Published in final edited form as:

NMR Biomed. 2017 July ; 30(7): . doi:10.1002/nbm.3715.

CEST imaging of fast exchanging amine pools with corrections for competing effects at 9.4 T

Xiao-Yong Zhang^{1,2}, Feng Wang^{1,2}, Hua Li^{1,2}, Junzhong Xu^{1,2,3,4}, Daniel F. Gochberg^{1,2,3}, John C. Gore^{1,2,3,4,5}, and Zhongliang Zu^{1,2}

¹Vanderbilt University Institute of Imaging Science, Vanderbilt University, Nashville, Tennessee, USA

²Department of Radiology and Radiological Sciences, Vanderbilt University, Nashville, Tennessee, USA

³Department of Physics and Astronomy, Vanderbilt University, Nashville, Tennessee, USA

⁴Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, USA

⁵Molecular Physiology and Biophysics, Vanderbilt University, Nashville, Tennessee, USA

Abstract

Chemical exchange saturation transfer (CEST) imaging of fast exchanging amine protons at 3 ppm offset from the water resonant frequency is of practical interest but quantification of fast exchanging pools by CEST is challenging. To effectively saturate fast exchanging protons, high irradiation powers need to be applied, but these may cause significant direct water saturation (DS) as well as non-specific semi-solid magnetization transfer (MT) effects and thus decrease the specificity of the measured signal. In addition, the CEST signal may depend on the water longitudinal relaxation time (T_{1w}), which likely varies between tissues and with pathology, further reducing specificity. Previously, an analysis of the asymmetry of saturation effects (MTR_{asym}) has been commonly used to quantify fast exchanging amine CEST signals. However, our results show that MTR_{asym} is greatly affected by the above factors, as well as asymmetric MT and nuclear Overhauser enhancement (NOE) effects. Here, we instead applied a relatively more specific inverse analysis method, named AREX that has previously been applied only to slow and intermediate exchanging solutes. Numerical simulations and controlled phantom experiments show that although MTR_{asym} depends on T_{1w} and semi-solid content, AREX acquired in steady state does not, which suggests that AREX is more specific than MTR_{asym} . By combining with a fitting approach instead of using the asymmetric analysis to obtain reference signals, AREX can also avoid contaminations from asymmetric MT and NOE effects. Animal experiments show that these two quantification methods produce differing contrasts between tumors and contralateral normal tissues in rat brain tumor models, suggesting that conventional MTR_{asym} applied *in vivo* may be influenced by variations in T_{1w} , semi-solid content, or NOE effect. Thus, the use of MTR_{asym} may lead to misinterpretation, while AREX with corrections for competing effects likely enhances the specificity and accuracy of quantification to fast exchanging pools.

Graphical abstract

CEST imaging of fast exchanging amine protons at 3 ppm offset from the water resonant frequency is of practical interest but quantification of fast exchanging pools by CEST is challenging. In the present work, we evaluated the specificity of conventional MTR_{asym} and an AREX metric. Our results show that MTR_{asym} may lead to misinterpretation, while AREX with corrections for competing effects likely enhances the specificity of quantification to fast exchanging pools.

Keywords

Chemical exchange saturation transfer (CEST); fast exchanging pools; apparent exchange-dependent relaxation with asymmetric analysis (AREX); magnetization transfer ratio with asymmetric analysis (MTR_{asym})

INTRODUCTION

Chemical exchange saturation transfer (CEST) is an MRI technique that is being used for detecting low-concentration metabolites and molecules with exchangeable protons of specific resonance frequencies that are sometimes difficult to detect using conventional MRI (1–3). CEST imaging is achieved by applying a long irradiation pulse at the resonance frequency of solute protons, and detecting the reduction of the water signal due to chemical exchange between water protons and the saturated solute protons. The long irradiation and cumulative exchange cause detectable alterations in the water signal, and enhance the sensitivity for indirectly detecting the solute molecules via the water signal (2). In biological tissues, CEST effects have been observed for a number of endogenous metabolites and molecules, including proteins/peptides (4), creatine (5–8), glutamate (9–11), glucose (12,13), myo-inositol (14), glycogen (15), and glycosaminoglycans (16). In principle, CEST can provide high-sensitivity and high-spatial resolution imaging of selected proton pools *in vivo*, which complements some of the limitations of conventional magnetic resonance spectroscopy (MRS).

CEST effects can be roughly divided into three categories based on how fast the chemical exchange rates (k_{sw}) of the protons are relative to their resonance frequency offsets (ν_r): amide protons on the backbone of proteins/peptides ($k_{\text{sw}} = \sim 30 \text{ s}^{-1}$ and $\nu_r = 3.5 \text{ ppm}$ (4)) are in a slow exchange regime; guanidinium amine protons ($k_{\text{sw}} = 500 \text{ to } 1000 \text{ s}^{-1}$ and $\nu_r = 2 \text{ ppm}$ (5–8)) are in an intermediate exchange regime; most other amine protons (e.g. glutamate amine (9,10) and protein lysine amine protons (17,18)) and hydroxyl protons (k_{sw} is several thousands s^{-1} and $\nu_r = 1 \text{ to } 3 \text{ ppm}$) are in a fast exchange regime. Imaging of fast exchanging amine pools at 3 ppm at high field strength is of special interest because these

protons are part of important molecules such as glutamate and proteins (9–11,17,19–25). However, these pools are not well suited to produce CEST signals because they break the CEST condition that exchange should be slow-intermediate on the NMR time scale (i.e. $k_{\text{ex}} \gg k_{\text{sw}}$) (2). To effectively saturate fast exchanging solute spins, high irradiation powers are required. However, this in turn causes more significant direct water saturation (DS) and non-specific semi-solid magnetization transfer (MT) effects which may reduce the detection specificity of CEST measurements to fast exchanging pools.

To remove the DS and semi-solid MT effects, a reference signal that ideally has the same contributions from DS and non-specific semi-solid MT effects but without chemical exchange is required to compare to the exchange-labeled signal. The magnetization transfer ratio (MTR) may then be calculated as the difference in the labelled and reference signals normalized to a control signal with no saturating pulses (2) or a reference signal (9,26), which assumes CEST, DS, and semi-solid MT effects add linearly. MTR_{asym} describes this ratio when the reference signal is obtained from the offset frequency symmetric about the water resonance. MTR_{asym} has been previously used to quantify fast exchanging amine CEST signals at high irradiation powers (9–11,19,20). However, recent studies have shown that the CEST, DS, and non-specific semi-solid MT effects have mutual interactions, and do not add linearly (27). Thus MTR including MTR_{asym} cannot fully remove the DS and semi-solid MT effects (27–29) and they may detract from the accuracy for measuring true CEST effects. In addition, the water longitudinal relaxation time (T_{1w}), which often varies with pathology, scales the CEST effects (28,30–33) and hence induces additional confounding signal variations (34,35). Furthermore, contaminations from asymmetric MT effect and relayed nuclear Overhauser enhancement (rNOE) saturation transfer effect from mobile macromolecules to the MTR_{asym} , which were shown to be significant at low irradiation powers, have not been evaluated at high irradiation powers up to date. Thus, the specificity of previous measurements of fast exchanging amine CEST effects using MTR_{asym} is doubtful.

A novel analysis of CEST data which subtracts the reciprocals of the label and reference signals obtained in steady state, was previously described which addresses the non-specific factors associated with the use of MTR and was termed AREX (27–29). This method has been successfully applied to quantify slow exchanging CEST signals (e.g. amide proton transfer (APT) (36,37)) and intermediate exchanging pool CEST signals (e.g. creatine (creCEST) (7)), but has not to date been applied to quantify fast exchanging CEST signals. In this work, we applied AREX and a fitting approach to quantify fast exchanging amine CEST signals. Specifically, we first obtained the reference signal by fitting the DS and semi-solid effects based on Henkelman's two-pool MT model, and then inversely subtracted the reference and label signals, with corrections for the apparent water longitudinal relaxation rate ($R_{1\text{obs}}$). The specificities of conventional MTR_{asym} , MTR with reference signals obtained using the fitting approach (named MTR_{fit} here), and the AREX method were evaluated and compared through numerical simulations and controlled samples in which the magnitudes of the confounding signal contributions were altered. Since it has been reported that imaging contrast of fast exchanging amine pools reaches maximum when the spin system is in non-steady state (i.e. the irradiation time is much less than water longitudinal relaxation time (T_{1w})) (38), we also evaluate these metrics with signals acquired in both

steady state and non-steady state. The values of MTR_{asym} , MTR_{fit} , and AREX were also examined *in vivo* in animal models by comparing signals between healthy and pathological tissues.

METHODS

MTR and AREX

MTR was defined to be the subtraction of the labelled signal (S_{lab}) from the control signal (S_0) with normalization by S_0 (2). In a complex biological system with significant DS and semi-solid MT effects, the MTR should be more precise by the subtraction of S_{lab} from a reference signal (S_{ref}) which represents signal only from DS and semi-solid MT. Here, we redefine it as,

$$MTR = \frac{S_{\text{ref}} - S_{\text{lab}}}{S_0} \quad (1)$$

It is also named MTR_{asym} when the reference signal is obtained from the offset frequency symmetric about the water resonance. An alteration of the MTR_{asym} (named Rel_{asym} (39)), which is obtained by subtracting downfield labelled signal from the upfield reference signal with normalization by the upfield reference signal, is also used to increase the specificity (9,16,26,40),

$$Rel_{\text{asym}} = \frac{S_{\text{ref}} - S_{\text{lab}}}{S_{\text{ref}}} \quad (2)$$

Here, we also applied a fitting approach to get a more accurate reference signal for calculating MTR and name it as MTR_{fit} .

The metric AREX was defined as

$$AREX = \left(\frac{S_0}{S_{\text{lab}}} - \frac{S_0}{S_{\text{ref}}} \right) R_{\text{Iobs}} (1 + f_m) \quad (3)$$

where f_m is a measure of the semi-solid MT pool concentration. Depending on how the reference signals are determined, we define two AREX metrics: $AREX_{\text{asym}}$ obtained with the reference signal obtained at the offset frequency symmetric about the water resonance; and $AREX_{\text{fit}}$ obtained with reference signals obtained using a fitting approach mentioned below.

Fitting reference signal

Recently, a fitting approach, which extrapolated semi-solid MT reference (EMR) signals to obtained the reference signals, was proposed to quantify APT and rNOE saturation transfer signals (41,42). Here, we implement this method to obtain the reference signals for $AREX_{\text{fit}}$ to quantify fast exchanging amine CEST signals. Specifically, steady state CEST data

acquired with offsets from −6500 Hz to −3500 Hz, −100 Hz to 100 Hz, and 3500 Hz to 6500 Hz and irradiation power of 3.6 μT, and with offsets from −4000 Hz to −2500 Hz, −100 Hz to 100 Hz, and 2500 Hz to 4000 Hz and irradiation power of 1.0 μT were fitted to Henkelman's two-pool MT model with a Lorentzian lineshape:

$$\frac{S}{S_0} = \frac{\frac{1}{T_{1m}}(k_{mw}f_m T_{1w}) + R_{\text{rfm}} + \frac{1}{T_{1m}} + k_{mw}}{(k_{mw}f_m T_{1w})(R_{\text{rfm}} + \frac{1}{T_{1m}}) + [1 + (\frac{\omega_1}{2\pi\Delta})^2 (\frac{T_{1w}}{T_{2w}})](R_{\text{rfm}} + \frac{1}{T_{1m}} + k_{mw})} \quad (4)$$

where k_{mw} is the exchange rate between semi-solid and water protons; T_{1w} and T_{2w} are the longitudinal and transverse relaxation times of the water pool, respectively; T_{1m} and T_{2m} are the longitudinal and transverse relaxation times of the semi-solid pool, respectively; Δ is the RF frequency offset; and f_m is the semi-solid component concentration. R_{rfm} is the RF absorption rate, which depends on the absorption lineshape, $g_m(2\pi\Delta)$ through the relationship $R_{\text{rfm}} = \omega_1^2 \pi g_m(2\pi\Delta)$,

$$g_m(2\pi\Delta) = \frac{1}{\frac{\pi}{T_{2m}}(1 + T_{2m}^2(\Delta - \Delta_m)^2)} \quad (5)$$

where Δ_m is the central resonance frequency of semi-solid pool. Although the semi-solid MT pool has a super-Lorentzian lineshape, it has been approximated as a Lorentzian lineshape near water resonance (37). Here, we assumed a Lorentzian lineshape for the semi-solid MT pool to avoid the singular point in the super-Lorentzian function. Five independent semi-solid MT model parameters (k_{mw} , T_{2m} , $k_{mw}f_m T_{1w}$, T_{1w}/T_{2w} , Δ_m) were obtained by fitting CEST data to Eq. (4), based on the nonlinear least squares fitting approach, using the Levenberg-Marquardt algorithm. In the fitting, T_{1m} was set to 1 s (41,42). Table 1 lists the starting points and boundaries of the fit of semi-solid MT model parameters. The reference signals for quantifying fast exchanging amine pools at irradiation power of 3.6 μT in an offset range from −5 ppm to 5 ppm were then estimated using Eq. (4) with the fitted MT model parameters.

Animal preparation

All animal experiments were approved by the Animal Care and Usage Committee of Vanderbilt University. Three healthy rats and three rats bearing 9L tumors were included in this study. For brain tumor induction, each rat was injected with 1×10^5 9L glioblastoma cells in the right brain hemisphere, and was then imaged after 2 to 3 weeks. All rats were immobilized and anesthetized with a 2%/98% isoflurane/oxygen mixture during data acquisition. 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, USA).

Sample preparation

A series of glutamate samples served to evaluate the specificity of MTR and AREX to individual parameters, i.e., solute concentration, solute pH (exchange rate), T_{1w} , and semi-

solid concentration. The pH of each sample was titrated by using NaOH/HCl, and was confirmed with a pH meter. Measurements on these samples were performed at 37°C. All chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Study 1, to study the sensitivity to solute concentration, three samples were made of 10, 20, and 30 mM glutamate solution in 1 × phosphate buffered saline (PBS) buffer and pH titrated to 7.0.

Study 2, to study the sensitivity to pH, three samples were made with 20 mM glutamate and pH titrated to 6.8, 7.0, and 7.4.

Study 3, to study the influences of T_{1w} , two samples were made with 20 mM glutamate and different concentrations of $MnCl_2$ (0.05mM and 0.1mM, pH = 7.0).

Study 4, to study the influences of semi-solid MT effects, two samples were made with 20 mM glutamate and 1%, and 2% (w/w) agarose (pH = 7.0).

MRI

All measurements were performed on a Varian DirectDrive™ horizontal 9.4T magnet with a 38-mm Litz RF coil (Doty Scientific Inc. Columbia, SC, USA). CEST measurements were performed by applying a continuous wave (CW) irradiation with power of 1 μ T and 3.6 μ T before acquisition. This 3.6 μ T power has been previously used in an important application of fast exchanging pool: glutamate CEST (gluCEST) (10). So we evaluated the specificity of CEST quantification methods at 3.6 μ T here. An 8 s irradiation pulse for steady state and a 2 s irradiation pulse for non-steady state with TR of 10 s were used in the phantom experiments, while a 5 s and a 1 s irradiation pulses with TR of 7 s were used for steady state and non-steady state, respectively, in the animal experiments. Z-spectra with power of 1 μ T were acquired with RF offsets from -4000 Hz to -2500 Hz with a step size of 500 Hz (-10 ppm to -6.25 ppm with a step size of 1.25 ppm at 9.4 T), -2000 Hz to 2000 Hz with a step size of 50 Hz (-5 ppm to 5 ppm with a step size of 0.125 ppm at 9.4 T), and 2500 Hz to 4000 Hz with a step size of 500 Hz (6.25 ppm to 10 ppm with a step size of 1.25 ppm at 9.4 T). Z-spectra with power of 3.6 μ T were acquired with RF offsets from -6500 Hz to -3500 Hz with a step size of 500 Hz (-16.25 ppm to -8.75 ppm with a step size of 1.25 ppm at 9.4 T), -2000 Hz to 2000 Hz with steps of 50 Hz (-5 ppm to 5 ppm with a step size of 0.125 at 9.4 T), and 3500 Hz to 6500 Hz with a step size of 500 Hz (8.75 ppm to 16.25 ppm with a step size of 1.25 at 9.4 T). Control images were acquired with RF offsets of 100,000 Hz (250 ppm at 9.4 T). CEST signals on phantoms were obtained by a free induction decay (FID) acquisition, while CEST signals on animals were obtained by a single-shot SE-EPI acquisition. All CEST images on animals were acquired with matrix size 64 × 64, field of view 30 mm × 30 mm, and one average.

R_{1obs} and f_m in animal experiments were obtained using a selective inversion recovery (SIR) quantitative MT method (43). Specifically, a 1-ms inversion hard pulse was applied to invert the free water pool and the subsequent longitudinal recovery times were set to be 4, 5, 6, 8, 10, 12, 15, 20, 50, 200, 500, 800, 1000, 2000, 4000, and 6000 ms. Spin-echo Echo Planar Imaging (SE-EPI) was used for the readout followed by a saturation pulse train to shorten

total acquisition time as described previously (44). A constant delay time of 3.5 s was set between the saturation pulse train and the next inversion pulse. SIR quantitative MT images were acquired with matrix size 64×64 , field of view $30 \text{ mm} \times 30 \text{ mm}$, and one average. $R_{1\text{obs}}$ in phantom experiments were obtained using an inversion recovery fast spin echo (FSE) sequence with TR of 15 s and inversion time of 0.01, 0.028, 0.076, 0.21, 0.58, 1.6, 4.4, and 12 s. Apparent water transverse relaxation time ($T_{2\text{obs}}$) in phantom experiments were also obtained using a FSE sequence with TR of 15 s and multiple echo time (TE) at 20, 40, 80, 100, 200, 300, 500, and 1000 ms. All FSE images on phantoms were acquired with matrix size 32×32 , field of view $30 \text{ mm} \times 30 \text{ mm}$, and one average.

Numerical simulations

Z-spectra were simulated using one-pool (water pool), two-pool (fast exchanging solute and water pools) and three-pool (fast exchanging solute, water, and symmetric semi-solid pools) model numerical simulations with the same RF offsets and irradiation powers as those used in experiments. To evaluate the specificity of MTR and AREX to individual parameters, we varied the solute pool concentration f_s (0.0005, 0.001, **0.0015**, 0.002, 0.0025), solute-water exchange rate k_{sw} (1000, 3000, **5000**, 7000, 9000 s^{-1}), T_{1w} (0.5, 1.0, **1.5**, 2.0, 2.5 s), T_{2w} (0.02, 0.04, **0.06**, 0.08, 0.10 s), and f_m (0.03, 0.06, **0.09**, 0.12, 0.15) in the three-pool model simulations. Each parameter was varied individually, with all other parameters remaining at the values in bold. To evaluate the contaminations from the asymmetric MT and mobile macromolecular rNOE saturation transfer effects to the asymmetric analysis, we also performed numerical simulations using a four-pool (fast exchanging solute, water, asymmetric semi-solid, and mobile macromolecular rNOE pools) model numerical simulation. The irradiation time is 5 s for steady state and 1 s for non-steady state. Other simulation parameters are listed in Table 2. Different from experiments, where $R_{1\text{obs}}$ was fitted from measurements, $R_{1\text{obs}}$ in all simulations was calculated based on T_{1w} and f_m by using the following Eq. (6) according to Ref (27),

$$R_{1\text{obs}} \approx (R_{1w} + f_m R_{1m}) / (1 + f_m) \quad (6)$$

where $R_{1w} = 1/T_{1w}$ and $R_{1m} = 1/T_{1m}$. The calculated $R_{1\text{obs}}$ together with f_m was then used to calculate AREX using Eq. (3) in all simulations.

The coupled Bloch equations can be written as $\frac{d\mathbf{M}}{dt} = \mathbf{A}\mathbf{M} + \mathbf{M}_0$, where $\mathbf{A}$ is a 3×3 , 6×6 , 7×7 , or 10×10 matrix for the one-, two-, three-, or four-pool model, respectively. The water and solute pools each has three coupled equations representing their x, y, and z components. The semi-solid MT pool has a single coupled equation representing the z component, with a Lorentzian absorption line shape. All numerical calculations of the Z-spectra integrated the differential equations through the sequence using the ordinary differential equation solver (ODE45) in MATLAB 2013b (Mathworks, Natick, MA, USA).

Data analysis and statistics

Both glutamate amine protons and lysine amine protons in proteins have resonance frequency offsets around 3 ppm and CEST signals at this frequency offset have attracted much interest (9–11,17,19,20). For this reason, the current work focuses on this offset. The three-pool model systems assume that the semi-solid MT effect is symmetric and there is no mobile macromolecular rNOE saturation transfer effect, and hence the asymmetric analysis of these CEST data should provide accurate reference signals. Therefore, in the study to evaluate the specificity of MTR and AREX by three-pool model simulations and phantoms, we used the asymmetric analysis (MTR_{asym} and $AREX_{\text{asym}}$) to quantify the CEST signals at 3 ppm. In the study to evaluate the contamination from mobile macromolecular rNOE saturation transfer effect in the four-pool model simulations and *in vivo* experiments, we used MTR_{fit} at 3 ppm to quantify the CEST signals and used MTR_{fit} at -3 ppm to quantify the mobile macromolecular rNOE saturation transfer signals. Comparison between the two MTR_{fit} values would show the relative contamination from the mobile macromolecular rNOE saturation transfer effect. Since the fitted reference signals represent the DS and semi-solid MT effects, asymmetric analysis of the fitted reference spectra would remove the symmetric DS effect but isolate the asymmetric MT effect. Hence, in the study to evaluate the contamination from asymmetric MT effect in the four-pool model simulations and *in vivo* experiments, we compared asymmetric analysis of the fitted reference signals at 3 ppm.

ROIs for tumors and contralateral normal tissues were outlined from each tumor rat brain based on the $T_{1\text{obs}}$ map. Student's t-tests were employed to evaluate the signal differences, which were considered to be statistically significant when $P < 0.05$. All data and statistical analyses were performed using MATLAB 2013b.

RESULTS

MTR and AREX spectra

Fig. 1 shows the two-pool (fast exchanging solute and water pools) model simulated Z-spectra, MTR_{asym} spectra, and $AREX_{\text{asym}}$ spectra and the experimental Z-spectra, MTR_{asym} spectra, and $AREX_{\text{asym}}$ spectra on phantoms containing glutamate. Different from the MTR_{asym} spectra in Fig. 1c and 1d, there is a rapid increase in the $AREX_{\text{asym}}$ spectra in Fig. 1e and 1f at low offsets (e.g. < 2 ppm). We performed more simulations with one-pool (containing water without any fast exchanging amine pool) model. The black lines in Fig. 1a show the simulated Z-spectrum of this one-pool model in steady state (solid line) and in non-steady state (dashed line). Note that the downfield CEST effect from the fast exchanging amine pool disappears. Black lines in Fig. 1c and 1e show the MTR_{asym} and $AREX_{\text{asym}}$ spectra calculated from the one-pool simulated Z-spectrum according to Eq. (1) and Eq. (2), respectively. Note that the $AREX_{\text{asym}}$ values (as well as the MTR_{asym} values) become zero, indicating that the rapid increase in the $AREX_{\text{asym}}$ spectrum for the two-pool system at low offsets is not from non-specific signals, but reflects only the solute-water exchange effect. The different curves shown from Fig. 1c to 1f indicate that the two quantification methods provide different results.

MTR and AREX specificities

Fig. 2 shows the three-pool model (fast exchanging solute, water, and symmetric semi-solid pools) simulated MTR_{asym} (red), Rel_{asym} (gray), and $AREX_{\text{asym}}$ (blue) values at 3 ppm in steady state (solid) and non-steady state (dashed) as a function of several tissue parameters. It was found that all these three metrics depend on both f_s and k_{sw} , whereas $AREX_{\text{asym}}$ in non-steady state, MTR_{asym} , and Rel_{asym} at 3 ppm also depend on other non-solute parameters such as T_{1w} , T_{2w} , and f_m , suggesting that AREX in steady state is immune to competing factors, and thus is a more specific indicator than $AREX_{\text{asym}}$ in non-steady state and other metrics to detect fast exchanging protons.

Fig. 3 shows the experimental MTR_{asym} (red), Rel_{asym} (gray), and $AREX_{\text{asym}}$ (blue) values at 3 ppm in steady state (solid) and non-steady state (dashed) as a function of several tissue parameters. The amine proton of glutamate is predominantly base-catalyzed, so the glutamate-water exchange is faster for higher pH. Similar to simulations in Fig. 2, $AREX_{\text{asym}}$ in steady state depends on both glutamate concentration and the pH (or exchange rate), but not on other tissue properties, while $AREX_{\text{asym}}$ in non-steady state and other metrics depend on the other (non-solute) tissue parameters.

Asymmetric MT and mobile macromolecular rNOE saturation transfer effects

To investigate the asymmetric MT and mobile macromolecular rNOE saturation transfer effects on the quantification of the fast exchanging amine pools, we further performed four-pool model (fast exchanging solute, water, asymmetric semi-solid, and mobile macromolecular rNOE pools) simulations and animal experiments. As shown in Fig. 4, our results show that the simulations are roughly in agreement with the experimental results, except two small peaks ranging from 3 to 4 ppm and from -1 to -2 ppm in Fig. 4d. The two small peaks may arise from APT and other rNOE saturation transfer effects (45,46) in biological tissues. Compared with simulations, the two big peaks centered at approximately 2 ppm and -3.5 ppm on the MTR_{fit} curves in Fig. 4d should have contributions from the fast exchanging amine and the mobile macromolecular rNOE pools, respectively. The mean MTR_{fit} values at 3 ppm and -3 ppm in Fig. 4d are around 0.06 and 0.03, respectively, indicating that the contamination from the upfield mobile macromolecular rNOE saturation transfer effect to the fast exchanging amine signal cannot be neglected. The mean value of the asymmetric analysis of the fitted references at 3 ppm in Fig. 4d is around -0.01, indicating the asymmetric MT effect could be another contamination factor. The MTR_{asym} value at 3 ppm acquired in steady state (solid red) is thus a complicated contrast from multiple sources, i.e. CEST, asymmetric MT, and mobile macromolecular rNOE saturation transfer effects. Strictly speaking, Henkelman's MT model applies to steady state condition only, and hence we cannot obtain the reference signals from non-steady state acquisitions and then evaluate the asymmetric MT and the mobile macromolecular rNOE saturation transfer effects in non-steady state. However, the negative MTR_{asym} values in non-steady state with $\omega > 4$ ppm indicate that there are still contaminations from these factors. The match of the fitted reference spectra and the measured spectra beyond 5 ppm and -5 ppm (16.25 ppm to 8.75 ppm and -8.75 ppm to -16.25 ppm) in Fig. 4b indicates the success of the fitting approach.

MTR_{asym}, Rel_{asym}, MTR_{fit}, and AREX_{fit} contrasts in tumors and contralateral normal tissues

Fig. 5 shows the maps of these metrics from a representative rat brain. Note that all MTR metrics (Fig. 5a to 5e) show significant hyperintense signal in tumor, but AREX_{fit} (Fig. 5f) shows moderate hypointense signal in tumor. The negative values in Fig. 5a and 5b may be due to the asymmetric MT or mobile macromolecular rNOE saturation transfer effects. In addition, we quantitatively compared the contrast generated by these metrics between tumors and contralateral normal tissues in rat brain tumor model. Fig. 6 demonstrates that while all MTR signals at 3 ppm (Fig. 6a to 6e) increase in tumors, AREX_{fit} signal at 3 ppm (Fig. 6f), by contrast, decreases in tumors. The higher MTR values at 3 ppm in tumors may be partially caused by the smaller f_m (Fig. 6h) or longer T_{1w} in tumors.

DISCUSSION

CEST imaging indirectly detects solute molecules by observing water signal changes caused by chemical exchange with solute molecules, but often also depends on multiple other competing factors, including T_{1w} , DS effect, semi-solid MT effect, and CEST effects from other pools. Therefore, comparison of CEST signals between normal and pathological tissues may result in inaccurate conclusions if there are also changes in these confounding factors. Two of these effects (semi-solid MT and DS) increase with irradiation powers and can potentially cause significant variations in CEST measurements of fast exchanging protons.

In both simulations and phantom experiments in Fig. 2 and Fig. 3, all the metrics depend linearly on f_s (or glutamate concentration) and inversely on k_{sw} (or pH) in the physiological range, which is in agreement with a previous report (10). The simulated MTR_{asym} at 3 ppm in steady state in Fig. 2g increases with T_{1w} when it is smaller than 1.0 s, and decreases when it is larger than 1.0 s. This non-monotonic dependence is caused by two competitive T_{1w} effects. First, the CEST MTR_{asym} metric increases with T_{1w} when there are no DS and semi-solid MT effects. This can be shown from the two-pool model (water and solute) Bloch-McConnell equations in a weak saturation pulse approximation that assumes no RF irradiation applied on water (Eq. 8 in Ref (2)). Second, T_{1w} inversely scales the CEST signal when there are significant DS and semi-solid MT effects. For longer T_{1w} , both DS and semi-solid MT increase, but the CEST decreases. These two opposite T_{1w} effects result in a maximal MTR_{asym} value when $T_{1w} \approx 1$ s in the simulations. The experimental MTR_{asym} at 3 ppm on phantoms in Fig. 3g depends monotonically on T_{1w} that is different from the simulations in Fig. 2g. This different dependence may be caused by the DS and semi-solid MT effects. There are weaker DS effect and no semi-solid MT effect in the sample experiments, but significant DS and semi-solid MT effects in simulations (Sup. Fig. S1). Thus, the MTR_{asym} at 3 ppm in Fig. 3g is mainly scaled by T_{1w} , but not the DS or semi-solid MT, and thus monotonically increases with T_{1w} . Similarly, the simulated MTR_{asym} at 3 ppm in non-steady state also has less DS and semi-solid MT effects compared with that in steady state, and thus monotonically increases with T_{1w} . Furthermore, it was reported that Rel_{asym} can reduce the DS effect (16,26). However, Rel_{asym} is still scaled by T_{1w} , and thus also monotonically increases with T_{1w} in Fig. 2h and Fig. 3h. Shorter T_{2w} can also cause

greater DS effect and thus the decreased MTR_{asym} at 3 ppm in Fig. 2j. The MTR_{asym} and Rel_{asym} in Fig. 2m, 2n, 3j, and 3k depend inversely on semi-solid component (agarose) concentration, which is caused by signal saturation (via semi-solid MT) destroying the solute exchange information in MTR_{asym} and Rel_{asym} . The complex dependences on multiple tissue parameters indicate that the MTR metrics are not specific to fast exchanging protons.

In the present work, we applied AREX together with a fitting approach to quantify fast exchanging amine CEST signals. The relative specificities of AREX and several MTR metrics were evaluated through numerical simulations and experiments on controlled samples. Our results show that AREX in steady state is not susceptible to changes in T_{1w} , T_{2w} , and f_m , while MTR is sensitive to these parameters. Therefore, contributions from changes in T_{1w} , T_{2w} , and f_m , which vary in different pathologies, such as tumors vs. contralateral normal tissues, may contaminate MTR based contrast but are unlikely to significantly affect AREX contrast. Hence, the differing MTR and AREX contrasts at 3 ppm between tumors and contralateral normal tissues in rat brain tumor model (Fig. 5) are consistent with the prediction that *in vivo* MTR is not specific for detecting solute exchange effects. In addition, influences from upfield mobile macromolecular rNOE saturation transfer effects ranging from -2 to -5 ppm at low irradiation powers (e.g. 1 μ T) have been reported (35,47). However, because the rNOE 'coupling' rate is significantly slower than fast chemical exchange (48), the mobile macromolecular rNOE saturation transfer effect at high irradiation powers were neglected in previous studies (9–11). But our results show that the mobile macromolecular rNOE saturation transfer effect acquired at 3.6 μ T, especially in steady state, is still significant compared with the CEST signal from fast exchanging amines. Our results also show that the asymmetric MT may be another contamination factor for *in vivo* measurements. The asymmetric MT effect, although is not present in the agarose, is significant *in vivo* which may arise from the lipid CH_2 groups. Corrections for those competing effects are thus necessary for quantifying fast exchanging CEST signals *in vivo*. Here, a fitting approach, instead of the conventional asymmetric analysis, was used to avoid the contaminations from the mobile macromolecular rNOE saturation transfer and asymmetric MT effects. Fig. 4 indicates that the fitting approach may provide better quantification of CEST effect than the asymmetric analysis. Some slow to intermediate exchanging pools (e.g. APT at 3.5 ppm, creatine amine at 2 ppm (5), and downfield aromatic NOE at 3.5 ppm and 2 ppm (49)) may also cause contaminates at 3 ppm. But their CEST peaks are narrow and should have weak contributions at 3 ppm at high field strength. Fast exchanging hydroxyl pools (e.g. myo-inositol at 0.6 ppm (14)) are far from 3 ppm, and thus their contaminations at 3 ppm should be also weak (see Sup. Fig. S2).

The AREX from rat brains (around 0.58 s^{-1} in Fig. 4f) is an order of magnitude larger than that from 20 mM glutamate phantom (around 0.04 s^{-1} in Fig. 3c), indicating that glutamate may not be the main contributor to the CEST signal at 3 ppm. Although a previous study validated that the gluCEST signal at 3 ppm dominates other metabolites in the brain at high irradiation powers (10), another study showed that the CEST signal from proteins at 3 ppm can be much higher than the gluCEST signal (17). Thus, it is possible that the fast exchanging amine CEST signal at 3 ppm mainly comes from proteins. However, these two conclusions were based on phantom experiments only, in which the chemical environment

may be different from the *in vivo* environment. Further *in vivo* and/or *in vitro* validation of the molecular origins of the fast exchanging amine CEST signals at 3 ppm, e.g. correlations between CEST signals and biochemical measurements of glutamate and/or proteins, is thus required. Serving as a specific method to quantify fast exchanging CEST signals, AREX may enhance the detection specificity *in vivo*.

Previous reports showed that the slow and intermediate exchanging CEST signals quantified by AREX are Lorentzian lineshapes with central frequencies at the solute resonances (7,27,28). However, our simulations and phantom experiments in Fig. 1e and 1f and *in vivo* experiments in Fig. 4f do not show Lorentzian lineshapes centered at 3 ppm for the fast exchanging amine CEST signals. This may be due to the coalescence of the fast exchanging pool and water pool, and thus the interference of their MRI signals. The lineshape of fast exchanging CEST signals has been previously modeled by a Lorentzian function multiplied by a term named ‘a-peak’ (see Eq.(23) in Ref (50)). The ‘a-peak’, which peaks at the water resonance, shifts the CEST peak towards the water resonance and makes the CEST signal non-Lorentzian. Interestingly, although the fast exchanging amine pool is coalesced with water pool and its lineshape is influenced by the coalescence, Fig 2i and 2l indicate that the AREX at 3 ppm is independent of T_{1w} and T_{2w} . In addition, at the solute resonance frequency, this ‘a-peak’ is close to 1 and the AREX only depends on solute parameters, similar to that in the slow and intermediate exchange regimes (50). Thus, the fast exchanging amine pool quantified by AREX should have no influence from the coalescence. The rapid increase in the AREX spectrum can also be understood as an exchange related apparent water transverse relaxation effect or an on-resonance longitudinal relaxation rate in the rotating frame ($R_{1\rho}$)-type of signal. The disappearance of the rapid increase in the AREX spectrum at low offsets from simulations without the fast exchanging amine pool (black lines in Fig. 1e) indicates that this is the true characteristic of chemical exchange effect between exchanging amine and water pools. In MTR_{asym} spectra, this rapid increase at low offsets disappears and a pseudo peak at around 3 ppm in the simulation (solid line in Fig. 1c) or 2 ppm in the phantom experiment (solid line in Fig. 1d) appears, which may be caused by the increased effect from DS near the water resonance. The different shift of the MTR_{asym} peak thus cannot represent the solute resonance frequency. Previously, Jin *et al.* has shown that the shift of MTR_{asym} peak depends on solute concentration and pH (51). Our simulations in Sup. Fig. S3 show that the shift of MTR_{asym} peak also depends on T_{2w} and irradiation powers.

The AREX can be quantified by an exchange-dependent relaxation rate in the rotating frame (R_{ex}) (27,28,50). Jin *et al.* has also evaluated the non-specificity of CEST effect in the fast exchange regime, and has used another R_{ex} with different definition to quantify specific CEST effect (51). Different from the AREX spectrum, Jin’s R_{ex} spectrum has a Lorentzian lineshape centered at the solute frequency offset, and thus shows the distinct feature of CEST effect. By inspection of the two R_{ex} , AREX can also be quantified by Jin’s $R_{ex} \times \omega_1^2/(\omega_1^2 + \omega_{ex}^2)$. Therefore, a Lorentzian function can also be obtained by normalizing $\omega_1^2/(\omega_1^2 + \omega_{ex}^2)$ in the AREX spectrum. Different from Jin’s work that calculated R_{ex} from a simple two-pool model Z-spectrum, and thus might be challenging to be applied *in vivo*, here we applied a fitting approach to analyze our *in vivo* data. Another approach using asymmetric analysis of $R_{1\rho}$ has also been used to quantify specific CEST effect in the fast

exchanging regime (39). This method does not require steady state. However, the asymmetric analysis of $R_{1\rho}$ would be problematic *in vivo*.

Previously, AREX was defined to be the inverse subtraction of the labelled signal from the reference signal, with corrections for the water longitudinal relaxation rate (R_{1w}) (52,53). However, simulations in Ref (27) show that correction with $R_{1obs}(1 + f_m)$ would make AREX more specific. Both R_{1obs} and f_m can be fitted from an inversion recovery technique (43,44,54), and thus the determination of f_m would not take additional acquisition time. In the CEST imaging of fast exchanging pools, although the optimal irradiation time is reported to be less than 2 s during which the spin system cannot reach steady state (55), our results indicate that the AREX method requires a steady state CEST signal acquisition, which increases the imaging time. Interestingly, a recent study showed that the AREX can also be applied to the transient state after some modifications (56). Further studies based on this approach hold the possibility of shortening the scan time and reducing the specific absorption rate (SAR).

In this work, tumor model with variation of T_{1w} and f_m was used to evaluate the specificity of MTR and AREX metrics. Fig. 5f and 6f show no significant contrast between tumor and contralateral normal tissue using $AREX_{fit}$, indicating that this metric may not be a useful contrast for tumor diagnosis. However, this metric may be applied in other pathologies in which glutamate or/and proteins vary. Although this work focused on irradiation power of 3.6 μ T which was used in the first report of gluCEST (10), our conclusion may be extended to other irradiation powers as well as other high field strength (e.g. 7 T) (see simulations in Sup. Fig. S4–S8). With higher irradiation powers, the AREX would be more sensitive or specific to fast exchanging pools. However, S_{lab} and S_{ref} would be significantly reduced due to the increased semi-solid MT and DS effects, which might result in lower signal to noise ratio for the AREX.

CONCLUSION

Our results show that the conventional MTR metrics are susceptible to several non-specific tissue parameters, and thus may not be appropriate methods for quantifying fast exchanging solutes. It should be prudent to interpret MTR contrasts, especially in tissues with pathological variations, because the concurrent alterations in semi-solid MT and relaxation may contaminate MTR contrasts. By contrast, $AREX_{fit}$ provides a more specific quantification of fast exchanging amine CEST effects with minimized influences from some competing factors, i.e. DS, semi-solid MT, and mobile macromolecular rNOE saturation transfer effects.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Grant Sponsor: R21 EB17873, R01CA109106, R01CA184693, R01EB017767

Abbreviations

CEST	chemical exchange saturation transfer
k_{sw}	solute-water exchange rate
DS	direct water saturation
MT	magnetization transfer
MTR	magnetization transfer ratio
MTR_{asym}	MTR asymmetry analysis
AREX	apparent exchange-dependent relaxation
APT	amide proton transfer
R_{1obs}	apparent water longitudinal relaxation rate
T_{2obs}	apparent water transverse relaxation time
f_m	semi-solid MT pool concentration
f_s	solute concentration
CW	continuous wave
rNOE	relayed nuclear Overhauser enhancement
ROIs	region of interests
R_{ex}	exchange-dependent relaxation rate in the rotating frame
R_{1ρ}	longitudinal relaxation rate in the rotating frame
TE	echo time

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FIG. 1.

Two-pool (fast exchanging solute and water pools) model simulated and experimental Z-spectra (green) (a, b), MTRasym spectra (red) (c, d), and AREXasym spectra (blue) (e, f), respectively in steady state (solid) and non-steady state (dashed). Black lines in (a, c, and e) are the corresponding one-pool model simulations. (b, d, and f) show the experimental results on 20 mM glutamate sample with pH of 7.0.

FIG. 2.

Three-pool model (fast exchanging solute, water, and symmetric semi-solid pools) simulated MTR_{asym} (red lines), Rel_{asym} (gray lines), and $AREX_{asym}$ (blue lines) at 3 ppm in steady state (solid) and non-steady state (dashed) as a function of f_s (a, b, c), k_{sw} (d, e, f), T_{1w} (g, h, i), T_{2w} (j, k, l), and f_m (m, n, o), respectively.

FIG. 3.

Experimental MTR_{asym} (red lines), Rel_{asym} (gray lines), and AREX_{asym} (blue lines) at 3 ppm in steady state (solid lines) and non-steady state (dashed lines) as a function of glutamate concentration (a, b, c), solute pH (d, e, f), T_{1obs} and T_{2obs} (g, h, i), and agarose concentration (j, k, l), respectively. T_{1obs} was measured to be around 4 s for all samples except those with MnCl₂. In calculating AREX_{asym}, f_m was set to be 0 for all samples without semi-solid components. In all agarose samples, f_m was also set to be 0 due to its low value (around 0.0066 for 2% agarose (58)) and thus (1 + f_m) ≈ 1.

FIG. 4.

Four-pool (fast exchanging solute, water, asymmetric semi-solid, and mobile macromolecular rNOE pools) model simulated and experimental Z-spectra (green) (a, b), MTR_{fit} spectra (gray) and MTR_{asym} spectra (red) (c, d), and AREX_{fit} spectra (blue) (e, f), respectively. Solid lines and dashed lines represent the corresponding metrics in steady state and non-steady state, respectively. Black lines in (a) and (b) are the fitted reference signals. Black lines in (c) and (d) are the asymmetric analysis of the fitted reference signals. Experimental results were from the whole brain of three healthy rats. The data points beyond 5 ppm and -5 ppm are from 16.25 ppm to 8.75 ppm and -8.75 ppm to -16.25 ppm.

Fig. 5.

Maps of MTR_{asy} and Rel_{asy} at 3 ppm in steady state (a, b), MTR_{asy} and Rel_{asy} at 3 ppm in non-steady state (c, d), MTR_{fit} (e), $AREX_{fit}$ (f), R_{1obs} (g), f_m (h), and anatomy image (i) from a representative rat brain bearing 9L tumor. Red arrow in (i) indicates the tumor.

Fig. 6.

Statistics of MTR_{asy} and Rel_{asy} at 3 ppm in steady state (a, b), MTR_{asy} and Rel_{asy} at 3 ppm in non-steady state (c, d), MTR_{fit} (e), $AREX_{fit}$ (f), R_{1obs} (g), and f_m (h) from tumors (red) and contralateral normal tissues (blue). Error bars represent the standard deviations across subjects. (* $P<0.05$, ** $P<0.01$)

Table 1

Starting points and boundaries of MT model parameters.

	Start	Lower	Upper
k_{mw} (s^{-1})	25	0	100
T_{2m} (μs)	16	1	100
$k_{mw}f_mT_{1w}$	2	0	10
T_{1w}/T_{2w}	40	0	100
δ_m (ppm)	0	-3	3

Table 2

Parameters for the multiple-pool numerical simulations with pool concentration (f), exchange rate (k), longitudinal relaxation time (T_1), transverse relaxation time (T_2), and resonance frequency offset for each pool (ν_r). Water content is set to be 1. Simulations with fewer pools were performed by setting the corresponding pool concentration to zero.

	water	Fast exchanging amine	mobile macromolecular rNOE	Semi-solid
f	1	0.0015	0.005	0.09
k (s^{-1})	–	5000	25	25
T_1 (s)	1.5	1.5	1.5	1.5
T_2 (ms)	60	15	1	0.015
ν_r (ppm)	0	3	–3.5	–2.3 ^a

^a _Ref (57)