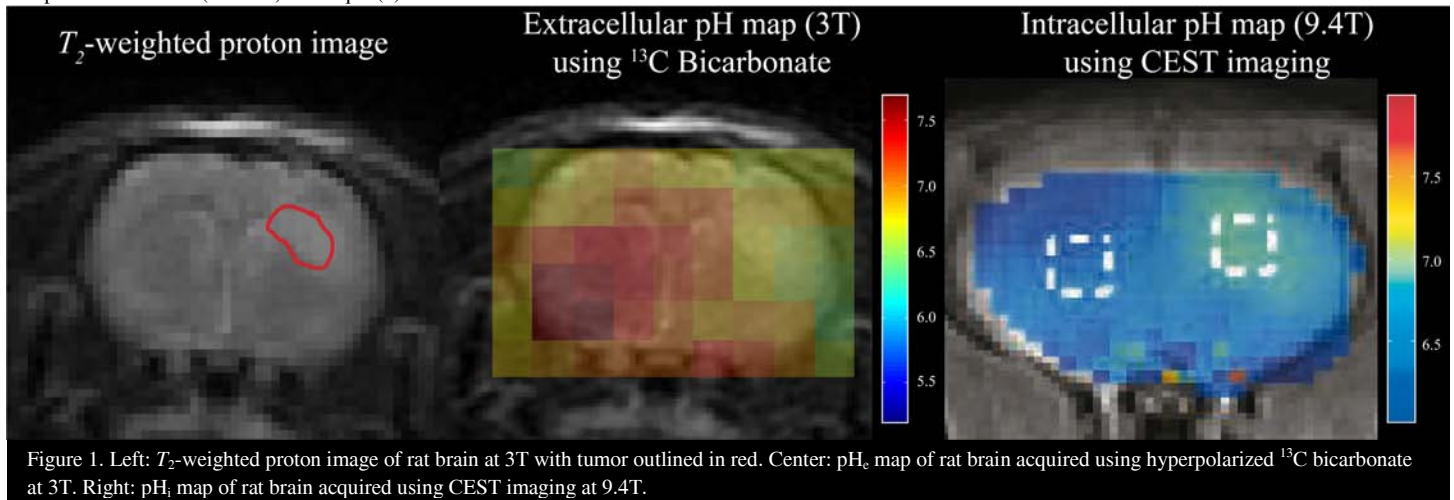


Figure 1. Left: T_2 -weighted proton image of rat brain at 3T with tumor outlined in red. Center: $p\text{H}_e$ map of rat brain acquired using hyperpolarized ^{13}C bicarbonate at 3T. Right: $p\text{H}_i$ map of rat brain acquired using CEST imaging at 9.4T.

Results: Intracellular ($p\text{H}_i$) and extracellular pH ($p\text{H}_e$) maps of the rat brain are shown in Figures 1 respectively. In contralateral tissue, the average $p\text{H}_e$ was 7.09 ± 0.06 and the average $p\text{H}_i$ was 6.75 ± 0.12 ($\Delta\text{pH} = (p\text{H}_i - p\text{H}_e) = -0.34 \pm 0.13$). In the tumor, the average $p\text{H}_e$ was 6.91 ± 0.11 and the average $p\text{H}_i$ was 7.05 ± 0.09 ($\Delta\text{pH} = 0.14 \pm 0.14$). Errors represent one standard deviation of the mean.

Discussion: Endogenous buffers such as bicarbonate (extracellular) and phosphate (intracellular) normally maintain tight control of the acid-base balance in mammalian tissue. The maintenance of an alkaline tumor $p\text{H}_i$ increases the activity of several metabolic enzymes that drive cellular proliferation (5). Acidic $p\text{H}_e$ in tumors occurs due to increased lactic acid production in tumor cells and the subsequent active transport of H^+ out of the cell. Results of the current study are consistent with previous measurement of $p\text{H}_e$ in C6 gliomas using pH-sensitive T_1 relaxivity, which found a heterogeneous pH distribution with a mean value of 6.87(1.6). Also ^{31}P -MRSI studies of glioma report $p\text{H}_i$ values ranging between 7.12 and 7.24 compared with 6.99 to 7.05 in normal human brain.

Conclusion: We have non-invasively measured $p\text{H}_i$ and $p\text{H}_e$ in a rat glioma and contralateral brain tissue. A large transmembrane pH gradient ($\Delta\text{pH} \approx 0.14 \pm 0.14$) was observed in the glioma. Since most therapeutic agents are weak acids or bases, *a priori* knowledge of the transmembrane pH gradient might be an indicator to guide choice of therapeutic agent (7,8).

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