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Spin-lock imaging of early tissue pH changes in ischemic rat brain

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Abstract

We have previously reported that the dispersion of spin lattice relaxation rates in the rotating frame ($R_{1\rho}$) of tissue water protons at high field can be dominated by chemical exchange contributions. Ischemia in brain causes changes in tissue pH which in turn may affect proton exchange rates. Amide proton transfer (APT, a form of chemical exchange saturation transfer) has been shown to be sensitive to chemical exchange rates and able to detect pH changes non-invasively following ischemic stroke. However, the specificity of APT to pH changes is decreased because of the influence of several other factors that affect magnetization transfer. $R_{1\rho}$ is less influenced by such confounding factors and thus may be more specific for detecting variations in pH. Here, we applied a spin-locking sequence to detect ischemic stroke in animal models. Although $R_{1\rho}$ images acquired with a single spin-locking amplitude (ω_1) have previously been used to assess stroke, here we use $\Delta R_{1\rho}$, which is the difference in $R_{1\rho}$ values acquired with two different locking fields to emphasize selectively the contribution of chemical exchange effects. Numerical simulations with different exchange rates and measurements of tissue homogenates with different pH were performed to evaluate the specificity of $\Delta R_{1\rho}$ to detect tissue acidosis. Spin-lock and APT data were acquired on five rat brains after ischemic strokes induced via middle cerebral artery occlusions (MCAO). Correlations between these data were analyzed at different time points after the onset of stroke. The results show that $\Delta R_{1\rho}$, (but not $R_{1\rho}$ acquired with a single ω_1) was significantly correlated with APT metrics consistent with $\Delta R_{1\rho}$ varying with pH.

Graphical Abstract

$\Delta R_{1\rho}$, the difference of $R_{1\rho}$ acquired with low and high locking amplitudes, can emphasize selectively the contribution of chemical exchange effects, and thus provide pH-weighted imaging.

Experiments on MCAO induced cerebral ischemia in rats indicate that $R_{1\rho}$ has strong correlation with APT, but shows better image quality.

$\Delta R_{1\rho}$ (s^{-1}), 0.5 – 1 h after stroke

Keywords

Relaxometry; Chemical exchange saturation transfer; Brain ischemia and degeneration; Spin-lock

INTRODUCTION

During ischemia, anaerobic respiration leads to the production of lactate which usually causes tissue acidosis. Imaging of such tissue pH changes would be valuable for the diagnosis, staging, and monitoring of ischemic strokes. For example, pH sensitive imaging has been applied to identify the salvageable penumbra after ischemic events in the brain (1–4). Development of non-invasive and higher sensitivity pH imaging methods could significantly enhance the clinical role of MRI in stroke studies.

Magnetic resonance spectroscopy (MRS) has been used to assess tissue pH during stroke (5). However, due to its low spatial and temporal resolutions, its practical applicability is limited. The chemical exchange rate between solute protons and water protons also depends on physico-chemical factors such as pH and temperature. Imaging methods that produce parametric maps that are sensitive to chemical exchange could provide alternative means to assess variations in tissue pH. Previously, amide proton transfer (APT), a variation of chemical exchange saturation transfer (CEST) from protein and peptide amides, has been shown to be sensitive to tissue pH (6) and has been applied to assess tissue acidosis following ischemic stroke. However, CEST approaches such as APT (7) and amine-water exchange effects (8,9) are not specific solely for chemical exchange but are inherently sensitive to changes in water relaxation time (T_{1w}), which also starts to increase at later time points after ischemia (10,11).

Recently, we reported that chemical exchange effects from fast exchanging pools may provide dominant contributions to the spin-lattice relaxation rate in the rotating frame, $R_{1\rho}$, at high fields (12). Judicious selection of spin locking powers, and combinations of data

acquired with different powers, can isolate the contribution of chemical exchange effects from those of non-chemical exchange related effects (12–14). Moreover, we have confirmed that spin-locking techniques can be made sensitive to changes in pH (13,15), yet remain relatively insensitive to other tissue parameters (16), and thus may provide relatively specific pH imaging. Here, we applied spin-lock sequences to monitor tissue acidosis in an animal stroke model.

MATERIALS AND METHODS

Contributions to $R_{1\rho}$

In biological tissues, $R_{1\rho}$ is the superposition of several terms (14),

$$R_{1\rho} \approx R_{1\rho}^{Diff} + R_{1\rho}^{Ex} + R_{2w} \quad (1)$$

where $R_{1\rho}^{Diff}$ and $R_{1\rho}^{Ex}$ are the relaxation rates due to water diffusion in the presence of gradients induced by intrinsic susceptibility variations and chemical exchange effects, respectively. R_{2w} is the tissue water intrinsic transverse relaxation rate which is due mainly to dipolar relaxation effects. Here, we call R_{2w} in Eq. (1) the intrinsic R_{2w} to distinguish it from the apparent R_{2w} that is measured from multiple-echo sequences. Previous studies have shown that $R_{1\rho}^{Diff}$ contributes at very low spin-locking amplitudes ($\omega_1 < 100$ Hz) whereas $R_{1\rho}^{Ex}$ is significant up to relatively high ω_1 (several hundreds or thousands Hertz), but disperses as the locking field increases (12,14,17,18). In contrast, the intrinsic R_{2w} is largely independent of ω_1 (19). These different dependencies on ω_1 provide a means to separate contributions of chemical exchange from those of other mechanisms. Fig. 1 gives an illustration based on a previous publication (18) to show the behavior of $R_{1\rho}^{Diff}$, $R_{1\rho}^{Ex}$, and R_{2w} as a function of locking power.

Quantification of spin-locking and APT signals

A complete characterization of the dispersion of $R_{1\rho}$, from which explicit exchange parameters may be extracted (12,14), would require measurements at multiple locking fields, but simpler approaches are more practical yet capture the essential features required to assess pH (13,15). We used the difference of $R_{1\rho}$ at high and moderately low locking powers to quantify the contribution of chemical exchange in spin-lock imaging.

$$\Delta R_{1\rho} \approx R_{1\rho}(low) - R_{1\rho}(high) \quad (2)$$

A low value of $\omega_1 = 316$ Hz and high value of $\omega_1 = 5620$ Hz were chosen to effectively remove the diffusion and non-chemical exchange related effects. To optimize the high power so that the spin-locking techniques are more sensitive to pH, we defined a metric,

$$\Delta R_{1\rho}(\omega_1) \text{ difference} = (R_{1\rho}(316 \text{ Hz}) - R_{1\rho}(\omega_1))|_{\text{lesion}} - (R_{1\rho}(316 \text{ Hz}) - R_{1\rho}(\omega_1))|_{\text{normal}} \quad (3)$$

APT was quantified by a conventional asymmetric analysis (MTR_{asym}) as well as one normalized by T_{1w} , which has been shown to be relatively more specific to chemical exchange effects than the conventional quantity MTR_{asym} (20).

$$\text{MTR}_{\text{asym}} = \left(\frac{S_- - S_+}{S_0} \right) \quad (4)$$

$$\text{Normalized MTR}_{\text{asym}} = \left(\frac{S_- - S_+}{S_0} \right) / T_{1w} \quad (5)$$

where S_+ and S_- are the labelled signals (acquired with RF irradiation on the solute protons) and the reference signal (acquired with the opposite frequency offset) respectively; S_0 is the control signal acquired with no RF irradiation or with RF irradiation very far from the solute resonance frequency.

Sample and animal preparation

One rat was anesthetized with 5% isoflurane (ISO) and euthanized by decapitation. The brain was taken from the freshly sacrificed rat immediately. The intact tissues were washed quickly in phosphate buffered saline (PBS) to remove residual blood. Then after addition of 4 x PBS (w/w), the tissues were homogenized with a motor-driven blade-type homogenizer (Brinkmann Polytron PT3000, Kinematica AG) at max speed ~23000 rpm for 2×30 s bursts. The homogenates were divided into two aliquots and pH was titrated to 7.0 and 6.6 at room temperature. The pHs of the two samples were measured with a pH meter before and after MRI measurements to ensure they were stable during the experiments. MRI measurements on the samples were performed as described below at 37 °C.

Five animals were subjected to middle cerebral artery occlusions (MCAO) as described. Anesthesia was induced with 4 % ISO in an induction chamber and maintained at 2–2.5 % during surgery. Lubricant ophthalmic ointment was applied to both eyes prior to surgery and a rectal temperature probe was used to maintain the animals core temperature. Depth of anesthesia was monitored using the paw pinch technique. Under an operating microscope, the left common carotid artery was carefully separated and isolated from the vagus nerve. A 30 mm, silicon-coated, 4-0 nylon suture (Docol Corporation, Redlands, CA, USA) was routed into the left internal carotid artery and advanced until it occluded the MCA at a depth of 18–20 mm. A suture was tightened around the filament and the incision closed. The surgery took roughly 7 mins. Immediately after surgery, the rats were taken from the surgery room to the MRI scanner. The rats were secured on a custom-built holder using adhesive tape and a bite bar. A breathing sensor (SA instruments Inc., NY, USA) was placed under the

ventral surface of the rat body. Breathing rate was monitored throughout the experiments. Animals were anesthetized with 2–3 % ISO for induction and 2 % for maintenance. A warm-air feedback system was used to ensure that the rat rectal temperature was maintained at approximately 37 °C throughout the experiments. At around 30 mins after surgery, the first MRI acquisitions were performed. All procedures were reviewed and approved by the Vanderbilt University Institutional Animal Care and Use Committee.

MRI

Spin-lock experiments on tissue homogenates and spin-lock and APT images of rats *in vivo* were performed using a horizontal 7T Varian small animal magnet. The spin-lock preparation cluster has an initial 90° flip, a pair of on-resonance locking pulses applied along the direction of the transverse magnetization with selectable amplitudes and durations and separated by a 180° refocusing pulse, followed by a –90° flip back to the z direction. $R_{1\rho}$ dispersion curves were acquired with ω_1 from 316 Hz to 5620 Hz. Spin-locking time (SLT) was varied as 1, 25, 50, 75, 100 ms and TR was 4 s. Spin-locking signals were acquired with varied ω_1 for each SLT to reduce the potential for RF heating effects at high locking fields. The total acquisition time was roughly 4 mins to acquire data to derive each $R_{1\rho}$ dispersion and roughly 80 s to acquire spin-locking signals with two locking powers to calculate only $R_{1\rho}$.

The APT sequence contained a selective off-resonance rectangular irradiation pulse with amplitude of 1 μ T, duration of 5 s and TR of 7 s. S_+ , S_- , and S_0 were acquired with RF irradiation frequency offsets at 1050 Hz, –1050 Hz, and 100,000 Hz, respectively (corresponding to 3.5 ppm, –3.5 ppm, and 333.3 ppm at 7 T). The total acquisition time for acquiring CEST signals to calculate MTR_{asym} was roughly 42 s, which is a half of the 80 s taken for acquiring spin-locking signals to calculate only $R_{1\rho}$.

T_{1w} ($1/R_{1w}$) was obtained using a selective inversion recovery (SIR) method (21). Apparent R_{2w} was obtained using five echo times of 30, 50, 70, 90, and 110 ms. Apparent diffusion coefficient (ADC) was also measured using a pulse gradient spin echo sequence with gradients applied simultaneously on three axes (gradient duration = 6 ms, separation = 12 ms, five b-values between 0 and 1000 s/mm²).

All images were acquired using single-shot spin-echo Echo Planar Imaging (EPI) readouts with triple references for phase correction and with matrix size 64 × 64, field of view 30 mm × 30 mm, and one acquisition. Before data acquisition, shimming was carefully performed so that the root mean square (RMS) deviation of B_0 was less than 5 Hz. All images were acquired in 30 mins. Baseline and later images were acquired at 1–3 days before and at 0.5–1 h, 1–1.5 h, 1.5–2 h, and 2–2.5 h after the MCAO surgery.

Numerical simulation

Simulations were performed of the expected behavior of a two-pool (solute and water) model using coupled Bloch equations (16). Simulation parameters included: solute longitudinal and transverse relaxation times (1.5 s and 15 ms); water longitudinal and transverse relaxation times (1.5 s and 60 ms); solute concentration and resonance frequency offset (0.005 and 3 ppm). The solute-water exchange rates were set to be 10 kHz and 5 kHz

to mimic normal and acidic tissues respectively. Sequence parameters (e.g. ω_1 , SLT, and B_0) were the same as those used in the MRI experiments. The spin-locking signals were obtained numerically by integrating the appropriate differential equations through the sequence using the ordinary differential equation solver (ODE45) in Matlab 2013b (Mathworks, Natick, MA, USA).

Data analysis

All data analyses were performed using Matlab R2013b. Spin-locking and APT images were smoothed by a 3×3 median filter before data processing. $R_{1\rho}$ was obtained by fitting the spin-locking data with a mono-exponential function voxel by voxel. The standard deviations and mean values of $R_{1\rho}$ and ADC from the normal tissues in the right hemispheres were measured. Regions of interests (ROIs) in baseline maps were manually drawn on $R_{1\rho}$ maps. ROI within the stroke in each rat obtained at 0.5–1 h were selected in the left hemisphere with $R_{1\rho}$ values more than one standard deviation of those from normal tissues. ROIs of contralateral normal tissue were chosen to mirror the stroke ROIs. These ROIs were also used in other maps acquired at different time point after stroke. Data from those ROIs were used for statistical analysis. Student's t-tests were employed to evaluate differences in parameters, which were considered to be statistically significant when $P < 0.05$. To compare the lesion boundary between $R_{1\rho}$ map and ADC map, we also drew the lesion boundary on ADC map at 0.5–1 h with ADC values more than one standard deviation of those from normal tissues.

RESULTS

Tissue homogenates

We used numerical simulations with different exchange rates and diluted tissue homogenates with different pH to evaluate the dependence of the spin-locking technique on pH. Fig. 2a and 2c show the simulation results and Figs. 2b and 2d show the measured $R_{1\rho}$ dispersion curves for the homogenates. $R_{1\rho}$ dispersion is clearly influenced by exchange rate or pH; the $R_{1\rho}$ value at low $\omega_1 = 316$ Hz ($R_{1\rho}(316 \text{ Hz})$) increases after acidification, whereas the $R_{1\rho}$ value at higher ω_1 (e.g. 5620 Hz) decreases after acidification (with a not quite significant p value 0.09 in Fig. 2c). This result is in agreement with a previous report of the effects of pH on exchange rates in the intermediate to fast exchange regime (22) and consistent with the theoretical expression of Chopra et al. (23) discussed below. Fig. 2b and 2d also show that $R_{1\rho}$ values increased with decreasing exchange rate or pH.

Animal experiments

Fig. 3 shows the $R_{1\rho}$ dispersion curves, $R_{1\rho}(\omega_1)$ difference, $R_{1\rho}$, $R_{1\rho}(316 \text{ Hz})$, $R_{1\rho}(5620 \text{ Hz})$, MTR_{asym} , normalized MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC on five rat brains before and at different time points after onset of stroke. Fig. 3a shows that the $R_{1\rho}$ dispersion curves from the lesion ROIs changed significantly at 0.5–1 h after onset of stroke. In particular $R_{1\rho}$ decreases at higher locking fields, corresponding to a shift in the inflexion point of the dispersion consistent with a decrease in the exchange rate or a shift to lower pH. Fig. 3c shows that $R_{1\rho}$ values increased significantly consistent with the simulations for relatively fast exchanging protons and effects of decreasing pH in tissue homogenates in Fig. 2. Fig.

3b shows that the optimal high power to make the spin-locking techniques more sensitive to pH is in a range from 1778 Hz to 5620 Hz (note the dip in the curve at 1778 is interpreted as an artifact). $R_{1\rho}(5620 \text{ Hz})$, MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC change significantly after onset of stroke, but changes in $R_{1\rho}(316 \text{ Hz})$ and the normalized MTR_{asym} did not reach significance, though the latter showed similar trends to MTR_{asym} . The significant variation of R_{1w} in lesion after onset of stroke is in agreement with previous reports (8,24–26), which suggests the necessity to use the normalized MTR_{asym} to increase the specificity of CEST detection of exchange effect for our experimental settings (see following discussions). In addition, slightly elevated R_{1w} in contralateral normal tissue can be also observed. This can be also found in a previous publication (10).

We quantified the correlations between the three spin-locking parameters ($R_{1\rho}$, $R_{1\rho}(316 \text{ Hz})$, and $R_{1\rho}(5620 \text{ Hz})$) and both MTR_{asym} metrics from the ischemic regions at different time points after onset of stroke, and results are shown in Fig. 4. Significant correlations with the normalized MTR_{asym} were found for $R_{1\rho}$ (Fig. 4a, $p=0.0007$), but not for $R_{1\rho}(316 \text{ Hz})$ (Fig. 4b, $p=0.0882$) or $R_{1\rho}(5620 \text{ Hz})$ (Fig. 4c, $p=0.9804$), confirming that $R_{1\rho}$, but not $R_{1\rho}$ acquired with a single ω_1 , is sensitive to the same parameters as the exchange measured by APT. We also correlated the spin-lock parameters with MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC. Significant correlations were found between $R_{1\rho}$ and R_{1w} (Fig. 4g, $p=0.0002$); between $R_{1\rho}(316 \text{ Hz})$ and R_{1w} , apparent R_{2w} , and ADC (Fig. 4h, 0.0020; Fig. 4k, 0.0019; and Fig. 4n, 0.0053, respectively), and between $R_{1\rho}(5620 \text{ Hz})$ and apparent R_{2w} and ADC (Fig. 4l, $p=0.0019$ and Fig. 4o, $p=0.0000$, respectively).

Figures 5 shows parametric images of $R_{1\rho}$, normalized MTR_{asym} , MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC, respectively, from a representative rat brain before and at different time points after onset of stroke. Parametric images from other rats are shown in Sup. Fig. S1–S4. The fitting of $R_{1\rho}$ is good which can be seen from the mono-exponential fitting of spin-locking signals in Sup. Fig. S5. It can be seen that lesion regions detected by $R_{1\rho}$ are larger than seen in ADC images. This spatial mismatch suggests that the pH-weighted imaging quantified by $R_{1\rho}$ may be potentially used in detecting the ischemic penumbra (2).

DISCUSSION

This study reports the application of spin-locking techniques at high field for the early characterization of ischemic stroke. Through subtraction of $R_{1\rho}$ values acquired at two selected ω_1 , the contribution of chemical exchange effects can be isolated from those of other non-chemical exchange related effects. Chopra et al. (23) have provided a theoretical expression for the contribution of chemical exchange to $R_{1\rho}$. The relevant exchange-

dependent term depends directly on the ratio $\frac{k\Delta\omega_0^2}{k^2 + \Delta\omega_0^2 + \omega_1^2}$, where k is the proton exchange rate and ω_0 is the chemical shift of the exchanging species relative to water. At high locking fields, this increases with k or pH, whereas at low locking fields and high exchange rates (high pH) it can decrease with k . This behavior was seen in tissue homogenates and areas of stroke. Moreover, $R_{1\rho}$ is then expected to increase as k or pH decrease. The precise behavior will depend on the relative values of k and ω_0 . From the dispersion curves of the homogenates, the decrease in $R_{1\rho}$ from its low field value to the high locking field

value reaches halfway at a locking field of ≈ 1800 Hz, suggesting the mean exchange rate of the protons ≈ 11 kHz. This would be typical of hydroxyls and justifies the choice of parameters in the simulation.

Based on the definition of MTR_{asym} (27) and $R_{1\rho}$ (23), the normalized MTR_{asym} under weak saturation pulse approximation (no direct water saturation (DS) effect) mainly depends on exchange effect, and the $R_{1\rho}$ mainly depends on exchange and R_{2w} . At 9.4 T and with relatively low irradiation power, the DS effect is weak and thus our CEST experiments roughly obey the weak saturation pulse approximation. To evaluate the relative contributions of exchange and R_{2w} , we examined correlations between the three spin-locking parameters and the normalized MTR_{asym} . Significant correlations between $R_{1\rho}$ and the normalized MTR_{asym} in Fig. 4a suggests that $R_{1\rho}$ and APT are sensitive to similar factors affecting exchange rates, which in this case are likely changes in tissue pH. In Fig. 3, both $R_{1\rho}$ and the normalized MTR_{asym} from areas of stroke change at 0.5–1 h, and then return slowly toward the values of contralateral normal tissues, which differs from the behaviors of R_{1w} and $R_{1\rho}(5620 \text{ Hz})$ which have larger differences between lesions and contralateral normal tissues at longer time after stroke. This further suggests that $R_{1\rho}$ may reflect the same process as the normalized MTR_{asym} . There is no significant difference between lesion regions and contralateral normal tissues for the normalized MTR_{asym} in Fig. 3g, which may be due to the T_{1w} normalization, which enhances specificity to exchange.

Although spin-locking has been previously applied for detecting ischemic stroke (28–31), most of these studies acquired $R_{1\rho}$ values at only a single locking field ω_1 , but not $R_{1\rho}$. Our studies indicate that $R_{1\rho}$ values acquired with a single ω_1 do not correlate well with the normalized MTR_{asym} and by themselves cannot isolate the effects of exchange or pH. $R_{1\rho}$ values at any single locking field reflect exchange and dipolar contributions and also scale with the amount of solute within the tissues. After ischemia there are often changes in tissue water caused by edema and/or cellular swelling. The correlations of $R_{1\rho}$ at low and high frequencies with R_{2w} suggest water content did change. Subtraction of $R_{1\rho}$ values from different regions of the dispersion curve will emphasize contributions from exchange that are more specific to pH, but still reflect total tissue composition. Normalization of $R_{1\rho}$ by e.g. the mean $R_{1\rho}$ or some other combination may remove the influence of changes in water content as described in our earlier works (13,14).

Recently, Sun *et.al.* evaluated the pH-dependence of APT via correlation between the normalized MTR_{asym} and MRS measurements of tissue acidosis (20). Here we performed correlations between spin-locking parameters and the normalized MTR_{asym} to compare their changes over time. The method used to measure MTR_{asym} can be influenced by relayed nuclear overhauser enhancement (rNOE), MT, and MT asymmetry effects (32,33). As a result, the normalized MTR_{asym} images are inhomogeneous and their values are negative in both lesions and contralateral normal tissues. However, rNOE and MT are relatively stable in the acute stage of stroke at sufficiently high irradiation power and long irradiation time, where the dipolar effects dominate the exchange effects (10,34), so the normalized MTR_{asym} is less influenced by potential changes in rNOE and MT due to tissue acidification. Although other quantification methods have been suggested for increasing the specificity of APT imaging, they either require longer acquisition times or reduce the sensitivity. For

example, the multiple-pool Lorentzian fit of CEST Z-spectra requires the acquisition of complete Z-spectra at multiple frequencies, which lengthens the total acquisition time and is not appropriate for some practical applications (11,35–40). In addition, some protons, e.g. fast exchanging amine protons, do not produce Lorentzian lineshapes and their effects coalesce with the water peak, which causes errors in multiple-pool Lorentzian fitting (33). Specially designed pulsed saturation schemes e.g. chemical exchange rotation transfer (CERT) (32,41) and variable-delayed multi-pulse saturation (VDMP) (42,43), usually do not need to acquire whole Z-spectra, but they increase specificity at the expense of sensitivity. Here, we performed a 5 s irradiation with power of 1 μ T to obtain $\sim 1.5\%$ MTR_{asym} contrast between the lesion and the contralateral normal tissue. In other studies with different sequence parameters (2,6,8,34,44), the MTR_{asym} contrast between the lesion and the contralateral normal tissue was usually higher. However, optimizing APT sequence parameters *in vivo* is challenging due to overlapping signals from other exchanging pools. For example, the irradiation power may be limited by nearby amines which are more significant at higher powers and contaminate APT signals (33). The inhomogeneous $R_{1\rho}$ and the normalized MTR_{asym} signals in some rat brains in baseline measurements may be caused by inhomogeneous B_1 fields to which both methods are sensitive.

Our results disagree with a previous study (45) which found that MTR_{asym} is strongly correlated with MRS-measured pH values in acute stroke models. This discrepancy may be due to the different experimental settings. A recent study indicates that MTR_{asym} is sensitive to T_{1w} with lower irradiation powers, but is roughly insensitive to T_{1w} with higher irradiation powers (46). Longer T_{1w} causes greater MTR_{asym} , but also causes greater DS which decreases MTR_{asym} (47). Therefore, at higher irradiation powers, MTR_{asym} should have reduced dependency on T_{1w} . In the previous study (45) at 4.7 T and with short-pulse irradiation (6.6 ms) and thus broad bandwidth, MTR_{asym} may be affected less by variations in T_{1w} and thus correlated well with pH. By contrast, in our study at 7 T and in Sun et.al.'s study (20) the longer irradiation pulse and lower power used had less DS effect, so MTR_{asym} then depends more on T_{1w} , reducing its correlation with pH. After normalizing for T_{1w} , the normalized MTR_{asym} showed significant correlation with pH. The previous study (45) also showed that $R_{1\rho}$ acquired with ω_1 of 2250 Hz correlated with MRS measured pH. At this locking power, $R_{1\rho}$ has contributions from both the apparent R_{2w} and chemical exchange, based on our simulation in Fig. 2a, and thus should depend on pH. However, in principle, a stronger correlation with pH is achieved by removing the apparent R_{2w} .

$R_{1\rho}$ is sensitive to fast exchanging pools such as glutamate, glucose, and proteins. During ischemia, the content of these molecules may change which may confound the interpretation of spin lock imaging. Although the mean exchange rate can be derived from the $R_{1\rho}$ dispersion, a simple two-pool model may not be appropriate for use in complex biological tissues. Another drawback of $R_{1\rho}$ is that it requires high ω_1 and thus its specific absorption rate (SAR) is relatively high. In addition, the exchange contribution to $R_{1\rho}$ decreases at lower fields as used in clinical scanners (12), so spin-locking techniques for detecting pH changes may be limited to preclinical applications.

CONCLUSION

We show that $R_{1\rho}$ can be used to provide pH-weighted imaging, and can be applied for characterizing acute ischemic stroke in rat brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

$R_{1\rho}$	spin-lattice relaxation rate in the rotating frame
APT	amide proton transfer
CEST	chemical exchange saturation transfer
$R_{1\rho}^{Diff}$	spin-lattice relaxation rate in the rotating frame due to diffusion through susceptibility gradients
$R_{1\rho}^{Ex}$	spin-lattice relaxation rate in the rotating frame due to chemical exchange effect
MCAO	middle cerebral artery occlusion
SIR	selective inversion recovery
ADC	apparent diffusion coefficient
EPI	echo planar imaging
ROI	regions of interest
MTR_{asym}	magnetization transfer ratio with asymmetric analysis
rNOE	relayed nuclear overhauser enhancement
CERT	chemical exchange rotation transfer
VDMP	variable-delayed multi-pulse saturation
SAR	specific absorption rate
SLT	spin-locking time

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FIG. 1.

Illustration to show the behavior of $R_{1\rho}^{Diff}$, $R_{1\rho}^{Ex}$, and R_{2w} as a function of locking power.

FIG. 2.

$R_{1\rho}$ dispersion (a and c) and $\Delta R_{1\rho}$ (b and d) from numerical simulations with different k_{sw} (a and b) from measurements of tissue homogenates (diluted with 4 x PBS (w/w)) with different pH (c and d). Error bars in (c and d) represent the standard deviations of 5 measurements on one sample (* $p < 0.05$).

FIG. 3.

$R_{1\rho}$ dispersion (a), $R_{1\rho}(\omega_1)$ difference (b), $R_{1\rho}$ (c), $R_{1\rho}(316 \text{ Hz})$ (d), $R_{1\rho}(5620 \text{ Hz})$ (e), MTR_{asym} (f), normalized MTR_{asym} (g), R_{1w} (h), apparent R_{2w} (i), and ADC (j) from stroke lesions (red) and contralateral normal tissues (blue). Error bars represent the standard deviations across subjects (* p < 0.05).

Fig 4.

Summarized correlations of three spin-locking parameters ($R_{1\rho}$, $R_{1\rho}$ (316 Hz), and $R_{1\rho}$ (5620 Hz)) with five conventional MRI parameters (normalized MTR_{asym} , MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC). The circles represent the mean values of each ROI from stroke lesion acquired at 0.5–1 h (red), 1–1.5 h (blue), 1.5–2 h (green), and 2–2.5 h (magenta) after onset of stroke. The Spearman's rank correlation coefficient (r) and p value of the correlation are provided. The solid line represents the linear regression of all data points.

Fig. 5.

Maps of $R_{1\rho}$, normalized MTR_{asym} , R_{1w} , apparent R_{2w} , and ADC from one representative rat brain before and at different time point after onset of stroke. ROIs of lesion and contralateral normal tissue are shown in $R_{1\rho}$ maps.