Published in final edited form as:

NMR Biomed. 2016 October ; 29(10): 1364–1372. doi:10.1002/nbm.3578.

Towards Longitudinal Mapping of Extracellular pH in Gliomas

Yuegao Huang^{1,2}, Daniel Coman^{1,2}, Peter Herman^{1,2}, Jyotsna U. Rao^{1,2}, Samuel Maritim³, and Fahmeed Hyder^{1,2,3}

¹Magnetic Resonance Research Center, Yale University, New Haven, CT, USA

²Department of Radiology and Biomedical Imaging, Yale University, New Haven, CT, USA

³Department of Biomedical Engineering, Yale University, New Haven, CT, USA

Abstract

Biosensor Imaging of Redundant Deviation in Shifts (BIRDS), an ultrafast chemical shift imaging technique, requires infusion of paramagnetic probes like 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis(methylene phosphonate) (DOTP⁸⁻) complexed with thulium (Tm³⁺) ion (i.e., TmDOTP⁵⁻), where the pH-sensitive resonances of hyperfine-shifted nonexchangeable protons contained within the paramagnetic magnetic resonance probe are detected. While imaging extracellular pH (pH_e) with BIRDS meets an important cancer research need by mapping the intratumoral-peritumoral pH_e gradient, the surgical intervention used to raise the probe's plasma concentration limits longitudinal scans on the same subject. Here we describe using probenecid (i.e., an organic anion transporter inhibitor) to temporarily restrict renal clearance of TmDOTP⁵⁻, thereby facilitating molecular imaging by BIRDS without surgical intervention. Co-infusion of probenecid with TmDOTP⁵⁻ increased the probe's distribution into various organs, including the brain, compared with when infusing TmDOTP⁵⁻ alone. In vivo BIRDS data using probenecid/TmDOTP⁵⁻ co-infusion method in rats bearing RG2, 9L, and U87 brain tumors showed intratumoral-peritumoral pH_e gradients that were unaffected by the probe dose. This co-infusion method can be used for pH_e mapping with BIRDS in preclinical models for tumor characterization and therapeutic monitoring given the possibility of repeated scans with BIRDS (e.g., over days and even weeks) in the same subject. The longitudinal pH_e readout by probenecid/TmDOTP⁵⁻ co-infusion method for BIRDS adds translational value in tumor assessment and treatment.

Keywords

CEST; extravasation; metastasis; permeability; renal ligation; uric acid reducer

INTRODUCTION

Gliomas are the most common central nervous system tumors (1,2). Tumor cells exhibit increased glucose uptake and elevated lactate generation despite presence of sufficient oxygen for oxidative phosphorylation. This phenomenon is known as aerobic glycolysis, but

Address correspondence and reprint requests to: Yuegao Huang / D. S. Fahmeed Hyder, N143 TAC (MRRC), 300 Cedar Street, Yale University, New Haven, CT 06520, USA, Tel: +1-203-785-6205, Fax: +1-203-785-6643, yuegao.huang@yale.edu / fahmeed.hyder@yale.edu.

it is also referred to as the Warburg effect after its discoverer (3). Elevated aerobic glycolysis of tumor cells generates excessive protons (and other acids), which have to be excreted out of the cell, and as a result, the extracellular pH (pH_e) becomes acidic in most solid tumors. Given that pH_e for normal tissue is close to neutral, the pH_e inside tumor and outside tumor tissue can differ, thereby generating an intratumoral-peritumoral pH_e gradient. Extracellular acidosis promotes tumor growth, cell invasion, metastasis, and resistance to therapy (4–6). Thus, mapping intratumoral-peritumoral pH_e gradient quantitatively is an important biomarker in tumor diagnosis and prognosis after treatment (7,8).

Noninvasive magnetic resonance (MR) methods are ideal for cancer imaging, and various tumor properties have been targeted, e.g., diffusion or perfusion (9–11). In particular, pH_e mapping is possible with both MR imaging (MRI) - e.g., contrast based on relaxation or chemical exchange - and spectroscopy (MRS) (12–14). *Biosensor Imaging of Redundant Deviation in Shifts (BIRDS)* is an ultrafast CSI platform for molecular imaging in which paramagnetically-shifted non-exchangeable protons (i.e., CH_x) on DOTA-based macrocyclic complexes are directly detected (15–21). BIRDS has some unusual properties. Both longitudinal (T_1) and transverse (T_2) relaxation times of protons of the complexes are very short (i.e., only in ms range) due to their close proximity to the paramagnetic metal ion. Short T_1 allows fast averaging, whereas short T_2 means insensitivity to static magnetic field inhomogeneity. T_2 and T_1 equivalence leads intrinsically to higher MR sensitivity. Because the proton resonances of the agent are sufficiently shifted away from the water proton resonance, there are no overlaps with signals from other metabolites. Finally, the pH_e readout does not depend on the agent concentration because the readout is purely based on shift changes (i.e., not peak heights).

Previously we reported capability of using DOTA phosphonates (i.e., TmDOTP^{5-} and TmDOTA-4AmP^{5-}) to map the intratumoral-peritumoral pH_e gradient of brain tumors (22). However to raise concentration of the agents in brain tissue, we surgically stopped renal clearance of the agents. While this method provided sufficient local concentration of agents for molecular imaging, it required surgical intervention - thus preventing longitudinal survival studies.

Probenecid inhibits organic anion transporters (OATs) and it has been used clinically to decrease the renal excretion of drugs, such as penicillin, bumetanide, and zidovudine (23–25). While probenecid can be used as adjuvant agent to increase systemic level of drugs, it also serves as an OATs3 inhibitor mediating efflux of drugs across the blood brain barrier (26). Hence, probenecid can significantly increase drug concentration in plasma and target tissues for prolonged treatment. For example, the distribution of fluorescein in brain was enhanced by 2-fold when probenecid was co-administered with the drug (27). Probenecid has a half-life of 4–12 h (28), and although the effect is temporary, it allows sufficient time for imaging. We hypothesized that pH_e imaging of gliomas with BIRDS can be achieved when probenecid and TmDOTP^{5-} are co-infused. We show that the co-infusion method is a safe and fast; and thus is a viable alternative to renal ligation for BIRDS. Thus, longitudinal monitoring of tumor growth and assessment of therapeutic response will be possible with the probenecid/ TmDOTP^{5-} co-infusion method for BIRDS.

MATERIALS AND METHODS

Probenecid was purchased from Sigma-Aldrich (St. Louis, MO) and TmDOTP⁵⁻ was obtained from Macrocyclics (Dallas, TX). Tumor cell lines RG2, 9L, and U87 were purchased from American Type Culture Collections (Manassas, VA). Adult male Sprague-Dawley rats (200–250 g), male Fischer 344 rats (200–250 g) and nude rats (150–200 g) were obtained from Yale University vendors and maintained according to approved animal care protocols. All animal experiments were performed under NIH guidelines and approved by Institutional Animal Care and Use Committee (IACUC) at Yale University. In vivo MR scans were conducted on an 11.7 T Agilent (Santa Clara, CA) horizontal-bore spectrometer (bore size = 21 cm; maximum gradient strength = 400 mT/m) using custom built ¹H surface RF probe (1.4 cm diameter). Ex vivo samples were scanned on an 11.7 T Bruker (Billerica, MA) vertical-bore spectrometer.

Biodistribution and Survival Experiments

Sprague-Dawley rats were anesthetized with isoflurane (~2%). A femoral vein and an artery were cannulated for agent/drug infusion and physiological monitoring (pCO₂, pO₂, pH and blood pressure) during the experiment, respectively. Rats were separated into three groups and in each group we infused either 0.1 or 1 mmol/kg TmDOTP⁵⁻ for 120 minutes (n = 6 in each case). In group I we infused TmDOTP⁵⁻ (0.1 and 1 mmol/kg) without probenecid or renal ligation. In group II we co-infused TmDOTP⁵⁻ (0.1 and 1 mmol/kg) and probenecid (100 mg/kg). In group III we infused TmDOTP⁵⁻ (0.1 and 1 mmol/kg) after renal ligation. The concentration of probenecid stock solution was 100 mg/mL at pH 7.4. The concentrations of the TmDOTP⁵⁻ stock solutions were 150 mM and 15 mM for 1 mmol/kg and 0.1 mmol/kg doses, respectively, dissolved in 100 mg/kg probenecid. The infusion rate was 15 μ L/min for probenecid and TmDOTP⁵⁻.

Blood plasma and urine samples were collected throughout the experiment from rats in groups I and II. Plasma concentrations were used for calibrating the agent concentration in the tissue using tissue-plasma volumes (29). After the infusion rats were euthanized with focused microwave irradiation (30), immediately after which various organs (i.e., brain's cortex and subcortex, lung, heart, liver, spleen, muscle and kidney) were removed, weighed and homogenized for agent concentration analysis by ¹H NMR. We used 0.1 mM TmDOTMA⁻ as an internal reference. NMR spectra were collected using standard pulse-acquire experiments on an 11.7T vertical bore magnet using fast acquisition times and intensity of H6 proton of TmDOTP⁵⁻ was measured. The final tissue concentrations of TmDOTP⁵⁻ were calculated after reference and plasma corrections.

A few rats (n = 4) were anesthetized (~2% isoflurane) and the tail vein was cannulated for co-infusion of TmDOTP⁵⁻ (1 mmol/kg) and probenecid (100 mg/kg) for 120 minutes. A water-heating pad maintained the body temperature in the physiological range (36–37 °C). The rats were survived after the co-infusion. The rats' activity (e.g., sleep and eating cycles) was monitored for 48 hours before the co-infusion study was repeated and then revived again.

Tumor Rat Preparation

RG2 and 9L cells were cultured in T75 flasks in DMEM media containing 10% heat inactivated FBS and 1% antibiotics at 37°C and 5% CO₂, whereas U87 cells were cultured in T75 flasks in MEM media containing 10% heat inactivated FBS and 1% antibiotics at 37°C and 5% CO₂.

Tumor cells were harvested at 60–80% confluence, washed and suspended in serum-free media for implantation in Fischer 344 rats (5 µl 2×10⁴ RG2 cells and 5 µl 1×10⁵ 9L cells) and nude rats (5 µl 5×10⁵ U87 cells). Rats were anesthetized with isoflurane (~2%) and positioned in a stereotaxic system. A water-heating pad was used to maintain the body temperature in the physiological range (36–37 °C). Tumor cells were injected with a 25 µL Hamilton syringe into the right thalamus at coordinates 3 mm laterally to the right of bregma and 3 mm ventral to the dura. A 5 µL volume of the cell suspension was injected over the course of 5 minutes and the needle was left in place for an additional 5 minutes before slow withdrawal.

On the day of scanning, rats (n=6 for RG2 and 9L tumors each and n=3 for U87 tumor) [R3.1] were anesthetized (~2% isoflurane), tracheotomized, and artificially ventilated (70% N₂O/30% O₂). A femoral vein and an artery were cannulated for agent/drug infusion and physiological monitoring (pCO₂, pO₂, pH and blood pressure) during the experiment. A water-heating pad was used to maintain the body temperature in the physiological range (36–37 °C) throughout the experiment. Body temperature was measured with a rectal probe and no further adjustments in the water-heating pad were made for the entire duration of the experiment (~150 minutes). Probenecid (100 mg/kg) and TmDOTP⁵⁻ (1 mmol/kg) were co-infused for a maximum of 120 minutes.

BIRDS Analysis

Spin-echo MRI datasets (128×128 resolution, 1 mm slice thickness, field of view (FOV) of 25×25 mm², a recycle time (TR) of 6 s, echo time (TE) of 10 ms) were obtained for tumor localization and visualization. In vivo 3D CSI for BIRDS was acquired 30 minutes after probenecid/TmDOTP⁵⁻ co-infusion began, using dual-banded refocused 90° Shinnar-Le Roux (SLR) RF pulse (35 kHz bandwidth and 90 kHz separation with 205 µs duration) for selective excitation of the H2/H3 and H6 protons of TmDOTP⁵⁻. The 3D CSI datasets were acquired with a TR of 5 ms and a FOV of 25×25×25 mm³, spectral window of 250 kHz and the acquisition time of 4.1 ms. The total time of each 3D CSI data set acquisition was 12 minutes. The 3D CSI data were reconstructed to 25×25×25 resolution resulting in a nominal voxel resolution of 1 µL. Each ¹H spectrum was line broadened (500 Hz), phased (zero order), and baseline corrected (first order). The data were analyzed in Matlab (MathWorks, Inc., Natick, MA). The pH for each voxel was calculated from the chemical shifts of the H2, H3 and H6 protons of TmDOTP⁵⁻ according to

$$pH = a_0 + \sum_{k=2,3,6} a_1^k \delta_k + \sum_{k=2,3,6} \sum_{j=2,3,6} a_2^{kj} \delta_k \delta_j \quad [1]$$

where the coefficients a_0 , a_1^k and a_2^{kj} were calculated from linear least-squares fit of pH as a function of chemical shifts δ_2 , δ_3 and δ_6 , as previously described (22). The tumor and its margin were localized by MRI contrast and pH_e measured by BIRDS was assessed for voxels inside the tumor (intratumoral) and outside the tumor (peritumoral). Intratumoral and peritumoral voxels were defined as all voxels inside and outside the tumor boundary defined by MRI, respectively. Voxels that were partly inside or outside of the tumor were excluded in the analysis. For single voxel region-of-interest analysis, an intratumoral voxel was selected in the center of the tumoral voxels and compared to a contralateral peritumoral voxel. Significance between intratumoral and peritumoral pH_e was assessed by Student's t -test, where P -values less than 0.05 were considered to be significant.

RESULTS

Probenecid is a nonselective OAT inhibitor used to raise the plasma concentrations of drugs (31). We examined the effect of probenecid/TmDOTP⁵⁻ co-infusion in rats. Plasma concentration was higher for probenecid/TmDOTP⁵⁻ co-infusion than TmDOTP⁵⁻ infusion alone (Figure S1), but the highest plasma concentration was achieved for infusion with renal ligation. On the contrary, urine concentration was lower for probenecid/TmDOTP⁵⁻ co-infusion than TmDOTP⁵⁻ infusion alone. These results suggest that probenecid inhibited the renal clearance of TmDOTP⁵⁻.

Biodistribution data of TmDOTP⁵⁻ in the rat's body is presented in Figures 1 and S2. TmDOTP⁵⁻ concentrations in various organs (brain cortex, brain subcortex, liver, lung, heart, muscle, spleen, and kidney) were quantitatively measured and compared for the three different infusion protocols: infusion of TmDOTP⁵⁻ without any intervention, co-infusion of TmDOTP⁵⁻ with probenecid, and TmDOTP⁵⁻ infusion with renal ligation.

Two infusion doses of TmDOTP⁵⁻ were used. At low dose of 0.1 mmol/kg (Figure 1A), TmDOTP⁵⁻ concentrations in tissues for co-infusion method were slightly higher than infusion of TmDOTP⁵⁻ alone, but comparable with TmDOTP⁵⁻ concentrations detected using the renal ligation method. The enhancement in TmDOTP⁵⁻ concentration in various tissue is small at low dose and becomes larger at large dose with both co-infusion and ligation methods. At high dose of 1 mmol/kg (Figure 1B), the differences in TmDOTP⁵⁻ concentration in each tissue for the three infusion methods were larger. As noted by the H6 intensities in TmDOTP⁵⁻ depicted in Figures 1C and 1D, co-infusion of TmDOTP⁵⁻ with probenecid increased TmDOTP⁵⁻ concentrations both in kidney and brain. Rats revived after co-infusion of TmDOTP⁵⁻ with probenecid showed no overt behavioral signs that were different from rats revived from anesthesia. Although probenecid/TmDOTP⁵⁻ co-infusion method did not result in an increase of TmDOTP⁵⁻ concentration in the tissue as remarkably as the renal ligation method, it was approximately double compared to infusion of TmDOTP⁵⁻ alone. Therefore, co-infusion of TmDOTP⁵⁻ with probenecid provides a good alternative to renal ligation to raise the TmDOTP⁵⁻ concentration in various tissues, including brain.

Representative BIRDS data of a brain bearing RG2 tumor in a Fischer rat after co-infusion of TmDOTP⁵⁻ with probenecid is shown in Figure 2. T₂-weighted MRI identified the tumor

upon TmDOTP⁵⁻ infusion (Figure 2A). The tumor region was slightly darkened by T₂ contrast as a result of TmDOTP⁵⁻ extravasation due to higher permeability of the tumor vasculature. While the tumor border may not be clearly distinguishable by endogenous MRI contrast (e.g., see Figure S2 in ref. (22)), endogenous MRI contrast is needed for arterial spin labeling (ASL) to measure blood flow differences within and beyond tumor boundary. Future studies with ASL and BIRDS in the same subject will provide novel relations between gradients of intratumoral-peritumoral blood flow and pH_e. The TmDOTP⁵⁻ CSI dataset in Figure 2B shows the resonances H2, H3 and H6 of TmDOTP⁵⁻ in intratumoral and peritumoral voxels. pH_e values of each voxel were differentially determined by chemical shifts of TmDOTP⁵⁻ resonances according to eq. 1. As an example, a selected intratumoral voxel ($\delta_2=58.96$ ppm, $\delta_3=76.97$ ppm and $\delta_6=-141.09$ ppm) had a pH_e of 6.64 (Figure 2C) and a selected peritumoral voxel ($\delta_2=59.75$ ppm, $\delta_3=77.52$ ppm and $\delta_6=-140.42$ ppm) had a pH_e of 7.27 (Figure 2D), where the pH_e values were determined from eq. 1. Consistent with our previous results, the intratumoral pH_e values were distinctly lower than peritumoral pH_e values for rat brains bearing RG2 tumors (22). In this rat brain with RG2 tumor, the average pH_e values of intratumoral voxels were 6.73 ± 0.02 while those of peritumoral voxels were 7.11 ± 0.01 . We showed previously that large intratumoral-peritumoral pH_e gradient for aggressive tumors (e.g., RG2 vs. 9L) matched the increased presence of Ki-67 (proliferation marker) positive cells beyond the tumor border (see Figures 1 and S1 in ref. (22)).

The overall intensities of proton resonances for intratumoral voxels were higher than those for peritumoral voxels, indicating higher TmDOTP⁵⁻ accumulation in the intratumoral region of the RG2 tumor. As an example shown in Figure 3A, the intensity of the H6 proton resonance of a selected intratumoral voxel was higher than that of a selected peritumoral voxel. Intensities of the resonances across the brain increased during co-infusion. However the concentration decreased immediately after the co-infusion suggesting agent clearance. In support of this both urine and plasma signals showed decrease of TmDOTP⁵⁻ signal immediately after end of infusion (Figure S1). Although the intensities of each resonance of TmDOTP⁵⁻ varied across either intratumoral or peritumoral voxels, pH_e determination by BIRDS was independent of TmDOTP⁵⁻ concentration. Thus, even though lower concentration of TmDOTP⁵⁻ was present in the peritumoral region, the pH_e of each voxel were accurately determined. As shown in Figures 3B and 3C, the pH_e maps obtained ~12 minutes apart during the probenecid/TmDOTP⁵⁻ co-infusion were nearly identical, indicating dose-independent pH_e imaging with BIRDS. The correlation between these two pH_e maps was excellent (Figure S3).

Probenecid/TmDOTP⁵⁻ co-infusion was tested in rats bearing different types of gliomas (Figure 4). More aggressive xenograft U87 tumors and less aggressive gliosarcoma 9L were similarly localized using T₂-weighted MRI images (left column of Figure 4). The TmDOTP⁵⁻ CSI datasets for U87 and 9L tumors with probenecid/TmDOTP⁵⁻ co-infusion showed higher signals inside the tumor core (middle column of Figure 4). The pH_e map of U87 tumor showed low values in intratumoral region as well as tumor margin (top, left column of Figure 4), whereas the pH_e map of 9L tumor showed low values in intratumoral region and high values in peritumoral region (bottom, left column of Figure 4).

The intratumoral and peritumoral pH_e values for each tumor type measured by BIRDS are summarized in Figure 5. The histograms were fitted to a Gaussian distribution to obtain the most probable (or expected) pH_e value and the full width at half maximum, FWHM (Table 1) [R3.3][R3.5][R3.6]. The results show that the intratumoral-peritumoral pH_e gradients for brains with 9L and U87 tumors were larger compared to that of brains bearing RG2 tumors, suggesting that the pH_e boundary between intratumoral and peritumoral regions were more clearly separated for 9L and U87 tumors.

For brains bearing 9L tumors (Figure 5A), the expected pH_e values of intratumoral and peritumoral regions were 6.84 ± 0.01 and 7.32 ± 0.0 , respectively, indicating that the intratumoral-peritumoral pH_e gradient was about 0.5. However the FWHM of the intratumoral distribution (0.24 ± 0.01) [R3.6] was similar compared to the peritumoral distribution (0.25 ± 0.01) [R3.6], indicating that the pH_e values were homogeneous in both regions. Thus the pH_e boundary between intratumoral and peritumoral regions was well resolved for 9L tumors (Table 1) [R3.3][R3.5][R3.6].

For brains with U87 tumors (Figure 5B), the expected pH_e values of intratumoral and peritumoral regions were 6.84 ± 0.02 and 7.34 ± 0.02 , respectively, indicating that the intratumoral-peritumoral pH_e gradient was also about 0.5. But the FWHM of the intratumoral distribution (0.37 ± 0.02) [R3.6] was larger than the peritumoral distribution (0.20 ± 0.01) [R3.6], indicating the pH_e values in intratumoral voxels were more spread. Thus the pH_e boundary for U87 tumors was more heterogeneous than 9L tumors (Table 1) [R3.3] [R3.5][R3.6].

For brains with RG2 tumors (Figure 5C), the expected pH_e values of intratumoral and peritumoral regions were 6.83 ± 0.02 and 7.22 ± 0.01 , respectively, indicating that the intratumoral-peritumoral pH_e gradient was about 0.4 for RG2 tumors. Moreover the FWHM of the intratumoral distribution (0.17 ± 0.01) [R3.6] was smaller than the peritumoral distribution (0.42 ± 0.03) [R3.6], and the peritumoral distribution overlaps partially with the intratumoral distribution, indicating that low pH_e values were measured in peritumoral regions. Thus the pH_e boundary between intratumoral and peritumoral regions was not clearly separated for RG2 tumors (Table 1) [R3.3][R3.5][R3.6].

Because different tumors grow at different rates, we could not image all tumor types at the same size. We have estimated the tumor volume for each tumor type (9L: $111.7 \pm 37.7 \mu\text{L}$; RG2: $83.9 \pm 35.7 \mu\text{L}$; U87: $68.1 \pm 17.6 \mu\text{L}$). The measured intratumoral pH_e was independent on tumor volume (Table 1; Figure S4). However it should be noted that the peritumoral pH_e also changes for aggressive tumors (22). [R3.2] For each given tumor cell line, the pH_e values were statistically significant between intratumoral and peritumoral voxels ($p < 0.05$). For intratumoral pH_e , an Anova showed that there was significant differences across three tumor types ($p=0.011$); post-hoc Bonferroni test revealed significant differences between 9L and U87 tumors ($p=0.023$), and RG2 and U87 tumors ($p=0.019$). For peritumoral pH_e , an Anova showed that there was significant differences across three tumor types ($p=0.000$); post-hoc Bonferroni test revealed significant differences between 9L and RG2 tumors (0.000), and RG2 and U87 tumors ($p=0.019$). [R3.4]

DISCUSSION

Acidic pH_e is one of the primary cancer hallmarks and thus a target for cancer imaging (32). Mapping intratumoral and peritumoral pH_e with BIRDS is valuable in assessment of both tumor growth and possible therapeutic response. Previous work with BIRDS has shown promise for pH_e mapping of brain tumors (22). The urinary excretion is the major pathway to eliminate most MRI contrast agents, including TmDOTP^{5-} . Thus, to inhibit rapid clearance of TmDOTP^{5-} , previous BIRDS imaging required surgical intervention (i.e., renal ligation) to raise the agent's concentration in the plasma as well as in the tissue. Longitudinal monitoring of pH_e using BIRDS on tumors is an unmet need and could be achieved if the renal system is only temporarily (and not permanently) inhibited. Probenecid has been widely used to temporarily decrease the renal clearance of drugs. Hence, probenecid can be used as an alternative to renal ligation to allow longitudinal assessment of tumor microenvironments with BIRDS.

Compared to conventional water-based proton MRI, BIRDS is a molecular imaging technique that detects non-exchangeable protons on the paramagnetic agent in response to variations in microenvironment. Because BIRDS readouts rely on the chemical shift changes of hyperfine-shifted proton resonance, their molecular sensitivity is independent on agent's concentration without any background overlapping signals. Moreover, BIRDS does not interfere with conventional proton MRI method, for example, BIRDS signals and sensitivities are not affected by the presence of superparamagnetic iron oxide (SPIO) or even gadolinium-based contrast agents, which are widely used to localize brain tumor in patients (21).

Tissue distribution of TmDOTP^{5-} is the key to successful BIRDS experiments. Probenecid was efficient to raise the plasma and tissue concentrations of TmDOTP^{5-} at the infusion dose range of 0.1 to 1 mmol/kg (Figures 1A and 1B). While TmDOTP^{5-} concentrations in kidney was higher for co-infusion method than TmDOTP^{5-} infusion alone (Figures 1C and 1D), TmDOTP^{5-} concentrations in brain cortex were slightly higher for co-infusion and ligation methods compared to infusion of TmDOTP^{5-} alone. As the infusion dose increased, biodistribution data showed that the enhancement in tissue concentrations for probenecid/ TmDOTP^{5-} co-infusion method was more noticeable, although non-survival renal ligation method results in larger tissue concentrations. In addition, the effect was universal and non-selective for specific organs, including the brain. Delivery of TmDOTP^{5-} to the brain is in general more difficult than to other organs because the blood brain barrier prevents the agent entering the brain readily (33). Thus, a non-surgical method (i.e., probenecid/ TmDOTP^{5-} co-infusion) that can increase the life-time of the agent in the blood circulation and thus raise the concentration of the agent in the brain is preferable.

Probenecid-mediated elevated agent concentration in brain tissue can be attributed to inhibitions of OATs in both kidney and brain (34). Thus, probenecid markedly increased TmDOTP^{5-} concentration in brain most probably by two mechanisms: increased plasma concentration and/or half-life of TmDOTP^{5-} by inhibition of TmDOTP^{5-} excretion in the kidney, and reduced TmDOTP^{5-} elimination by inhibition OATs (35,36). More experiments are required to further illustrate the mechanism of probenecid-mediated TmDOTP^{5-}

enhancement in tissues. Other OATs inhibitors (e.g., sulfinpyrazone and benzbromarone) could possibly be used to enhance tissue concentration of TmDOTP⁵⁻ (37,38).

Tumor has immature (i.e., leaky) vascular structure, which may facilitate the permeability of the contrast agent into tumor and poor perfusion may increase retention time (3). The strong T₂-weighted contrast observed due to higher accumulation of TmDOTP⁵⁻ in the tumor was consistent with our previous study when renal ligation was used (22). Using probenecid/TmDOTP⁵⁻ co-infusion, higher concentration of TmDOTP⁵⁻ was observed for intratumoral voxels compared to peritumoral voxels, as shown by their MR resonance intensities (Figure 3). Ex vivo quantitative analysis agreed with the in vivo results, showing that the intratumoral region had higher TmDOTP⁵⁻ concentration (0.36 ± 0.02 mmol/kg) compared to the peritumoral region (0.13 ± 0.02 mmol/kg). In addition, the concentrations in both intratumoral and peritumoral regions with probenecid/TmDOTP⁵⁻ co-infusion were higher than similar infusion dose of DyDOTP⁵⁻ without probenecid (14).

A typical experiment of p*H*_e mapping with BIRDS using probenecid/TmDOTP⁵⁻ co-infusion was acquired within 12 minutes. We demonstrated in our previous work (with renal ligation) that for typical in vivo detection of TmDOTP⁵⁻ protons (i.e., H₂, H₃, and H₆) with SNR of 15 the error in the p*H*_e measurement was 0.0013 (18). Although the SNR is slightly lower in these newer experiments (17,22), the estimation of p*H*_e error is still less than 0.01. p*H*_e maps acquired at different time points of co-infusion and therefore corresponding to different TmDOTP⁵⁻ doses (Figures 3B and 3C) showed that the maps are very similar. This observation is presented in Figure S3 by plot of p*H*_e maps obtained ~12 minutes apart. The plot has a slope equal or close to unity, suggesting the reliability of the BIRDS method and the dose-independent p*H*_e mapping with BIRDS using probenecid/TmDOTP⁵⁻ co-infusion. In addition, validation of pH determination of phantoms containing BSA samples (i.e., 5%) with BIRDS shows accurate measurements and good correlation with pH values expected in physiology measured with a pH electrode (Figure S5). [R2.1] For future experiments, improved signal to noise ratio (SNR) for BIRDS can be achieved by shorter acquisition times through hardware improvement (e.g., faster digitizer) and alternative pulse sequence design (e.g., ultra-short TE sequences) (39). Methylated BIRDS agents could be synthesized to provide significant SNR increase (17). In addition, these paramagnetic agents also have exchangeable protons (-NH_y), which can be used for CEST imaging to provide higher spatial resolution images and multi-modality with BIRDS (16,19). Furthermore, hypoxia (i.e., low pO₂) is also a hallmark of tumor microenvironment. Simultaneous imaging p*H*_e and pO₂ with BIRDS is possible when both p*H*_e- and pO₂-sensitive chelates are attached to the cyclen-based probes (40,41).

Three tumor models (i.e., RG2 as a glioblastoma, 9L as a gliosarcoma, and U87 as a human GBM xenograft) were used in this study to test the probenecid/TmDOTP⁵⁻ co-infusion method for p*H*_e mapping with BIRDS. All three tumor models have been widely studied for cancer imaging as they are good models to represent human-like gliomas (42). While 9L tumors are considered aggressive and infiltrative, RG2 and U87 tumors are more invasive models, mimicking human high-grade gliomas. However, microvessel studies by MRI and metabolite profiling by proton MRS show large variations in tumor properties, and thus are incapable to distinguish among tumor models (43,44). Simultaneous p*H*_e measurements

inside and outside the tumor using the probenecid/TmDOTP⁵⁻ co-infusion method enabled mapping of the intratumoral-peritumoral pH_e gradient, which may serve as an additional biomarker for tumor assessment, but also in monitoring the efficiency of tumor treatment over longitudinal studies in the same subject.

Tumor acidosis promotes invasion and resistance to therapy (45,46). The Warburg effect is believed to be the major mediator of the low pH_e observed in the tumor microenvironment. In addition, poor perfusion in tumors, which causes ineffective removal of excess H⁺ and lactate, cannot be neglected. Gillies and coworkers demonstrated that low pH_e of the tumor drives local invasion (6). H⁺ ions generated by tumor cells diffuse into adjacent normal tissues, causing tissue remodeling and promoting invasion. Various MR methods, including ³¹P MRS, T₁- and T₂- based MRI and CEST imaging, have been proposed for tumor imaging of pH_e (8,14,47,48). However, these methods mainly focus on intratumoral regions. Our previous study showed that intratumoral-peritumoral pH_e gradient was smaller in RG2 tumors than that in 9L tumors (22), suggesting a more invasive nature of the RG2 tumors. In agreement with previous studies, here we also measured an intratumoral pH_e lower than the peritumoral pH_e and therefore an intratumoral-peritumoral pH_e gradient was smaller in more invasive RG2 tumors. pH_e mapping with BIRDS provides a clear picture regarding the invasive properties of aggressive tumors by showing low pH_e in peritumoral voxels. Although intratumoral-peritumoral pH_e gradient of U87 tumors was similar to that of 9L tumors (Figure 5), 9L tumor has a more uniform boundary around the tumor core, while U87 tumor shows heterogeneous pH_e distribution. These results imply that pH_e readout of each tumor type at high spatial resolution is crucial to assess the extent of tumor invasion, which may be important when assessing their responses to treatments. Moreover, the similarity between the BIRDS results in this study and BIRDS results those of our previous study (22) suggests that pH_e measured by BIRDS is not affected by the method used to inhibit renal clearance of the contrast agent (probenecid/TmDOTP⁵⁻ co-infusion or renal ligation).

CONCLUSION

Our results show that probenecid/TmDOTP⁵⁻ co-infusion can be used for intratumoral and peritumoral pH_e mapping with BIRDS. We have demonstrated that intratumoral-peritumoral pH_e gradient mapping can be used to assess tumor invasion beyond tumor margin. Longitudinal monitoring of pH_e from early to late stages of tumorigenesis in the same animal (to gain insights into tumor growth) is now possible with the co-infusion method. Furthermore, this method enables the assessment of therapeutic response to acidic pH_e targeting treatments, such as proton pump inhibitors, sodium bicarbonate, and other pH-regulating drugs (49–51).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by NIH grants (R01 EB-011968, R01 CA-140102, P30 NS-052519).

Abbreviations

BIRDS	biosensor imaging of redundant deviation in shifts
CEST	chemical exchange saturation transfer
CSI	chemical shift imaging
DOTP⁸⁻	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetrakis methylene phosphonate
pH_e	extracellular pH
FOV	field of view
FWHM	full width at half maximum
GBM	glioblastoma multiforme
OAT	organic anion transporter
RF	radio-frequency
SLR	Shinnar-Le Roux
SPIO	superparamagnetic iron oxide

REFERENCES

1. Ostrom QT, Gittleman H, Fulop J, Liu M, Blanda R, Kromer C, Wolinsky Y, Kruchko C, Barnholtz-Sloan JS. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008–2012. *Neuro-oncology*. 2015; 17(Suppl 4):iv1–iv62. [PubMed: 26511214]
2. Wen PY, Reardon DA. Neuro-oncology in 2015: Progress in glioma diagnosis, classification and treatment. *Nature reviews Neurology*. 2016; 12(2):69–70. [PubMed: 26782337]
3. Gatenby RA, Gillies RJ. Why do cancers have high aerobic glycolysis? *Nature Reviews Cancer*. 2004; 4(11):891–899. [PubMed: 15516961]
4. Webb BA, Chimenti M, Jacobson MP, Barber DL. Dysregulated pH: a perfect storm for cancer progression. *Nature Reviews Cancer*. 2011; 11(9):671–677.
5. Gerweck LE, Seetharaman K. Cellular pH gradient in tumor versus normal tissue: potential exploitation for the treatment of cancer. *Cancer research*. 1996; 56(6):1194–1198. [PubMed: 8640796]
6. Estrella V, Chen T, Lloyd M, Wojtkowiak J, Cornnell HH, Ibrahim-Hashim A, Bailey K, Balagurunathan Y, Rothberg JM, Sloane BF, Johnson J, Gatenby RA, Gillies RJ. Acidity generated by the tumor microenvironment drives local invasion. *Cancer research*. 2013; 73(5):1524–1535. [PubMed: 23288510]
7. Bhujwala ZM, Artemov D, Ballesteros P, Cerdan S, Gillies RJ, Solaiyappan M. Combined vascular and extracellular pH imaging of solid tumors. *NMR in biomedicine*. 2002; 15(2):114–119. [PubMed: 11870907]
8. Gillies RJ, Raghunand N, Garcia-Martin ML, Gatenby RA. pH imaging. A review of pH measurement methods and applications in cancers. *IEEE engineering in medicine and biology magazine : the quarterly magazine of the Engineering in Medicine & Biology Society*. 2004; 23(5): 57–64.
9. Lee SK. Diffusion tensor and perfusion imaging of brain tumors in high-field MR imaging. *Neuroimaging clinics of North America*. 2012; 22(2):123–134. ix. [PubMed: 22548924]

10. Ludemann L, Warmuth C, Plotkin M, Forschler A, Gutberlet M, Wust P, Amthauer H. Brain tumor perfusion: comparison of dynamic contrast enhanced magnetic resonance imaging using T1, T2, and T2* contrast, pulsed arterial spin labeling, and H2(15)O positron emission tomography. *European journal of radiology*. 2009; 70(3):465–474. [PubMed: 18359598]
11. Penet MF, Artemov D, Farahani K, Bhujwala ZM. MR - eyes for cancer: looking within an impenetrable disease. *NMR in biomedicine*. 2013; 26(7):745–755. [PubMed: 23784955]
12. Chen LQ, Howison CM, Jeffery JJ, Robey IF, Kuo PH, Pagel MD. Evaluations of Extracellular pH within In Vivo Tumors Using acidoCEST MRI. *Magnetic Resonance in Medicine*. 2014; 72(5): 1408–1417. [PubMed: 24281951]
13. De Leon-Rodriguez LM, Lubag AJ, Malloy CR, Martinez GV, Gillies RJ, Sherry AD. Responsive MRI agents for sensing metabolism in vivo. *Accounts of chemical research*. 2009; 42(7):948–957. [PubMed: 19265438]
14. Martinez GV, Zhang X, Garcia-Martin ML, Morse DL, Woods M, Sherry AD, Gillies RJ. Imaging the extracellular pH of tumors by MRI after injection of a single cocktail of T1 and T2 contrast agents. *NMR in biomedicine*. 2011; 24(10):1380–1391. [PubMed: 21604311]
15. Coman D, de Graaf RA, Rothman DL, Hyder F. In vivo three-dimensional molecular imaging with Biosensor Imaging of Redundant Deviation in Shifts (BIRDS) at high spatiotemporal resolution. *NMR in biomedicine*. 2013; 26(11):1589–1595. [PubMed: 23881869]
16. Coman D, Kiefer GE, Rothman DL, Sherry AD, Hyder F. A lanthanide complex with dual biosensing properties: CEST (chemical exchange saturation transfer) and BIRDS (biosensor imaging of redundant deviation in shifts) with europium DOTA-tetraglycinate. *NMR in biomedicine*. 2011; 24(10):1216–1225. [PubMed: 22020775]
17. Coman D, Trubel HK, Hyder F. Brain temperature by Biosensor Imaging of Redundant Deviation in Shifts (BIRDS): comparison between TmDOTP5- and TmDOTMA. *NMR in biomedicine*. 2010; 23(3):277–285. [PubMed: 19957287]
18. Coman D, Trubel HK, Rycyna RE, Hyder F. Brain temperature and pH measured by (1)H chemical shift imaging of a thulium agent. *NMR in biomedicine*. 2009; 22(2):229–239. [PubMed: 19130468]
19. Huang Y, Coman D, Ali MM, Hyder F. Lanthanide ion (III) complexes of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraaminophosphonate for dual biosensing of pH with chemical exchange saturation transfer (CEST) and biosensor imaging of redundant deviation in shifts (BIRDS). *Contrast media & molecular imaging*. 2014
20. Huang Y, Coman D, Hyder F, Ali MM. Dendrimer-Based Responsive MRI Contrast Agents (G1–G4) for Biosensor Imaging of Redundant Deviation in Shifts (BIRDS). *Bioconjugate chemistry*. 2015; 26(12):2315–2323. [PubMed: 26497087]
21. Maritim S, Huang Y, Coman D, Hyder F. Characterization of a lanthanide complex encapsulated with MRI contrast agents into liposomes for biosensor imaging of redundant deviation in shifts (BIRDS). *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry*. 2014; 19(8):1385–1398. [PubMed: 25304046]
22. Coman D, Huang Y, Rao JU, De Feyter HM, Rothman DL, Juchem C, Hyder F. Imaging the intratumoral-peritumoral extracellular pH gradient of gliomas. *NMR in biomedicine*. 2016; 29(3): 309–319. [PubMed: 26752688]
23. Overbosch D, Vangulpen C, Hermans J, Mattie H. The Effect of Probenecid on the Renal Tubular Excretion of Benzylpenicillin. *Brit J Clin Pharmacol*. 1988; 25(1):51–58.
24. Donovan MD, O'Brien FE, Boylan GB, Cryan JF, Griffin BT. The effect of organic anion transporter 3 inhibitor probenecid on bumetanide levels in the brain: an integrated in vivo microdialysis study in the rat. *The Journal of pharmacy and pharmacology*. 2015; 67(4):501–510. [PubMed: 25490899]
25. Hedaya MA, Elmquist WF, Sawchuk RJ. Probenecid inhibits the metabolic and renal clearances of zidovudine (AZT) in human volunteers. *Pharmaceutical research*. 1990; 7(4):411–417. [PubMed: 2362917]
26. Miyajima M, Kusuhara H, Fujishima M, Adachi Y, Sugiyama Y. Organic anion transporter 3 mediates the efflux transport of an amphipathic organic anion, dehydroepiandrosterone sulfate,

- across the blood-brain barrier in mice. Drug metabolism and disposition: the biological fate of chemicals. 2011; 39(5):814–819. [PubMed: 21325432]
27. Sun H, Miller DW, Elmquist WF. Effect of probenecid on fluorescein transport in the central nervous system using in vitro and in vivo models. *Pharmaceutical research*. 2001; 18(11):1542–1549. [PubMed: 11758761]
28. Cunningham RF, Israili ZH, Dayton PG. Clinical pharmacokinetics of probenecid. *Clinical pharmacokinetics*. 1981; 6(2):135–151. [PubMed: 7011657]
29. Lasher EP, Simmons BS, Everett NB. Effects of semistarvation on the distribution of erythrocytes and plasma in organs and tissues of the rat. *The Journal of nutrition*. 1958; 65(2):317–326. [PubMed: 13576216]
30. de Graaf RA, Chowdhury GM, Brown PB, Rothman DL, Behar KL. In situ 3D magnetic resonance metabolic imaging of microwave-irradiated rodent brain: a new tool for metabolomics research. *Journal of neurochemistry*. 2009; 109(2):494–501. [PubMed: 19200336]
31. Shitara Y, Sato H, Sugiyama Y. Evaluation of drug-drug interaction in the hepatobiliary and renal transport of drugs. *Annual review of pharmacology and toxicology*. 2005; 45:689–723.
32. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144(5):646–674. [PubMed: 21376230]
33. Gabathuler R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. *Neurobiology of disease*. 2010; 37(1):48–57. [PubMed: 19664710]
34. Robbins N, Koch SE, Tranter M, Rubinstein J. The history and future of probenecid. *Cardiovascular toxicology*. 2012; 12(1):1–9. [PubMed: 21938493]
35. Topfer M, Tollner K, Brandt C, Twele F, Broer S, Loscher W. Consequences of inhibition of bumetanide metabolism in rodents on brain penetration and effects of bumetanide in chronic models of epilepsy. *The European journal of neuroscience*. 2014; 39(4):673–687. [PubMed: 24251546]
36. VanWert AL, Gionfriddo MR, Sweet DH. Organic anion transporters: discovery, pharmacology, regulation and roles in pathophysiology. *Biopharmaceutics & drug disposition*. 2010; 31(1):1–71. [PubMed: 19953504]
37. Regina A, Koman A, Piciotti M, El Hafny B, Center MS, Bergmann R, Couraud PO, Roux F. Mrp1 multidrug resistance-associated protein and P-glycoprotein expression in rat brain microvessel endothelial cells. *Journal of neurochemistry*. 1998; 71(2):705–715. [PubMed: 9681461]
38. Strazielle N, Belin MF, Ghersi-Egea JF. Choroid plexus controls brain availability of anti-HIV nucleoside analogs via pharmacologically inhibitable organic anion transporters. *AIDS*. 2003; 17(10):1473–1485. [PubMed: 12824785]
39. Robson MD, Tyler DJ, Neubauer S. Ultrashort TE chemical shift imaging (UTE-CSI). *Magnetic resonance in medicine : official journal of the Society of Magnetic Resonance in Medicine / Society of Magnetic Resonance in Medicine*. 2005; 53(2):267–274.
40. Gulaka PK, Rojas-Quijano F, Kovacs Z, Mason RP, Sherry AD, Kodibagkar VD. GdDO3NI, a nitroimidazole-based T1 MRI contrast agent for imaging tumor hypoxia in vivo. *Journal of biological inorganic chemistry : JBIC : a publication of the Society of Biological Inorganic Chemistry*. 2014; 19(2):271–279. [PubMed: 24281854]
41. Kizaka-Kondoh S, Konse-Nagasawa H. Significance of nitroimidazole compounds and hypoxia-inducible factor-1 for imaging tumor hypoxia. *Cancer science*. 2009; 100(8):1366–1373. [PubMed: 19459851]
42. Barth RF, Kaur B. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. *Journal of neuro-oncology*. 2009; 94(3):299–312. [PubMed: 19381449]
43. Doblaz S, He T, Saunders D, Hoyle J, Smith N, Pye Q, Lerner M, Jensen RL, Towner RA. In vivo characterization of several rodent glioma models by 1H MRS. *NMR in biomedicine*. 2012; 25(4):685–694. [PubMed: 21954105]
44. Tropres I, Pannetier N, Grand S, Lemasson B, Moisan A, Peoc'h M, Remy C, Barbier EL. Imaging the Microvessel Caliber and Density: Principles and Applications of Microvascular MRI. *Magnetic Resonance in Medicine*. 2015; 73(1):325–341. [PubMed: 25168292]

45. Moellering RE, Black KC, Krishnamurty C, Baggett BK, Stafford P, Rain M, Gatenby RA, Gillies RJ. Acid treatment of melanoma cells selects for invasive phenotypes. *Clinical & experimental metastasis*. 2008; 25(4):411–425. [PubMed: 18301995]
46. Rofstad EK, Mathiesen B, Kindem K, Galappathi K. Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. *Cancer research*. 2006; 66(13):6699–6707. [PubMed: 16818644]
47. Garcia-Martin ML, Herigault G, Remy C, Farion R, Ballesteros P, Coles JA, Cerdan S, Ziegler A. Mapping extracellular pH in rat brain gliomas in vivo by ¹H magnetic resonance spectroscopic imaging: comparison with maps of metabolites. *Cancer research*. 2001; 61(17):6524–6531. [PubMed: 11522650]
48. McVicar N, Li AX, Goncalves DF, Bellyou M, Meakin SO, Prado MAM, Bartha R. Quantitative tissue pH measurement during cerebral ischemia using amine and amide concentration-independent detection (AACID) with MRI. *J Cerebr Blood F Met*. 2014; 34(4):690–698.
49. McSheehy PM, Troy H, Kelland LR, Judson IR, Leach MO, Griffiths JR. Increased tumour extracellular pH induced by Bafilomycin A1 inhibits tumour growth and mitosis in vivo and alters 5-fluorouracil pharmacokinetics. *Eur J Cancer*. 2003; 39(4):532–540. [PubMed: 12751386]
50. Robey IF, Baggett BK, Kirkpatrick ND, Roe DJ, Dosescu J, Sloane BF, Hashim AI, Morse DL, Raghunand N, Gatenby RA, Gillies RJ. Bicarbonate increases tumor pH and inhibits spontaneous metastases. *Cancer research*. 2009; 69(6):2260–2268. [PubMed: 19276390]
51. Silva AS, Yunes JA, Gillies RJ, Gatenby RA. The potential role of systemic buffers in reducing intratumoral extracellular pH and acid-mediated invasion. *Cancer research*. 2009; 69(6):2677–2684. [PubMed: 19276380]

Figure 1. Biodistribution of TmDOTP⁵⁻ in the rat body

Summary of TmDOTP⁵⁻ biodistribution (mmol/kg) in rats that were infused with (A) 0.1 mmol/kg TmDOTP⁵⁻ and (B) 1 mmol/kg TmDOTP⁵⁻ in three experimental conditions: infusion of TmDOTP⁵⁻ alone, co-infusion of 100 mg/kg probenecid and TmDOTP⁵⁻, infusion of TmDOTP⁵⁻ in renal ligated rats. Representative spectra of H6 proton in TmDOTP⁵⁻ from the kidney (top) and cortex (bottom) samples of rats that were infused with (C) 0.1 mmol/kg TmDOTP⁵⁻ and (D) 1 mmol/kg TmDOTP⁵⁻. The weight of each tissue sample is indicated below the corresponding spectrum. The concentration of TmDOTP⁵⁻ in each sample was estimated by the intensity of each spectrum after reference and plasma corrections.

Figure 2. Representative MRI and BIRDS data from a rat bearing RG2 tumor during probenecid/TmDOTP⁵⁻ co-infusion

(A) T₂-weighted image shows the tumor localization due to agent extravasation (tumor = blue outline; brain = orange outline). (B) A slice from a 3D CSI data set shows varying TmDOTP⁵⁻ levels throughout the brain (same slice as in A). (C) Examples of TmDOTP⁵⁻ resonances which indicate that the pH_e (according to eq. 1) of an intratumoral voxel (pH_e = 6.64) is lower than that of a peritumoral voxel (pH_e = 7.27).

Figure 3. pH_e maps by BIRDS are independent of TmDOTP⁵⁻ concentration

(A) Resonance intensities of H6 in TmDOTP⁵⁻ for intratumoral (red boxes in pH_e maps in B and C) and peritumoral (black boxes in pH_e maps in B and C) voxels at various time points during co-infusion of TmDOTP⁵⁻ with probenecid. Quantitative pH_e maps (according to eq. 1) were obtained at different TmDOTP⁵⁻ concentrations indicated by star and triangles respectively at (B) 66 minutes and (C) 78 minutes after start of probenecid/TmDOTP⁵⁻ co-infusion. See Figure S3 for correlation of these pH_e maps.

Figure 4. Representative MRI and BIRDS data of rats bearing U87 and 9L tumors during probenecid/TmDOTP⁵⁻ co-infusion

Left column shows the T_2 -weighted images, which depict the localized (A) U87 and (B) 9L tumors, respectively, due to agent extravasation (tumor = blue outline; brain = orange outline). The middle column shows representative slice from 3D CSI data sets showing varying TmDOTP⁵⁻ levels throughout the brain of (A) U87 and (B) 9L tumors, respectively. The right column shows the pH_e maps (according to eq. 1) calculated from TmDOTP⁵⁻ resonances in (A) U87 and (B) 9L tumors, respectively. Figure 2 shows similar data for RG2 tumor.

Figure 5. Distributions of intratumoral and peritumoral pH_e values

Histograms representing the distribution of intratumoral and peritumoral pH_e measured by BIRDS for (A) 9L, (B) U87, and (C) RG2 tumors. The fraction of voxels with a given pH_e value is shown for intratumoral voxels (open bars) and peritumoral voxels (filled bars).

[R3.5] [R3.6] Most probable pH_e values and the full width at half maximum (FWHM) obtained from the fit of the normal (Gaussian) distribution of pH_e shown in Figure 5 for intratumoral and peritumoral regions in the 9L, U87 and RG2 tumors. See Fig. S4 for relation between tumor size and tumor pH_e .

Table 1

Region	9L		U87		RG2	
	pH_e	FWHM	pH_e	FWHM	pH_e	FWHM
Intratumoral pH_e	6.84 ± 0.01	0.24 ± 0.01	6.81 ± 0.02	0.37 ± 0.02	6.83 ± 0.02	0.17 ± 0.01
Peritumoral pH_e	7.32 ± 0.01	0.25 ± 0.01	7.34 ± 0.01	0.20 ± 0.01	7.22 ± 0.01	0.42 ± 0.03
Intratumoral – Peritumoral pH_e Gradient	0.48 ± 0.01	-	0.53 ± 0.02	-	0.39 ± 0.02	-
Tumor size (μL)	111.7 ± 37.7	-	68.1 ± 17.6	-	83.9 ± 35.7	-