IN VIVO DETECTION AND CHARACTERIZATION OF 9L TUMORS IN RAT BRAIN BY BIRDS

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INTRODUCTION. Endogenous and exogenous MRI contrast agents provide outstanding ability to identify diseased tissue, while MRS of biochemicals provide insight into metabolic (i.e., molecular) bases of various diseases. However no magnetic resonance technology exists that can reliably extract quantitative molecular information from a region in the presence of a strong T1, T2 or T2* contrast agent. This is primarily because the water-based MRI signal and endogenous non-water MRS signals (e.g., glucose, glutamate, glutamine, GABA, etc. in the brain) are compromised by presence of conventional MRI contrast agents. The goal of this work is to go beyond the shades of gray in conventional MRI and extract biological/physiological information that can be used to follow the status of the contrasted tissue as a function of disease progression and treatment (e.g., extracellular pH and temperature inside and outside of a 9L tumor). We used ***b***iosensor ***i***maging of ***r***edundant ***d***eviation in ***s***hifts (BIRDS) in conjunction with 3D CSI to detect signals originating from the protons of the MRI contrast agent itself instead of agent's effect on water protons. We demonstrate in a rat brain tumor model that a commercially available contrast agent ($TmDOTP^{5-}$) provides similar contrast as other FDA approved contrast agents (Magnevist) but in addition allows true molecular reporting of cellular status of tumorous and non-tumorous brain tissue.

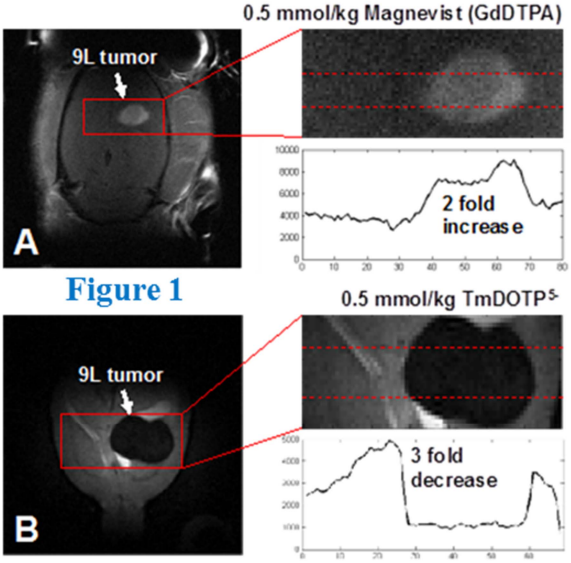

Figure 1

MATERIALS AND METHODS. ***Animal preparation.*** 9L cells (ATCC™) were cultured in T75 flasks in DMEM containing 10% heat inactivated FBS and 1% antibiotics. Cells were harvested at 60-80% confluence and suspended in serum-free DMEM for inoculation. Intracerebral tumors were induced in male Fisher344/DuCrl rats (200-300g) by implanting 9L cells (1×10^5 in 4 µl) in the frontal brain at 3 mm depth using a stereotactic device and allowed to grow for 28 to 36 days. For the MR experiments, the rats were tracheotomized and artificially ventilated (70% N_2O, 30% O_2). Four animals were used for Magnevist infusion and two for $TmDOTP^{5-}$. During the animal preparation, isoflurane (3-4%) was used for induction. An intraperitoneal line was inserted for administration of α-chloralose (46 ± 4 mg/kg/hr) and an intravenous line for administration of D-tubocurarine chloride (1mg/kg/hr), Magnevist (0.5mmol/kg) or $TmDOTP^{5-}$ (0.5mmol/kg). An arterial line was used for monitoring physiology (blood pH, pO_2, pCO_2) throughout the experiment. To prevent fast clearance of $TmDOTP^{5-}$, the anesthetized rats were prepared with renal ligation as previously described [1]. ***Data acquisition.*** Anatomical images and the 25x25x25 3D CSI datasets were obtained on a modified 11.7 T Bruker horizontal-bore spectrometer (Billerica, MA) using a ^1H resonator/surface coil RF probe. A double-banded refocused 90^0 Shinnar-Le Roux (SLR) RF pulse of 30 kHz bandwidth, 60 kHz separation and 204 µs duration was used for excitation. The data was acquired with 1743 spherical encoding steps, TR=5ms, 64 averages and FOV of 25x25x25 mm^3. The spectra were line broadened (300 Hz), phased (zero order) and baseline corrected (first order) in a similar fashion in Matlab. The pH and temperature maps **(Fig. 2C and 2D)** were calculated from the H2, H3 and H6 chemical shifts of TmDOTP [1,2].

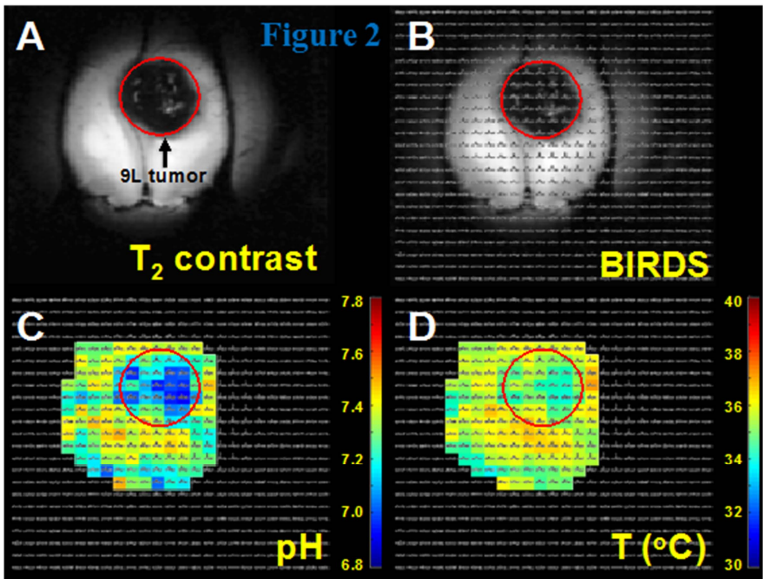

Figure 2

RESULTS. Analysis of the MRI data obtained after Magnevist infusion showed a 2-fold increase in the signal in the tumor region relative to the signal in normal tissue **(Fig. 1A)**. $TmDOTP^{5-}$ infusion resulted in a 3-fold decrease of signal in the tumor region compared to normal tissue **(Fig. 1B)**. In addition, 3D CSI datasets were obtained from the $TmDOTP^{5-}$ infused rats **(Fig. 2B)**, allowing both pH **(Fig. 2C)** and temperature **(Fig. 2D)** mapping. The results show that the pH in the tumor region is in the range from 6.9 to 7.2 while the pH in the rest of the brain is in the range from 7.3 to 7.5 **(Fig. 2C).** The temperature in the tumor region (34-35 ^0C) is also lower than in normal tissue (36-37 ^0C) **(Fig. 2D)**.

DISCUSSION. Magnevist is a well-known T_1 contrast agent used for tumor detection and its effect is an increase in the MR signal in the tumor region **(Fig. 1A)**. In contrast, $TmDOTP^{5-}$ infusion results in a decrease in the MR signal indicating that $TmDOTP^{5-}$ acts as a T_2 contrast agent **(Fig. 1B and 2A)**. Although the two contrast types are different, both agents give similar contrast ratios. The advantage of using $TmDOTP^{5-}$ is that in addition to conventional MRI contrast, BIRDS allows pH and temperature mapping. Similar contrast is expected for other BIRDS agents such as $TmDOTMA^-$, which provides temperature mapping only but with higher accuracy than $TmDOTP^{5-}$ [2]. In conclusion, we demonstrated in a rat brain tumor model that both contrast generation for tumor detection and molecular reporting (e.g. pH, temperature) in tumor and non-tumor regions are possible with contrast agents that can be used to make BIRDS measurements.

REFERENCES. [1] Coman D et al. (2009) *NMR in Biomed.* 22:229-239. [2] Coman D et al. (2009) *NMR in Biomed.* 23:277-285.

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