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Spin-lock imaging of exogenous exchange-based contrast agents to assess tissue pH

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Abstract

Purpose: Some X-ray contrast agents contain exchangeable protons that give rise to exchange-based effects on MRI, including chemical exchange saturation transfer (CEST). However, CEST has poor specificity to explicit exchange parameters. Spin-lock sequences at high field are also sensitive to chemical exchange. Here, we evaluate whether spin-locking techniques can detect the contrast agent iohexol *in vivo* after intra-venous administration, and their potential for measuring changes in tissue pH.

Methods: Two metrics of contrast based on $R_{1\rho}$, the spin lattice relaxation rate in the rotating frame, were derived from the behavior of $R_{1\rho}$ at different locking fields. Solutions containing iohexol at different concentrations and pH were used to evaluate the ability of the two metrics to quantify exchange effects. Images were also acquired from rat brains bearing tumors before and after intravenous injections of iohexol to evaluate the potential of spin-lock techniques for detecting the agent and pH variations.

Results: The two metrics were found to depend separately on either agent concentration or pH. Spin-lock imaging may thus provide specific quantification of iohexol concentration and the iohexol-water exchange rate, which reports on pH.

Conclusion: Spin-lock techniques may be used to assess the dynamics of intravenous contrast agents and detect extracellular acidification.

Keywords

MRI; spin-lock; X-ray agent; extracellular pH

INTRODUCTION

Iohexol is an iodinated X-ray contrast agent commonly used in clinical CT imaging. It is a non-ionic agent of low osmolality and low chemotoxicity which has demonstrated a very high safety profile in clinical practice (1,2). Some iodinated contrast agents have also been shown to generate MRI contrast in appropriate conditions. Different from CT imaging which exploits the increased X-ray atomic absorption of iodine to enhance images, MRI images may be affected by chemical exchange effects between the exchangeable protons of the iodinated agents and water protons. Iodinated contrast agents were first used to enhance T₂-weighted images (3), and more recently were used to produce more specific contrast in chemical exchange saturation transfer (CEST) images by applying saturation pulses at the resonance frequencies of the exchangeable protons and detecting a subsequent decrease in the water signal (4–10). Suggested applications of CEST imaging of iodinated contrast agents include dynamic contrast-enhanced imaging of tumors (11) and assessment of tumor acidosis (6,10,11).

Iodinated contrast agents (e.g. Iohexol, Ioversol, Iodixanol, and Iomeprol) often contain both amide and hydroxyl exchanging groups. The amide groups of iodinated contrast agents have a resonance frequency offset at around 4.3 to 4.4 ppm from water, and their exchange rates in physiological conditions are in the intermediate exchange regime (4). Previous CEST imaging of iodinated contrast agents applied saturation on these amide exchanging groups. The hydroxyl groups of iodinated contrast agents have resonance frequency offsets at around 0.6 ppm from water, and their exchange rates are in the fast exchange regime (12). Neither the amide nor hydroxyl protons in such X-ray agents are in slow exchange with water, so that high irradiation powers are required to effectively saturate these groups, resulting in non-specific direct saturation and semi-solid magnetization transfer effects in tissues.

We have previously shown that spin-lock sequences at high field are also sensitive to chemical exchange, and can be more suitable for detecting intermediate and fast exchanging groups than CEST (12). In addition, $R_{1\rho}$, the spin-lock relaxation rate obtained by comparing signals at a fixed locking power but at different locking times, is well estimated by linearly adding individual exchange contributions (13), whereas CEST signals from different exchanging pools have mutual interactions (14). These characteristics make the spin-lock technique more suitable for quantifying exogenous contrast agents in complex biological tissues, where multiple exchanging pools are present. Moreover, the variation of $R_{1\rho}$ with locking power (the $R_{1\rho}$ dispersion) can be used to estimate specific exchange parameters and emphasize the effects of protons of a specific exchange rate (15).

In this paper, we demonstrate the ability of spin-lock imaging to detect iohexol in tumor bearing rat brains. Two different metrics of MR contrast based on $R_{1\rho}$ are shown to depend separately on iohexol concentration and iohexol-water exchange rate respectively, and were evaluated for assessing the effects of iohexol administration and extracellular acidification in tumors.

METHODS

Spin-lock sequence and $R_{1\rho}$ -based contrasts

A conventional spin-lock preparation cluster consists of a 90° excitation pulse, followed by a single rectangular locking pulse with duration of τ and a -90° excitation pulse ($90_x - \tau_y - 90_{-x}$). To compensate for B_0 and B_1 field inhomogeneities (16), the single locking pulse is usually separated into two equal duration pulses ($\tau/2$) by an 180° refocusing pulse, and then each $\tau/2$ pulse is separated into two equal duration pulses ($\tau/4$) with opposite phase ($90_x - \tau_y/4 - \tau_{-y}/4 - 180_y - \tau_y/4 - \tau_{-y}/4 - 90_{-x}$). Fig. 1 shows the sequence diagram of the spin-lock preparation cluster used in this paper.

$R_{1\rho}$ values are sensitive to slow molecular motions and exchange processes on a time-scale corresponding to the Larmor precession about the spin-locking pulse amplitude (17,18) and at higher fields e.g. 7T, the latter exchange phenomena dominate in many samples. Observed variations in $R_{1\rho}$ with increasing spin-locking amplitude ($R_{1\rho}$ dispersion) provide explicit information on the relevant time scales involved, and in general vary from a low power value close to R_2 down to an asymptotic value close to R_1 (12,13,19–23). Dipolar interactions are not expected to vary over the range of locking powers available, so changes in $R_{1\rho}$ are dominated by exchange processes. Previously, we have shown how judicious selection of locking powers and combinations of data acquired with different powers can isolate chemical exchange effects from non-chemical exchange related effects (13,21,23). Here we define an $R_{1\rho}$ -based contrast simply as,

$$\Delta R_{1\rho} = R_{1\rho}(\text{low}) - R_{1\rho}(\text{high}) \quad (1)$$

to quantify chemical exchange effects, where $R_{1\rho}(\text{low})$ and $R_{1\rho}(\text{high})$ are the $R_{1\rho}$ values acquired with low and high locking powers. We adopt the theoretical model of Chopra *et al.* (15) to fit an exchange rate-weighted parameter S_ρ ,

$$R_{1\rho} = (R_2 + \frac{R_{1\rho}^\infty \omega_1^2}{S_\rho^2}) / (1 + \frac{\omega_1^2}{S_\rho^2}) \quad (2)$$

where $R_{1\rho}$, R_2 , and $R_{1\rho}^\infty$ are regarded as parameters in the fitting of $R_{1\rho}$ vs the spin-lock power (ω_1). In realistic cases, $S_\rho^2 \approx k_{sw}^2 + \Delta\omega_s^2$, where k_{sw} is the chemical exchange rate of protons with resonance frequency ω_s .

To calculate the contributions to $R_{1\rho}$ and S_ρ of iohexol *in vivo*, a $R_{1\rho}$ dispersion difference was obtained in two steps: first, the $R_{1\rho}$ value obtained with high locking power was subtracted from the value at low locking power to separate chemical exchange effects from non-chemical exchange related effects, which produces $R_{1\rho}$; second, $R_{1\rho}$ values before injection were subtracted from $R_{1\rho}$ values after injection to separate the effects of the iohexol agent from other endogenous exchanging pools in biological tissues. Eq. (3) shows the definition of $R_{1\rho}$ difference,

$$\Delta R_{1\rho} \text{ difference} = (R_{1\rho} - R_{1\rho}(\text{high}))\Big|_{\text{after injection}} - (R_{1\rho} - R_{1\rho}(\text{high}))\Big|_{\text{base line}} \quad (3)$$

Here, we used $R_{1\rho}$ and S_ρ fitted from $R_{1\rho}$ dispersion data to measure differences between iohexol samples and to quantify the effects of iohexol *in vivo*.

Phantom preparation

A series of iohexol samples served as test solutions to evaluate the dependence of $R_{1\rho}$ and S_ρ on pH and concentration. Four samples were made with 30 mM iohexol in $1 \times$ phosphate buffered saline (PBS) buffer and pH was titrated to 6.6, 7.0, 7.2, and 7.4, respectively, using NaOH/HCl. Three samples were made with 15, 30, and 45 mM iohexol in PBS, respectively, and pH was titrated to 7.0. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animal preparation

To demonstrate proof of concept, four rats bearing 9L tumors were used in this study. Each rat was injected with 1×10^5 9L glioblastoma cells in the right brain hemisphere to induce tumors, and was then imaged after 2 to 3 weeks when the tumor reached appropriate size. All rats were immobilized and anesthetized with a 2%/98% isoflurane/oxygen mixture during imaging. Respiration was monitored to be stable, and a constant rectal temperature of 37°C was maintained throughout the experiments using a warm-air feedback system (SA Instruments, Stony Brook, NY). The rat jugular vein was catheterized for intravenous injection. 2 ml 1.3 M iohexol solution (in saline), according to a previous publication (4), was injected to each rat in 1–2 min. This corresponds for a 250 g rat to a dose of approximately 4g I/kg body weight. All animal experiments were approved by the Animal Care and Usage Committee at Vanderbilt University.

MRI

Spin-lock signals were acquired with ω_1 varying from 100 to 3162 Hz. Spin-locking times were 1, 200, 400, 600, 800, 1000 ms for phantom or 1, 25, 50, 75, 100 ms for animal experiments. For the studies of iohexol injections, spin-lock signals were continuously acquired over 10 minute intervals before, during and after injection.

To assess the exchange parameters of both amide and hydroxyl exchanging groups of iohexol, CEST measurements were performed by applying an 8 s continuous wave (CW) irradiation with power of 0.5, 1, 2, 3, 4, and 5 μ T on a series of iohexol samples with different pH. Z-spectra were acquired with RF offsets from –2400 Hz to 2400 Hz with steps of 50 Hz (6 ppm to –6 ppm at 9.4T). Control images were acquired with RF offsets of 100,000 Hz (250 ppm at 9.4T). Water longitudinal relaxation rate (R_{1w}) was obtained using an inversion recovery method.

All measurements were performed on a Varian DirectDrive™ horizontal 9.4T magnet with a 38-mm Litz RF coil (Doty Scientific Inc. Columbia, SC). Spin-lock images on animals were acquired using single-shot spin-echo echo planar imaging (SE-EPI) readout and a repetition

time (TR) of 2 s. All images were acquired with matrix size 64×64 , field of view $30 \text{ mm} \times 30 \text{ mm}$, slice thickness 2 mm, and 1 acquisition. Spin-lock and CEST data on phantoms were acquired with free induction decay (FID) acquisitions.

Data analysis

$R_{1\rho}$ values were calculated by fitting the signal variation with spin-locking time to a three-parameter mono-exponential decay function in MATLAB (The MathWorks, Inc. Natick, MA). $R_{1\rho}$ and $R_{1\rho}$ difference were calculated by comparing values at low power (100 Hz) and high power (3162 Hz). The $R_{1\rho}$ dispersion or $R_{1\rho}$ dispersion difference data were fit to the three-parameter model of Chopra *et al.* (15) (shown in Eq. (2)) to estimate S_ρ .

To improve signal to noise ratio (SNR) a Gaussian spatial filter was used with a size of 3×3 adjacent pixels and standard deviation 1 to smooth the spin-lock images before $R_{1\rho}$ fitting. In addition, before fitting S_ρ , six images of $R_{1\rho}$ difference acquired at different times after injection were averaged, and pixels with no significant concentration of agent ($R_{1\rho}$ difference acquired with ω_1 of $100 \text{ Hz} < \text{threshold}$) were omitted. A threshold of 0.3 s^{-1} , which is half of the mean value of the $R_{1\rho}$ difference acquired with ω_1 of 100 Hz from the four rats, was chosen.

An asymmetric analysis of the magnetization transfer ratio (MTR_{asym}) (24) of the CEST data was performed to quantify the CEST effects from amide and hydroxyl exchanging groups. The CEST Z-spectra acquired with different powers were used to fit the iohexol amide-water exchange rate ($k_{\text{sw_amide}}$), amide concentration (f_{amide}), hydroxyl-water exchange rate ($k_{\text{sw_hydroxyl}}$), and hydroxyl concentration (f_{hydroxyl}) using a three-pool exchange model (amide, hydroxyl, and water) based on the Bloch-McConnell equations and a non-linear optimization algorithm. Other model parameters included the frequency offsets for iohexol amide (ω_{amide}) and iohexol hydroxyl (ω_{hydroxyl}) which were set to 4.3 and 0.6 ppm respectively; the water longitudinal relaxation time ($T_{1w}=1/R_{1w}$) was measured to be around 4.0s for our samples at 9.4T; the water transverse relaxation time (T_{2w}), solute longitudinal relaxation time (T_{1s}), and solute transverse relaxation time (T_{2s}) were set to 1 s, 1s, and 10 ms, respectively.

Voxel by voxel fittings of S_ρ and CEST parameters were performed to achieve the lowest root mean square (RMS) of residuals between the data and the model. Table 1 and Table 2 list the starting points and boundaries of the fit of S_ρ and CEST parameters, respectively.

$R_{1\rho}$ difference values in the time course were obtained by averaging signals from all pixels within each tumor and the contralateral normal brain. Regions of interest (ROIs) of tumor and contralateral normal brain were manually drawn from R_{1w} images. The average of the three measured $R_{1\rho}$ values acquired before injection was used as baseline.

To derive pH values from the fitted data, we assumed that k_{sw} can be modeled by a base-catalyzed exchange equation. To calibrate the pH dependence of S_ρ , we used the following equation:

$$S_\rho = \sqrt{(k_b \cdot 10^{pH - pH_w})^2 + \Delta\omega_s^2} \quad (4)$$

in which k_b , pH_w , and ω_s are fitting parameters.

RESULTS

Determination of chemical exchange parameters of iohexol

Fig. 2a and 2b shows the CEST Z-spectra and MTR asymmetry values for a series of irradiation powers on iohexol samples with pH 7.0. Arrows in Fig. 2b indicate the amide and hydroxyl groups on the MTR data. Table 3 lists the estimated amide and hydroxyl concentrations and exchange rates obtained by fitting the CEST spectra to the Bloch-McConnell equations for samples with pH of 6.6, 7.0, 7.2, and 7.4. It was found that k_{sw_amide} is in a range from 260 s^{-1} to 1472 s^{-1} , and $k_{sw_hydroxyl}$ is in a range from 1428 s^{-1} to 5062 s^{-1} . Therefore, neither amide nor hydroxyl exchanging groups of the iohexol are in the slow exchange regime. It was also found that the concentration of exchanging hydroxyls is three times larger than that of amides, which is in agreement with the chemical structure of iohexol. Both the range of the exchange rates and the relatively larger fraction of fast exchanging hydroxyl group suggest that spin-lock sequence may be more suitable to detect iohexol than CEST.

$R_{1\rho}$ and S_ρ

Fig. 3 shows the $R_{1\rho}$ dispersion, $R_{1\rho}$, and S_ρ for the four iohexol samples for different pH but constant agent concentration (a, c, and e), and the three iohexol samples for different agent concentrations but constant pH (b, d, and f). Note that $R_{1\rho}$ is sensitive to concentration, but not pH. In contrast, S_ρ is sensitive to pH, but not concentration. Therefore, $R_{1\rho}$ and S_ρ can provide images sensitive specifically to iohexol concentration and pH respectively. The red line in (e) is the best fit of Eq (4), which is used for pH calibration in the animal studies. k_b , pH_w , and ω_s in Eq. (4) are fitted to be 0.98, 3.45, and 2977 s^{-1} , respectively.

Animal experiments

Fig. 4a shows the $R_{1\rho}$ dispersion from rat brain tumors before (blue line), at 10 mins after (red line), and at 60 mins after (green line) injecting iohexol. The $R_{1\rho}$ dispersion acquired before injection was used as baseline. We found that the $R_{1\rho}$ values acquired 10 mins after injecting iohexol increased compared with baseline values, indicating that $R_{1\rho}$ is sensitive to the chemical exchange effects of iohexol. However, the $R_{1\rho}$ values acquired at 60 mins after injection returned nearly to baseline. At high locking power, the $R_{1\rho}$ values become even lower than the baseline values. A previous report showed that $R_{1\rho}$ changes after injecting the contrast agent could arise from both the increased concentration of exchanging groups and a contribution from non-chemical exchange related effects (e.g. decreased relaxation rates due to an increase in tissue water and/or a shift of water between blood and extravascular tissue) (25). They also validated that these non-chemical exchange related effects are independent of locking power through experiments on administration of mannitol which cannot be transported across blood-brain barrier (BBB) and thus its chemical exchange effects have very small contributions to $R_{1\rho}$, but can induce osmolality. Therefore, the decreased $R_{1\rho}$ dispersion acquired at 60 mins compared with the baseline may be due to the non-chemical exchange related effects. This decreased $R_{1\rho}$ dispersion should be also independent of

locking power and thus can be removed using $R_{1\rho}$ or $R_{1\rho}$ difference. Fig. 4b shows the $R_{1\rho}$ dispersion difference from tumor at 10 mins and 60 mins after injecting iohexol. It was found that although the $R_{1\rho}$ difference decreases at 60 mins compared with that at 10 mins after injection, most of the $R_{1\rho}$ difference values are still positive. $R_{1\rho}$ difference should reflect only exchange effects from iohexol. Fitting of this $R_{1\rho}$ dispersion difference using Chopra's model can quantify the exchange parameters of the agent.

Fig. 5a shows the mean time course of the $R_{1\rho}$ difference from four rat brains (time course of each rat is shown in Supporting Figure S1). It was found that $R_{1\rho}$ difference signals in tumors increase quickly after injection and decrease slowly up to 1 h, whereas intact brain showed little change. Fig. 5b, 5c, and 5d shows maps of R_{1w} and $R_{1\rho}$ before and at 10 mins after injection from a representative rat brain, respectively. Fig. 5e shows the $R_{1\rho}$ difference map which is obtained by subtracting Fig. 5c and 5d. Note that the $R_{1\rho}$ difference contrast in tumor is significantly enhanced. S_p in tumors was fitted to be 4282 ± 786 , 3384 ± 941 , 4328 ± 1524 , and $4614 \pm 356 \text{ s}^{-1}$ for the four rats. By calibrating pH using Eq. (4), these S_p values correspond to an extracellular pH ranging from 6.6 to 7.0. Fig. 6 shows the pH maps from the four rat brains. The heterogeneous distribution of pH may represent the heterogeneity of acidosis in the tumors, which has also been previously observed (26).

DISCUSSION

Administration of contrast agents can be used to assess vascular characteristics in tumors. Measurements of extracellular pH are potentially of interest to characterize tumors and to assess the effects of different therapies. Iohexol is a small molecule which leaks rapidly into the extravascular extracellular space and is not metabolized. Thus, administration of iohexol can be used to assess tumor perfusion and extracellular pH by using imaging techniques that are separately sensitive to its concentration and exchange rate, respectively. However, separating the effects of agent concentration and exchange rate in CEST imaging is challenging. Previously, fitting CEST signals as a function of irradiation power has been used to determine exchange rate (27–30). However, different direct saturation and semi-solid magnetization transfer effects that arise from different irradiation powers cause difficulties in quantifying chemical exchange parameters. Ratiometric CEST approaches using exogenous agents with two exchangeable groups (6,10,31,32) or using two exogenous agents (33) have also been used to determine exchange rates. However, these methods impose stringent requirements on the selection of potential contrast agents. Here, we show that spin lock can be used to derive quantities such as $R_{1\rho}$ and S_p which depend separately on these two parameters, and thus have some advantages over conventional relaxation agents and CEST.

Fig. 3a shows that $R_{1\rho}$ values acquired at low power (e.g. 100 Hz) and high power (e.g. 3162 Hz) are roughly insensitive to pH. $R_{1\rho}$ values at high power have contributions mostly from spin-spin relaxation, and have smaller contributions from chemical exchange effects, and thus should be relatively insensitive to pH. The insensitivity of $R_{1\rho}$ values at low power to pH may derive from the presence of two exchanging groups in iohexol which have inverse pH dependences (see Supporting Figure S2–S4). The subtraction of the two $R_{1\rho}$ values at high and low powers, $R_{1\rho}$, can thus provide pH-independent but concentration-weighted

imaging. Fig. 3a also shows that $R_{1\rho}$ values at intermediate powers are very sensitive to pH. Previously, we showed that the inflection point of the dispersion curve can quantify exchange rate, and combinations of spin-lock signals or $R_{1\rho}$ values acquired with judicious selection of low, intermediate, and high locking powers can provide concentration-independent but exchange rate-weighted imaging (13,21,23). Here, to increase SNR, we fitted the whole dispersion curve to Chopra's model to quantify an exchange rate-weighted parameter S_p . In the fitting of S_p , although we used a single-pool fit to a two-pool (amide and hydroxyl) exchanging system, the value of S_p of iohexol should depend on the resonance frequencies and the exchange rates of the two exchanging groups, so the derived value represents a form of average.

Fig. 5a shows that the $R_{1\rho}$ difference values increase rapidly, reach a peak at around 10 mins after injection and then slowly decrease. This is consistent with previous CEST imaging of administered X-ray agents in breast tumors (4). Previous reports have shown that the average extracellular pH value in solid tumors is proximately 6.4 – 7.2 (34,35). Our calibrated pH of the four rats is in this range, indicating the ability of the spin-lock sequence for assessing tumor acidosis.

The spin-lock technique is sensitive to B_0 shift at low locking power (see simulations in Supporting Figure S5), which induces $R_{2\rho}$ effects and may cause the increase of $R_{1\rho}$ with locking power from 100 Hz to 526 Hz in Fig. 4a. However, the $R_{2\rho}$ effect should not vary after injection of contrast agent, and thus it can be cancelled with the use of $R_{1\rho}$ difference. It can be found in Fig. 4b that the $R_{1\rho}$ dispersion difference has no increase at low locking power. Therefore, the B_0 shift does not influence our results. The accuracy in fitting S_p during agent administration depends on the signal to noise ratio (SNR) and physiological noise. Here, to improve the fitting accuracy and remove physiological noise, we used an image processing protocol that applied a Gaussian filter to the spin-lock images, averaged all the images of $R_{1\rho}$ difference acquired at different times after injection, and omitted voxels with no significant concentration of agent. Future studies may use readout techniques with higher SNR efficiency. In this paper, we used control phantoms (iohexol in PBS) to calibrate *in vivo* pH. However, the exchange rate could be influenced by buffer concentration and constitution, and thus the calibrated pH may not be accurate. Compared with CEST imaging of X-ray agent administration, the spin-lock technique requires higher powers, and thus its specific absorption rate (SAR) is relatively high. However, inspection of the dispersion data suggests there is little benefit to using the highest locking fields. Moreover, judicial choice of the timings of the locking pulses can substantially reduce SAR and we have previously demonstrated the ability to fit human spin lock images to derive exchange rates at 3 T (36). SAR will doubtless limit some applications but translation to high field human studies may be feasible.

CONCLUSIONS

We show that spin lock imaging can be applied to study the effects of exogenous agents with exchanging groups, and exchange dependent contrast may have some advantages over conventional relaxation agents and CEST.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.
Diagram of spin-locking sequence.

FIG2. CEST Z-spectra (a) and MTR_{asym} spectra (b) with irradiation power of 0.5, 1, 2, 3, 4, and 5 μ T on iohexol samples with pH 7.0. Note the two exchanging groups (amide and hydroxyl) on the MTR spectra.

FIG. 3.

Measured $R_{1\rho}$ dispersion, $R_{1\rho}$, and S_ρ on the four iohexol samples with a variety of pH but constant agent concentration (a, c, and e) and the three iohexol samples with a variety of agent concentration but constant pH (b, d, and f), respectively. Dots in all subfigures are measured data, solid lines in (a and b) are the fitted curves using Chopra's model, and the red line in (e) is the best fit using Eq. (4).

FIG. 4.

$R_{1\rho}$ dispersion (a) and $R_{1\rho}$ dispersion difference (b) from rat brain tumors before (used as baseline) and at 10 mins and 60 mins after injecting iohexol agent. Error bars are the standard deviations across four subjects.

FIG. 5.

(a) mean time course of $R_{1\rho}$ difference from four rat brains, (b) R_{1w} map, (c) $R_{1\rho}$ map before injection, (d) $R_{1\rho}$ map at 10 mins after injection, and (e) $R_{1\rho}$ difference map. Error bars in (a) are the standard deviations across four subjects.

FIG. 6.
pH maps from the four tumor bearing rat brains. Dark area represents the omitted voxels which have no significant concentration of agent.

Table 1.

Starting points and boundaries of S_p , R_2 , and R_{Ip}^∞ .

	Start	Lower	Upper
S_p	5000	0	20000
R_2	0	0	10
R_{Ip}^∞	0	0	10

Table 2.

Starting points and boundaries of k_{sw_amide} , f_{amide} , $k_{sw_hydroxyl}$, and $f_{hydroxyl}$.

	Start	Lower	Upper
$k_{sw_amide} (s^{-1})$	500	0	2000
f_{amide}	0.001	0	1
$k_{sw_hydroxyl} (s^{-1})$	5000	0	20000
$f_{hydroxyl}$	0.003	0	1

Table 3.

Numerically determined k_{sw_amide} , f_{amide} , $k_{sw_hydroxyl}$, and $f_{hydroxyl}$ values from CEST acquisitions.

pH	6.6	7.0	7.2	7.4
$k_{sw_amide} (s^{-1})$	260	586	963	1472
f_{amide}	0.0004	0.0004	0.0004	0.0004
$k_{sw_hydroxyl} (s^{-1})$	1428	2521	3590	5062
$f_{hydroxyl}$	0.0013	0.0013	0.0013	0.0013