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Insight into the Quantitative Metrics of Chemical Exchange Saturation Transfer (CEST) Imaging

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Abstract

Purpose—To evaluate the reliability of four CEST imaging metrics for brain tumors, at varied saturation power levels and magnetic field strengths (3–9.4 T).

Methods—A five-pool proton exchange model (free water, semi-solid, amide, amine, and NOE-related protons) was used for the simulations. For the *in vivo* study, eight glioma-bearing rats were scanned at 4.7 T. The CEST ratio (CESTR), CESTR normalized with the reference value (CESTR^{nr}), inverse Z-spectrum-based (MTR_{ReX}), and apparent exchange-related relaxation (AREX) were compared.

Results—The simulated CEST signal intensities using MTR_{ReX} and AREX were substantially increased at relatively high RF saturation powers at 3 T and 4.7 T, while CESTR and CESTR^{nr} metrics remained relatively stable. There were tremendously high MTR_{ReX} and AREX signals around the water frequency at all field strengths due to the small denominators. In the rat tumor study at 4.7 T, both CESTR and CESTR^{nr} showed clear contrasts in the tumor with respect to the normal tissue across all saturation power levels (0.5–3 μ T), while the AREX showed negligible to negative insignificant contrasts.

Conclusion—CEST metrics must be carefully selected based on the different experimental settings. CESTR and CESTR^{nr} are more reliable at 3 T (a clinical field strength) and 4.7 T.

Keywords

MTR; CEST; APT; NOE; brain tumor

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article.

INTRODUCTION

Chemical exchange saturation transfer (CEST) imaging is a novel molecular MRI technique that can generate a contrast unique from conventional MRI sequences (1–4). This contrast is usually created by selective saturation or labeling of water-exchangeable solute protons, followed by a chemical exchange of saturated magnetization from the labeled solute protons to free bulk water protons, resulting in a reduction in the free water magnetization. Using CEST MRI, endogenous and exogenous agents, such as amide protons (–NH) from proteins and peptides, amine protons (–NH₂) from glutamate and creatine, and hydroxyl protons (–OH) from glycosaminoglycans, glycogen, and glucose, can be detected indirectly through the bulk water signal used for clinical imaging (5–14). Amide proton transfer (APT) imaging is one of the CEST-based molecular MRI techniques that can detect various endogenous mobile proteins and peptides in tissue, such as those in the cytoplasm (15, 16). APT MRI has the potential to detect brain tumors (increased protein concentration) and to differentiate high-grade from low-grade gliomas (17–22), as well as to assess ischemic strokes (decreased pH) (23–25) and to simultaneously visualize hemorrhagic and ischemic strokes at the hyperacute stage in a single MRI scan (26). In addition, the APT-weighted signal has been used as a novel biomarker of the tumor response to chemotherapy, radiation, and high-intensity focused ultrasound (HIFU) (27–30).

However, APT imaging is prone to direct radiofrequency (RF) water saturation (DS, also called spillover) and broad semi-solid macromolecular magnetization transfer (MT) effects. To remove these DS and semi-solid MT effects, the MT ratio asymmetry (MTR_{asym}) analysis has been widely employed (5):

$$MTR_{\text{asym}}(3.5\text{ppm}) = MTR(+3.5\text{ppm, label}) - MTR(-3.5\text{ppm, reference}) \quad [1]$$

It is known that this MTR asymmetry analysis may be complicated by the intrinsic asymmetry of the Z-spectrum around the water resonance that essentially originates from the intramolecular and intermolecular nuclear Overhauser enhancement (NOE) effects of mobile proteins, lipids, and metabolites in tissue (11, 31–33). Nevertheless, it is important to keep in mind that the NOE is actually a positive confounding factor that can enhance the measured $MTR_{\text{asym}}(3.5\text{ppm})$ or APT-weighted image contrast in the tumor (34).

Several alternative methods have been proposed to isolate APT signals from other saturation effects, using novel post-processing approaches (non- MTR asymmetry analysis) or image acquisition schemes (35–44). In particular, two inverse Z-spectrum ($1/Z$)-based metrics, using the off-resonance spin-lock theory, were recently proposed: (i) the subtraction of the inverse Z-values (MTR_{Rex}) to correct the effects on the CEST signal from DS and semi-solid MT (45); and (ii) the apparent exchange-dependent relaxation (AREX) to remove the influence of the longitudinal water relaxation (T_{1w}) contribution (46, 47). Based on the inverse metric approach, it was found that the corrected APT signal in the tumor was not significantly different from that in normal tissue ($AREX_{\text{tumor}} \approx AREX_{\text{normal}}$) in a rat tumor study at 9.4 T and in a human tumor study at 7 T (47, 48). Their results disagreed with many previous studies in which the high APT-weighted signal intensities were always observed in

malignant (high-grade) tumors (17–22, 49). The APT quantification results depend on the CEST metrics, which is undesirable. Therefore, it is important to evaluate the reliability of these different CEST metrics at different experimental settings. In this study, the MTR_{Rex} and AREX signals at 3.5 ppm and 2 ppm were calculated and compared with the CESTR and $CESTR^{nr}$ signals, using five-pool Bloch equation-based simulations with varied RF saturation powers (0.5–3 μT) and magnetic field strengths (3, 4.7, 7, and 9.4 T), and in an *in vivo* rat tumor study at 4.7 T.

METHODS

CEST-MRI Metrics: CESTR, $CESTR^{nr}$, MTR_{Rex} , and AREX

The most commonly used CEST imaging metric is the subtraction of the MTR of the reference image from that of the label image (5, 36, 50–52), as shown in Eq. [1], using the definition: $MTR = 1 - S_{\text{sat}}/S_0 = 1 - Z$ (where S_{sat} and S_0 are the image signal intensities measured with and without RF saturation, respectively). Thus, one has:

$$CESTR = \frac{S_{\text{ref}} - S_{\text{lab}}}{S_0} = Z_{\text{ref}} - Z_{\text{lab}} \quad [2]$$

where S_{ref} and S_{lab} are the reference image signal intensity and the label image signal intensity, respectively, and Z_{ref} and Z_{lab} are the corresponding normalized image signal intensities with respect to S_0 . In the early APT imaging studies, Zhou et al. denoted this as $MTR_{\text{asym}}(3.5\text{ppm})$ or APT-weighted by selecting the saturation image at the upfield frequency offset of -3.5 ppm as the reference (5). Recently, we quantified CESTR by using the extrapolated semi-solid MT reference (EMR) signal (43, 44). An alternative approach is the normalization of CESTR by a reference scan (9, 11, 53, 54):

$$CESTR^{nr} = \frac{S_{\text{ref}} - S_{\text{lab}}}{S_{\text{ref}}} = \frac{Z_{\text{ref}} - Z_{\text{lab}}}{Z_{\text{ref}}} \quad [3]$$

An eigen-space approach for solving modified Bloch equations has been recently proposed, based on the similarity of spin-lock and CEST (45, 55). The intrinsic inverse metric for CEST quantification can be calculated by subtraction of the inverse Z-spectrum (45):

$$MTR_{\text{Rex}} = \frac{(S_{\text{ref}} - S_{\text{lab}})S_0}{S_{\text{ref}}S_{\text{lab}}} = \frac{1}{Z_{\text{lab}}} - \frac{1}{Z_{\text{ref}}} = \frac{Z_{\text{ref}} - Z_{\text{lab}}}{Z_{\text{ref}}Z_{\text{lab}}} \quad [4]$$

Furthermore, another intrinsic inverse metric, AREX, through multiplication by $R_{1w}(=1/T_{1w})$, was introduced to remove the influence of water longitudinal relaxation (46, 47):

$$AREX = \frac{MTR_{\text{Rex}}}{T_{1w}} = \frac{(S_{\text{ref}} - S_{\text{lab}})S_0}{S_{\text{ref}}S_{\text{lab}}T_{1w}} = \frac{Z_{\text{ref}} - Z_{\text{lab}}}{Z_{\text{ref}}Z_{\text{lab}}} \cdot \frac{1}{T_{1w}} \quad [5]$$

Simulations

Modified five-pool Bloch equation-based simulations (Supporting Theory) were performed to assess four CEST metrics (CESTR, CESTR^{nr}, MTR_{REX}, and AREX) for the quantification of CEST signals, incorporating the effects of pool concentrations, relaxation times, chemical shifts, and proton exchange rates. Frequency offsets of 3.5 ppm and 2 ppm were chosen for the simulation studies. The former is for the amide protons of mobile proteins and peptides, and the latter may actually have a complicated origin, including the protein and peptide side-chain amide protons (17) and various amine-related protons, as reported in the literature (36, 39, 42, 47). The APT (at 3.5 ppm) and CEST@2ppm effects were simulated as a function of magnetic field strength ($B_0 = 3\text{ T}–9.4\text{ T}$) and RF saturation power ($B_1 = 0.5\text{ }\mu\text{T}–3\text{ }\mu\text{T}$). The continuous-wave (CW) RF saturation time was 3 s for 3 T (6), 4 s for 4.7 T (5), and 5 s for 7 T and 9.4 T (47) for the efficient saturation according to water T_1 relaxation times. Furthermore, simplified analytical solutions (Eqs. [10] and [16] in (46), and Eqs. [13] and [14] in (47)), based on spin-lock theory, were compared with the full Bloch equation-based simulation with a five-pool model with varied magnetic field strengths and RF saturation power levels (46, 47). Reference signals (Z_{ref}) for CESTR, CESTR^{nr}, MTR_{REX}, and AREX metrics were taken from the simulated semi-solid MT signal. For the commonly used MTR_{asym}(3.5ppm) metric, Z_{ref} signals were taken from the saturation image at -3.5 ppm . Simulation parameters for the tumor case are shown in Table 1.

In Vivo MRI Experiments

Animal care throughout the experimental procedures in the study was in accordance with institutional guidelines. All MR experiments were performed on a 4.7 T Biospec animal imager (Bruker Biospin) with an actively decoupled cross-coil setup, which consists of a 70 mm body coil for RF transmission and a 25 mm surface coil for signal reception. Eight human glioblastoma-bearing adult nude rats were scanned at 45 days post-implantation. Conventional high-resolution T_2 -weighted images in the coronal plane were acquired with a fast spin-echo sequence to localize the tumor ($TR/TE = 3\text{ s} / 64\text{ ms}$, $FOV = 32 \times 32\text{ mm}^2$, slice thickness = 1.5 mm, matrix = 192×192 , NEX = 2, and five slices). A single slice passing through a maximum tumor region with T_2 -weighted hyperintensity was selected for water T_1 (T_{1w}^{obs}), T_2 (T_{2w}^{obs}), apparent diffusion coefficient (ADC), and CEST imaging, as described previously (44).

CEST image data were obtained using a fat-suppressed spin-echo pulse sequence, with a long CW RF saturation pulse and a single-shot, spin-echo, linear k-space encoded echo-planar imaging (EPI) readout, using the following parameters: $TR = 10\text{ s}$; $TE = 30\text{ ms}$; matrix size = 64×64 ; $FOV = 32 \times 32\text{ mm}^2$; spatial resolution = $500 \times 500\text{ }\mu\text{m}^2$; slice thickness = 1.5 mm; RF saturation time = 4 s; and single slice acquisition. Wide Z-spectra were acquired to quantify conventional semi-solid MT and CEST effects, using different RF saturation power levels (0.5, 0.9, 1.3, 2.1, 3.2, and $4.4\text{ }\mu\text{T}$ for $n = 3$, only $1.3\text{ }\mu\text{T}$ for $n = 5$). The frequency sweep corresponded to the full Z-spectrum with 62 frequency offsets: unsaturated (S_0), 0, ± 0.5 , ..., and $\pm 15\text{ ppm}$, in intervals of 0.5 ppm, which was acquired in 10 min 20 s (10 s for a single frequency offset). Water saturation shift-referencing (WASSR) data (26 frequency offsets from 0.6 to -0.6 ppm at intervals of 0.05 ppm including S_0 , and a

saturation power of 0.5 μ T) were acquired for B_0 corrections (56). The scan time for WASSR data was about 2 min 10 s (5 s for a single frequency offset).

CEST Processing

All simulations and CEST processing were performed using MATLAB (The MathWorks, Inc., Natick, MA). For the *in vivo* study, the CEST image data were first corrected for B_0 inhomogeneities by resetting the water signal to 0 ppm on a voxel-by-voxel basis, using the WASSR technique. Then, the EMR calculation was performed by the procedures based on the recently published papers (43, 44). To do this, the wide-offset MT data between 15 and 7.5 ppm and the data at the offset of 0.5 ppm (acquired from all six RF power levels) were fitted to Henkelman's two-pool model with a super-Lorentzian lineshape, based on the nonlinear least-squares fitting method, which implemented the Levenberg-Marquardt algorithm. All data points between 7 and -15 ppm were excluded to avoid a possible downfield CEST and an upfield NOE contribution, and the offset of 0.5 ppm was included to improve the fitting quality. The super-Lorentzian lineshape function was evaluated by the numerical integration. Fit parameter errors were estimated as the root of the sum of the signal-normalized squared difference between the fitted and experimental data, and the χ^2 goodness-of-fit metric. For convenience, T_{1m} was set as a constant value of 1.4 s (57), because it could not be determined well from fits. M_0^w was normalized to 1. Finally, Z_{ref} (namely, Z_{EMR}) and Z_{lab} were taken from the fitted two-pool MT model, and the B_0 -corrected experimental Z-spectra for CEST metric calculations, respectively.

To quantitatively compare the CEST signals in glioma, regions of interest (ROIs) were analyzed. Two ROIs, enclosing the normal-appearing gray matter and the glioma were carefully drawn. The noise values were compared in the different CEST metrics. The noise level was estimated from the standard deviation of the pixel signal intensity in the background (5×5 pixels). Preference was given to noise ROI away from EPI ghost artifacts. The contrast-noise-ratio (CNR) was calculated as follows: $CNR = (SI_{tumor} - SI_{normal})/noise$, where SI is the CEST signal intensity.

RESULTS

Simulation Results

The five-pool Bloch equation-based simulation results with six RF saturation power levels of 0.5, 1, 1.5, 2, 2.5, and 3 μ T are shown in Figs. 1 (for 4.7 T, with an RF saturation duration of 4 s (5)) and 2 (for 9.4 T, with an RF saturation duration of 5 s (47)), as well as in Supporting Figs. S1 (for 3 T, a typical clinical field strength, with an RF saturation duration of 3 s (6)) and S2 (for 7 T, with an RF saturation duration of 5 s). The magnetic field strength B_0 and RF saturation power B_1 dependencies of the DS, semi-solid MT, and CEST signals can be seen clearly. Particularly, at the relatively low RF power, the APT dip at 3.5 ppm in the Z-spectrum can be easily observed because of the small RF saturation width. However, the APT dip is no longer observable at the higher RF power (>2 μ T) because of the large RF saturation width (34, 58, 59), as well as the dilution effect induced by the DS and semi-solid MT, leading to a significantly reduced Z-spectrum.

Several interesting CEST signal features can be seen with the different CEST metrics (see also Figs. 3 and 4). (i) Clear CEST signal features in an offset range of 2–3.5 ppm can be seen with all four CEST metrics when a relatively low RF saturation power ($<1 \mu\text{T}$) is applied. (ii) The CEST signal intensities using the two inverse metrics (MTR_{Rex} and AREX) are noticeably increased as the RF saturation power increases up to $3 \mu\text{T}$, particularly at 4.7 T (e.g., $\text{MTR}_{\text{Rex}} \approx 42 \%$ and $\text{AREX} \approx 23 \%$ at 3.5 ppm, and $\text{MTR}_{\text{Rex}} \approx 156 \%$ and $\text{AREX} \approx 86 \%$ at 2 ppm, with a B_1 of $3 \mu\text{T}$) and 3 T (e.g., $\text{MTR}_{\text{Rex}} \approx 43 \%$ and $\text{AREX} \approx 31 \%$ at 3.5 ppm, and $\text{MTR}_{\text{Rex}} \approx 132 \%$ and $\text{AREX} \approx 95 \%$ at 2 ppm, with a B_1 of $3 \mu\text{T}$). However, both CESTR and CESTR^{nr} metrics remain relatively stable (decreased or increased), as compared to the inverse metrics (Figs. 3 and 4). Such an apparent tremendous increase in the inverse metrics at the high RF saturation powers and the typical clinical field strengths occurs simply because of the small denominators. (iii) The APT and CESTR@2ppm peaks can be well resolved in all CEST metrics at two ultra-high field strengths of 9.4 T (Fig. 2) and 7 T (Supporting Figs. S2), because of the high spectral resolution, but not at 4.7 T (Fig. 1) and particularly at the clinical field strength of 3 T (Supporting Figs. S1) for the relatively high RF saturation power levels ($>2 \mu\text{T}$). (iv) The inverse metric signal intensities around the water frequency are not reliable due to denominators approaching zero (Figs. 1 and 2) and a high vulnerability to confounding noise values.

Figure 5 shows the comparison between the simple analytical model based on the off-resonance spin-lock theory (colored dashed lines) (46, 47) and the CEST metrics based on the full Bloch equation-based simulation (colored solid lines) with a five-pool model (free water, semi-solid, amide, amine, and NOE-related protons). It was observed that the APT signal intensities obtained from the analytical model evidently differ from the results of the full Bloch equation-based simulation, particularly at the relatively high RF saturation power. Interestingly, CESTR and CESTR^{nr} at 3 T, as well as MTR_{Rex} and AREX at 9.4 T, from the simple analytical model are in a relatively good agreement with the full Bloch equation-based simulation results. Note that the exchange rate (k_{ws}) was set as a constant value of 0.12 s^{-1} ($k_{\text{ws}} = k_{\text{sw}} \cdot M_0^{\text{s}}/M_0^{\text{w}}$) and the $\cos^2\theta$ term was calculated with corresponding B_0 and B_1 at 3.5 ppm (46, 47).

Figure 6 shows a simulated relationship between the CESTR signal at 3.5 ppm and water T_1 with six RF saturation powers of 0.5, 1, 1.5, 2, 2.5, and $3 \mu\text{T}$. At the relatively low RF power ($<2 \mu\text{T}$), the CESTR signal at 3.5 ppm increased with $T_{1\text{w}}$, but the relationship is not linear due to complicated differential equations. Particularly, the positive correlation does no longer exist (increased $T_{1\text{w}}$, decreased APT) at the high RF saturation power ($>2 \mu\text{T}$), probably because the water direct saturation, semi-solid MT, and other CEST effects complicate the water T_1 contribution to the APT signal.

Experimental Results

Figure 7 shows the average two-pool, semi-solid MT fitted results and experimental data obtained from the normal tissue and tumor at six RF saturation powers (0.5, 0.9, 1.3, 2.1, 3.2, and $4.4 \mu\text{T}$), as well as the quantitative CEST and NOE signal features obtained from the four CEST metrics. As expected, downfield APT signals of the tumor from CESTR and CESTR^{nr} were significantly higher than those of the normal tissue across all power levels (p

< 0.05), while the upfield NOE signals of the tumor from CESTR and CESTR^{nr} were significantly lower than those of the normal tissue across all power levels ($p < 0.01$). The high MTR_{Rex} and the AREX peaks can be seen clearly around 1 ppm, which is very similar to the results from the Bloch equation-based simulation, as shown in Fig. 1. Note that large dips (negative) around the water resonance were observed in all CEST metrics due to an imperfect saturation at the water resonance, which was a result of the low signal-to-noise ratio and the local B₀ inhomogeneity.

Figure 8 compares the APT-weighted or APT image signal intensities (obtained from the four CEST metrics) and contrasts between the tumor and normal brain tissue. Based on this, several important results can be observed. (i) The MTR_{asym} signal intensities at 3.5 ppm increased with the RF power level and reached a maximum at the RF power of 3.2 μ T and 2.1 μ T for the normal tissue and tumor tissue, respectively (Fig. 8a). The MTR_{asym} signals of the normal tissue were negative at three relatively low RF power levels (0.51.3 μ T) due to the different power dependence of the APT and NOE peaks. (ii) The APT signals using the CESTR were positive across all RF power levels (Fig. 8c). As shown in Fig. 8b and d, the APT image contrasts measured by the MTR_{asym} and CESTR were quite similar, but the image contrast of the MTR_{asym} (due to a positive confounding effect from the NOE) was higher than that of the CESTR signal. (iii) Unlike using the CESTR, the APT signals using the CESTR^{nr} gradually increased with RF saturation power levels (Fig. 8e). Notably, the CESTR^{nr} image contrasts were similar across all RF power levels (Fig. 8f). (iv) The APT signal using the inverse metrics dramatically increased with RF saturation power levels due to denominators approaching zero (Fig. 8g and i). As shown in Fig. 8h and j, however, the APT image contrasts from the inverse metrics were not significant, except the MTR_{Rex} at RF powers of 0.5, 0.9, and 1.3 μ T.

Multiple quantitative MRI maps of a representative tumor-bearing rat are shown in Fig. 9. The T₂-weighted image, the ADC, the T_{2w}^{obs}, and the T_{1w}^{obs} maps showed a clear contrast that enabled tumor detection. Remarkably, the tumor was hyperintense on the MTR_{asym}, CESTR, CESTR^{nr}, and MTR_{Rex} maps at 3.5 ppm when an RF saturation power of 1.3 μ T was applied. On the CESTR, CESTR^{nr}, and MTR_{Rex} maps at 2 ppm, the tumor was slightly hyperintense. As reported previously (47, 48), the AREX maps at 3.5 and 2 ppm showed no contrast between the tumor and normal tissue. The noise values measured were 1.1 ± 0.1 , 1.2 ± 0.3 , 1.3 ± 0.5 , and 2.4 ± 1.1 for MTR_{asym}, CESTR, CESTR^{nr}, and MTR_{Rex}, respectively. The noise of MTR_{Rex} was roughly twice that of CESTR and CESTR^{nr} ($p < 0.01$), although the MTR_{Rex} showed the highest signal intensity in the tumor among four CEST metrics. Note that the noise of AREX was not estimated because T_{1w}^{obs} had an irrelevant noise value as a result of the fitting procedure ($I = A + Be^{-TI/T_1}$). Presumably, the noise of AREX was similar or a little higher than that of MTR_{Rex} due to the extra noise added from the T_{1w}^{obs}. The CNRs at the RF saturation power of 1.3 μ T were 5.5 ± 0.2 , 2.9 ± 0.7 , 3.0 ± 1.0 , 2.3 ± 1.7 , and -1.3 ± 8.1 for MTR_{asym}, CESTR, CESTR^{nr}, MTR_{Rex}, and AREX (the same noise value used as MTR_{Rex}), respectively.

DISCUSSION

CEST quantification depends on the choice of CEST metrics and reference images. In recent years, much effort has been focused on the measurement of pure APT and other CEST signals by using new CEST metrics (35–44). In particular, the new inverse Z-spectrum metrics (MTR_{Rex} and AREX) were recently proposed in order to remove several confounding factors, such as DS or spillover, water relaxation, and semi-solid MT effects (45–48). Here, we evaluated the reliability of the four CEST metrics and potential confounders, with varied magnetic field strengths and RF saturation power levels, through both the five-pool Bloch equation-based simulation and *in vivo* rat tumor experimental studies. Note that the simple analytical solution used for the inverse metric analysis is actually a poor approximation of the original Bloch-equation solution at high RF saturation power levels, particularly at 3 T (the clinical field strength) and 4.7 T (Fig. 5). The resulting deviations are much larger for fast exchange proton CEST measurements (such as the amine and hydroxyl groups) due to the larger DS and semi-solid MT effects. The exact five-pool Bloch equation-based simulations showed that all four metrics yielded the RF saturation power-dependent CEST signals. In this work, our simulated MTR_{Rex} and AREX signals with the five-pool Bloch equations at 9.4 T showed a consistent increase with B_1 (Fig. 3). In a recent experimental study at 9.4 T (46), the measured APT*, MTR_{Rex}^* , and AREX* signals showed an initial increase with B_1 , followed by a decrease when the RF saturation power was larger than 2 μT . The difference between these studies may be due to the fact that the other study used a three-offset method (36) that would underestimate the CEST signals with high RF powers (46), which the reference image was estimated by averaging of two boundary images acquired at 3 and 4.2 ppm. Notably, at 3 T (the clinical field strength (20–22)) and 4.7 T, CESTR and particularly CESTR^{nr} remained relatively stable in an RF saturation power range of 0.5 to 3 μT ; thus, CESTR and CESTR^{nr} should be more reliable and valid APT metrics. In addition, the CEST signals around the water frequency using MTR_{Rex} , AREX, and even CESTR^{nr} may not be reliable due to denominators approaching zero and relatively large confounding noise values for the inverse metrics. Therefore, care should be taken when interpreting various tremendously high CEST signals around the water resonance for hydroxyl groups at 1 ppm and amine groups at 2 ppm, as reported previously (60).

In the *in vivo* tumor model study at 4.7 T, the tremendous peaks around the water resonance from the inverse metrics were clearly observed (Fig. 7; also see Supporting Fig. S3 for an eggwhite phantom study), which is very similar to the results from the Bloch equation-based simulations (Fig. 1). Unlike the simulation result (Fig. 3), however, the APT signals using CEST^{nr} gradually increased with RF saturation power levels as shown in Fig. 8e, presumably due to other CEST contributions such as fast water-exchangeable hydroxyl protons.

Based on Eqs. [2]–[5], it is quite interesting to note that the four CEST metrics simply reflect the different normalizations and are thus associated with the different denominators. Unfortunately, it seems that the use of these metrics has resulted in some different CEST quantification results. Based on the new inverse Z-spectrum metric, AREX, the rat 9L tumor study at 9.4 T (47) and the human brain tumor study at 7 T (48) showed that there was no

APT contrast in the tumor ($\text{AREX}_{\text{tumor}} \approx \text{AREX}_{\text{normal}}$). This was inconsistent with numerous previous findings (using the $\text{MTR}_{\text{asym}}(3.5\text{ppm})$) that APT-weighted signals were higher in the tumor core than in peritumoral edema and normal tissue (17–22). The unexpected rat result was consistent with the total protein biochemical measurements, which showed that there was no significant enhancement of protein contents in the tumor, as measured with the Bradford and bicinchoninic acid assay (BCA) methods (47). However, one should keep in mind that the APT signal is related only to the mobile proteins and peptides in tissue (16), not to the total proteins. As discussed previously (5, 43, 44), the effects of the increasing T_{1w} and the increasing water content on the measured APT signal are mostly canceled out in many diseases. Further, our numerical simulation (Fig. 6) showed that the relationship between APT signals and T_{1w} was not linear. Thus, simply multiplying by R_{1w} as used in the AREX metric (Eq. [5]) would inspire an extra contribution of the water content, leading to the unexpected result ($\text{AREX}_{\text{tumor}} \approx \text{AREX}_{\text{normal}}$).

The choice of the reference images is critical for quantitative CEST measurements. For APT imaging, the commonly used reference image is acquired at the RF saturation frequency offset (-3.5 ppm) opposite of the APT signal, with respect to the water resonance, and the measured $\text{MTR}_{\text{asym}}(3.5\text{ppm})$ signal is called the APT-weighted signal. Ideally, except for APT, all other effects (such as DS, semi-solid MT) must be identical in both the reference image (Z_{ref}) and label image (Z_{lab}). However, the presence of the NOE effect at -3.5 ppm (including the so-called conventional MTR asymmetry) has complicated the interpretation of the quantified APT-weighted signal (based on MT asymmetry analysis). Nevertheless, the high APT-weighted signal consistently observed in patients with high-grade gliomas (18–22) and the ability to differentiate the tumor from radiation necrosis (61, 62) make the $\text{MTR}_{\text{asym}}(3.5\text{ppm})$ -based, APT-weighted MRI signal a powerful clinical biomarker for tumor detection.

Our simulation study compared four different CEST metrics based on CW RF irradiation schemes to approach the steady state with different B_0 and B_1 strengths. However, the RF irradiation schemes in human systems (particularly, 3 T and 7 T) are typically limited to the pulse train saturation, consisting of multiple short RF pulses (block, sinc-shaped, or Gaussian-shaped) due to scanner hardware constraints (particularly amplifier duty cycle) and specific absorption rate (SAR) requirements (63). The CEST effect from the pulse train scheme may be lower than that from the CW RF scheme due to inefficient saturation and CEST signal loss during the interpulse delay. However, a previous theoretical study by Sun et al. has shown that there is only a minor difference between CW and pulse train RF saturation schemes (64). Another potential issue in this work is the possible contribution of tumor pH to APT or APT-weighted contrast. Many studies have reported that malignant tumors have nearly neutral or slightly alkaline intracellular pH (an increase of about 0.1 pH unit) and acidic extracellular pH (a decrease of at least 0.5 pH unit) with respect to normal brain tissue (65–68), and the extracellular space volume fraction (regarding total tissue volume) is about 0.3–0.4 for high-grade human brain tumors (69, 70). It is thought previously (17, 18) that increased APT or APT-weighted contrast in the tumor is potentially caused by increased mobile protein and peptide concentration, and slightly increased intracellular pH, because most mobile proteins and peptides exist in the intracellular cytoplasm. However, the total tumor pH is definitely weighted by lower extracellular pH,

due to a large, acidic extracellular space volume fraction. The amide proton exchange rate is base-catalyzed in the physiological pH range, and one may see the negative APT contrast in tissue if the acidic extracellular pH is a dominant source (like in ischemic stroke (5)). However, APT or APT-weighted hyperintensities are always observed in the malignant tumors. Future studies are needed to determine the intracellular and extracellular pH gradient contribution to the APT contrast in tumor.

CONCLUSIONS

In this study, we evaluated the reliability of two inverse CEST metrics (MTR_{Rex} and AREX) compared with other CEST metrics, using a five-pool Bloch equation-based simulation and a rat tumor experiment at 4.7 T. The choice of CEST metrics must be carefully considered based on the RF saturation power levels, B_0 field strengths, and specific exchangeable solute protons. Based on the simulations and rat tumor experiments, all four metrics resulted in RF saturation power-dependent CEST signals. CESTR and CESTR^{nr} would be more reliable and valid for APT imaging at 3 T (the typical clinical field strength, including the optimal saturation power of 2 μ T used currently) and 4.7 T. MTR_{Rex} and AREX should be used with caution for amine and hydroxyl CEST measurements, particularly with a relatively higher saturation power level (with the large DS and semi-solid MT effect).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Simulated five-pool and two-pool Z-spectra, as well as CEST and NOE signals with four CEST metrics at various RF saturation power levels (B_1) at a B_0 magnetic field strength of 4.7 T. The five-pool model consists of bulk water protons, semi-solid macromolecular protons, amide protons at 3.5 ppm, amine protons at 2 ppm, and NOE-related protons at -3.5 ppm. The two-pool model consists of bulk water protons and semi-solid macromolecular protons.

Figure 2.

Simulated five-pool and two-pool Z-spectra, as well as CEST and NOE signals with four CEST metrics at various RF saturation power levels (B_1) at a B_0 magnetic field strength of 9.4 T. The five-pool model consists of bulk water protons, semi-solid macromolecular protons, amide protons at 3.5 ppm, amine protons at 2 ppm, and NOE-related protons at -3.5 ppm. The two-pool model consists of bulk water protons and semi-solid macromolecular protons.

Figure 3. Simulated APT signal (3.5 ppm) as a function of RF saturation power (0.5–3 μT) and magnetic field strength (3–9.4 T).

Figure 4. Simulated CEST@2ppm signal as a function of RF saturation power (0.5–3 μT) and magnetic field strength (3–9.4 T).

— CESTR — CESTR^{nr} — MTR_{Rex} — AREX — — — $K_{ws} (0.12 \text{ s}^{-1}) \times 100$

Solid: five-pool Bloch equation-based simulation
Dashed: simplified analytical simulation (46,47)

Figure 5.

Comparison of the simplified analytical solution-based (colored dashed lines) and five-pool full Bloch equation-based (colored solid lines) simulation results at various RF saturation powers (B_1) and magnetic fields (B_0). The inverse CEST metrics show high deviation when high RF saturation power is applied at 3 T and 4.7 T.

Figure 6.
Simulated CESTR signals at 3.5 ppm as a function of RF saturation power levels and water T_1 relaxation times.

Figure 7.

Average ROI-based experimental data (black dashed lines), extrapolated two-pool MT fitted curves (Z_{EMR} , black solid lines), CEST, and NOE signal features, obtained from normal tissue and from tumor at six RF saturation power levels of 0.5, 0.9, 1.3, 2.1, 3.2, and 4.4 μT . The reference signals were obtained from the Z_{EMR} data (black solid lines). Sky-blue solid lines: MTR_{REX} ; blue solid lines: AREX; orange solid lines: CESTR^{nr} ; red solid lines: CESTR.

Figure 8.

Average APT image intensities and contrasts between the normal tissue and tumor with four CEST metrics at 4.7 T. **a,b:** MTR_{asym} at 3.5 ppm; **c,d:** CESTR at 3.5 ppm; **e,f:** $CESTR^{nr}$ at 3.5 ppm; **g,h:** MTR_{Rex} at 3.5 ppm; **i,j:** AREX at 3.5 ppm. Error bars depict standard deviations. All values are statistically significant ($p < 0.05$), unless otherwise indicated. ns indicates not statistically significant.

Multiple MRI parametric maps overlaid on a corresponding EPI image for a representative tumor-bearing rat (45 days post-tumor implantation).

Figure 9. Multiple MRI parametric maps overlaid on a corresponding EPI image for a representative tumor-bearing rat (45 days post-tumor implantation).

Table 1

Five-pool model parameters used in the Bloch equation-based simulations

	Water		Amide	Amine		NOE	MT
	Exchange rate (k_{sw}) Concentration	M_0^w	30 Hz $1/250 M_0^w$	1 kHz $1/500 M_0^w$	20 Hz $1/300 M_0^w$	20 Hz $1/25 M_0^w$	
T_1	3 T (43)	1.4 s	1.4 s	1.4 s	1.4 s	1.4 s	1.4 s
	4.7 T (44, 15)	1.8 s	1.8 s	1.8 s	1.8 s	1.8 s	1.8 s
	7 T (48)	2.0 s	2.0 s	2.0 s	2.0 s	2.0 s	2.0 s
	9.4 T (71)	2.2 s	2.2 s	2.2 s	2.2 s	2.2 s	2.2 s
T_2	3 T (72)	100 ms	50 ms	50 ms	0.4 ms	20 μ s	20 μ s
	4.7 T (44)	65 ms	40 ms	40 ms	0.4 ms	20 μ s	20 μ s
	7 T	60 ms	38 ms	38 ms	0.4 ms	20 μ s	20 μ s
	9.4 T (71)	36 ms	36 ms	36 ms	0.4 ms	20 μ s	20 μ s
Chemical shift		0 ppm	3.5 ppm	2 ppm	-3.5 ppm	0 ppm	

Values were estimated based on the listed references, but adjusted for the field strength.