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Direct Saturation-Corrected CEST (DISC-CEST) MRI of Glioma: Simplified Decoupling of Amide Proton Transfer (APT) and Nuclear Overhauser Effect (NOE) Contrasts

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Abstract

Purpose—Chemical exchange saturation transfer (CEST) MRI has shown promise in tissue characterization in diseases like stroke and tumor. However, in vivo CEST imaging such as amide proton transfer (APT) MRI is challenging due to concomitant factors such as direct water saturation, macromolecular magnetization transfer and nuclear overhauser effect (NOE), which leads to a complex contrast in the commonly-used asymmetry analysis (MTRasym). Herein we proposed a Direct Saturation-Corrected CEST (DISC-CEST) analysis for simplified decoupling and quantification of in vivo CEST effects.

Methods—CEST MRI and relaxation measurements were performed on a classical 2-pool creatine-gel CEST phantom and normal rat brains (N=6) and a rat model of glioma (N=8) at 4.7T. The proposed DISC-CEST quantification was performed and compared with conventional MTRasym and the original three-offset method.

Results—We demonstrated the DISC-CEST contrast of the phantom had much stronger correlation with MTRasym than the three-offset method, which showed substantial underestimation. In normal rat brains, the DISC-CEST approach revealed significantly stronger APT effect in gray matter and higher NOE effect in white matter. Furthermore, the APT and NOE maps derived from DISC-CEST showed significantly higher APT effect in the tumors than contralateral normal tissue but no apparent difference in NOE.

Conclusion—The proposed DISC-CEST method, by correction of non-linear direct water saturation effect, serves as a promising alternative to both the commonly-used MTRasym and the simplistic three-offset analyses. It provides simple yet reliable in vivo CEST quantification such as APT and NOE mapping in brain tumor, which is promising for clinical translation.

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Keywords

Amide proton transfer (APT); Chemical exchange saturation transfer (CEST); Nuclear overhauser enhancement (NOE); Direct water saturation; RF spillover; Three-offset method; Tumor; Glioma

Introduction

Chemical Exchange Saturation Transfer (CEST) MRI emerges as a sensitive molecular imaging technique that can detect exchangeable groups via their interactions with bulk tissue water (1). The substantial sensitivity gain enables CEST MRI to map certain dilute metabolites (2–5) and physiological properties such as pH (6–8). It has been applied to study acute stroke (9–12), tumor (13–17) and epilepsy (18).

Amide proton transfer (APT) is a specific form of CEST imaging that is sensitive to pH and mobile protein/peptide content (19–21). Recently, APT imaging has shown promising results in tumor detection (19,22,23) and grading (24–26) and in the differentiation of tumor recurrence from radiation necrosis (27,28). APT effect is commonly quantified using asymmetry analysis (MTR_{asym}) to suppress direct water saturation (RF spillover) and magnetization transfer (MT) effects by taking the difference between a reference image (−3.5 ppm) and label image (+3.5 ppm). However, in vivo MTR_{asym} is contaminated by the asymmetry in MT and nuclear overhauser effect (NOE), which result in a negative and inhomogeneous shift across the brain (7,29–31). As such, in vivo MTR_{asym} has a mixed contribution from APT, NOE and asymmetric MT effects.

To improve in vivo CEST quantification, Z-spectral fitting has been employed to resolve the independent contributions from multiple origins (14,32,33). However, it requires densely-sampled Z-spectrum/spectra over a large range of frequency offsets, which will lead to extreme long scanning time. In addition, the reliability of multi-pool non-linear fitting depends strongly on the signal-to-noise ratio (SNR), initial guesses, and boundary values (34). A three-offset approach that subtracts the label image from the average of two boundary images was proposed to quantify APT/NOE effects (31,35). However, the linear correction of the baseline following this simplistic approach makes it limited to quantification of CEST peaks far from the water resonance (i.e., large chemical shift or high field) under the condition of weak RF saturation level. If the condition is not met such as at sub-high field strength ($1\text{ T} < B_0 < 7\text{ T}$), the direct saturation effect cannot be approximated by a linear function, which results in substantial underestimation of the CEST effect. In addition to APT, it has been postulated that NOE effect provides complementary information on tissue composition given its sensitivity to mobile macromolecules (36–39). It has been shown that the NOE image may provide information distinguishing brain gray matter (GM) and white matter (WM) (39,40), myelination (41) and protein unfolding (42). Nevertheless, in vivo NOE quantification remains challenging due to similar reasons as APT measurement. Therefore, it is necessary to develop processing techniques that can better quantify in vivo CEST effects with less confounding contaminations.

Recently, an analytical solution describing the steady state CEST echo planar imaging (EPI) signal with continuous wave RF saturation has been derived for optimization of CEST

experiments (43). For a given set of longitudinal and transverse relaxation times, this solution also simplifies direct water saturation calculation. Herein we propose a relaxation-based Direct Saturation-Corrected CEST (DISC-CEST) analysis that addresses the limitation of the simplistic three-offset calculation. We first evaluated the proposed approach in a classical 2-pool creatine-gel CEST phantom, and compared it with the standard MTR asymmetry and the three-offset methods. We then evaluated the DISC-CEST approach in both normal rat brains and a rat model of glioma, and demonstrated the advantage of the proposed analysis over the commonly used CEST analyses for quantification of in vivo CEST measurement.

Theory

For the CEST echo planar imaging (EPI) sequence with a continuous wave (CW) RF saturation, the control scan signal without RF irradiation can be described by (44)

$$S_0(TR, FA) = \frac{1 - e^{-TR \cdot R_{1w}}}{1 - \cos(FA)} \cdot \sin(FA) \cdot e^{-TE \cdot R_{2w}^*}, \quad (1)$$

where TR is the repetition time, TE is the echo time, R_{1w} and R_{2w}^* are the bulk water longitudinal and apparent transverse relaxation rates, respectively, and FA is the image excitation flip angle (In the case of spin echo acquisition, R_{2w}^* is replaced by R_{2w}) For the irradiated scans, the steady state signal can be given by (43,45,46)

$$S_{\text{sat}}(R_{1\rho}, TR, FA, Ts, B_1) = \frac{\left(1 - e^{-(TR-Ts) \cdot R_{1w}}\right) \cdot e^{-R_{1\rho} \cdot Ts} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}} \cdot \left(1 - e^{-R_{1\rho} \cdot Ts}\right)}{1 - \cos(FA) \cdot e^{-(TR-Ts) \cdot R_{1w}} \cdot e^{-R_{1\rho} \cdot Ts}} \cdot \sin(FA) \cdot e^{-TE \cdot R_{2w}^*} \quad (2)$$

where $R_{1\rho}$ is the longitudinal relaxation rate in the rotating frame, R_{1w} is the bulk water longitudinal relaxation rate, Ts is the saturation times, and $\theta = \tan^{-1}(\omega_1 / \omega)$ with ω_1 and ω being the RF irradiation level and offset. Therefore, the normalized CEST signal can be shown as $Z = S_{\text{sat}}/S_0$. Assuming no exchange and 90° excitation pulse, the symmetric direct saturation around the water frequency, also known as RF spillover, can be described by

$$Z' = \frac{S_{\text{sat}}(R_{1\rho}, TR, Ts, B_1)}{S_0(TR)} = \frac{\left(1 - e^{-(TR-Ts) \cdot R_{1w}}\right) \cdot e^{-R_{1\rho} \cdot Ts} + \frac{R_{1w} \cos^2 \theta}{R_{1\rho}} \cdot \left(1 - e^{-R_{1\rho} \cdot Ts}\right)}{(1 - e^{-TR \cdot R_{1w}})} \quad (3)$$

using $R_{1\rho}$ of the bulk water pool $R_{1\rho} = R_{1w} \cos^2 \theta + R_{2w} \sin^2 \theta$, where R_{2w} is the bulk water transverse relaxation rate. Subsequently, the direct saturation-corrected CEST signal can be

obtained by taking the difference between direct saturation calculated from Eq. 3 and experimentally measured CEST signal (i.e., $Z=Z'-Z$).

In previous work, a three-offset method to delineate APT or NOE effect is calculated as

$MTR(\Delta\omega_i) = \frac{Z(\Delta\omega_i+\delta)+Z(\Delta\omega_i-\delta)}{2} - Z(\Delta\omega_i)$, where $\omega_i \pm \delta$ are the frequency offsets of the two boundaries with equal offset shift δ from the peak of interest (ω_i) (31,35).

However, the method assumes linear baseline and hence its accuracy may be compromised by the nonlinear direct saturation. Here, we proposed a DISC-CEST analysis method that uses direct saturation-corrected CEST signal (Z) for three-offset analysis. Furthermore, the DISC-CEST method is generalized to accommodate asymmetric boundary offsets (upfield boundary $\omega_i - \delta_1$ and downfield boundary $\omega_i + \delta_2$), which is given by

$$MTR'(\Delta\omega_i) = \frac{\delta_1 \cdot \Delta Z(\Delta\omega_i + \delta_2) + \delta_2 \cdot \Delta Z(\Delta\omega_i - \delta_1)}{\delta_1 + \delta_2} - \Delta Z(\Delta\omega_i) \quad (4)$$

For accurate delineation of a CEST effect, the boundary offsets with minimum residual CEST effect from the peak are desired. The DISC-CEST analysis provides a baseline that can be relatively well approximated by a linear line, enabling objective determination of boundary offsets based on the first derivative test. The δ_{12} are identified as the smallest

values that meet $\frac{d\Delta Z(\Delta\omega_i - \delta_1)}{d\Delta\omega} = \frac{d\Delta Z(\Delta\omega_i + \delta_2)}{d\Delta\omega}$ and $\delta_{12} > 0$.

Methods

Phantom

We prepared a CEST phantom with creatine (CAS: 6020-87-7, C3630 SIGMA) and low gelling point agarose (CAS: 9012-36-6; gelling temperature 36° C; Sigma Aldrich, St. Louis, MO). Briefly, we added 1% agarose to phosphate-buffered saline (PBS) solution doped with 30 μ M gadolinium (Bayer HealthCare, Whippany, NJ). The mixture was microwave-heated until bubbles appeared and cooled to 50°C in a waterbath. We titrated its pH to 6.75 using a pH meter (EuTech Instrument, Singapore), and added creatine (Cr) to the gel solution to reach concentrations of 10, 20, 30, 40 and 50 mM, respectively. We transferred the Cr-gel solution at each concentration into separate 5 mm NMR tubes and let them solidify at room temperature. Afterward, the tubes were sealed and inserted into a phantom container filled with 1% empty gel.

Tumor Model

Animal experiments were performed in accordance with institutional guidelines, as approved by the Institutional Animal Care and Use Committees, Massachusetts General Hospital (IACUC, MGH). 2×10^5 cells of the non-infiltrating D74-rat glioma model were injected into the right frontal lobe of adult male Fischer 344 rats (N=8), as previously described (47). The animals were imaged 11–13 days after tumor implantation.

MRI

MRI scans were performed on a 4.7 Tesla scanner (Bruker Biospec, Ettlingen, Germany). For the phantom study, CEST MRI was implemented with single-slice, single-shot EPI (field of view (FOV) = 50×50 mm², matrix = 96×96, slice thickness = 5 mm). Z-spectrum was acquired from −3 ppm to 3 ppm with intervals of 0.0625 ppm and a series of RF irradiation power level corresponding to the RF field strength of 0.5, 0.75, 1, 1.25 and 1.5 μT. The repetition time (TR)/saturation time (TS)/echo time (TE) was 10 s/5 s/50 ms, number of average (NSA) = 2. For the in vivo study, multi-slice MRI (FOV = 20×20 mm², matrix = 64×64, 5 slices, slice thickness/gap = 1.8/0.2 mm) was performed by using single-shot EPI. We obtained Z-spectrum from −6 ppm to 6 ppm with intervals of 0.25 ppm and RF irradiation power level corresponding to 0.75 μT, TR/TS/TE = 10 s/5 s/15 ms, NSA = 2 and scan time = 8 min 20 s. In addition, T₁-weighted images were acquired with seven inversion delays ranging from 250 ms to 3,000 ms (TR/TE = 6,500/28 ms, NSA = 4); T₂-weighted images were obtained with two TE of 30 and 100 ms (TR = 3,250 ms, NSA = 16).

Data Analysis

Data were processed in MATLAB (MathWorks, Natick, MA). Parametric T₁ map was derived using least-squares fitting of the signal as functions of inversion time ($I = I_0[1 - (1 + \eta)e^{-TI/T_1}]$), where TI is the inversion time, and T₁, inversion efficiency η , and equilibrium signal I_0 are the three variables to be fitted. T₂ map was calculated as

$T_2 = \frac{TE_2 - TE_1}{\ln(I(TE_1)/I(TE_2))}$, where TE_{1,2} = 30/100 ms. Z-spectra ($Z = I/I_0$) were interpolated by smoothing splines, corrected for B₀-field inhomogeneity (48,49). The direct saturation corrected CEST effect was obtained by taking the difference between measured signal and simulated signal from Eq. 3 (i.e., $Z = Z' - Z$). In addition, $MTR_{asym} = Z(-\omega) - Z(\omega)$, where ω is the frequency offset of interest. For comparison, the three-offset method and DISC-CEST analysis were calculated using the same set of boundary offsets ($\omega - \delta_1$ and $\omega + \delta_2$), which can be determined based on the first derivative test. The regions of interest (ROIs) at WM and GM were used for analyzing the CEST data of normal brain. The ROIs of tumors were defined based on T₁ map and mirrored to the contralateral hemisphere after a slight adjustment for the shifted midline to form the ROIs of normal tissue.

Results

Phantom Study

Fig. 1a shows the experimentally measured Z-spectra (solid) for 5 creatine concentrations ($B_1 = 0.75 \mu T$) and the analytic estimation of direct water saturation effect (dashed) based on Eq. 3, with their difference (Z , dotted). Both MTR_{asym} from experimentally measured Z-spectra and Z reveal Cr guanidinium proton CEST effect at 1.9 ppm, which increases with Cr concentration. The Z approach provides satisfactory direct saturation correction, showing more distinct Cr CEST peaks than the Z-spectra. Fig. 1b compares Z , Z' and Z of one creatine concentration at varied saturation power. The Z suppressed the direct saturation effectively at different B_1 . More importantly, the Z revealed more distinguishable Cr peaks than the Z-spectra, particularly at large B_1 ($B_1 \geq 1 \mu T$).

We evaluated the three-offset analysis on the experimental data (Z) and the proposed DISC-CEST approach using the direct saturation corrected Z , and compared them with MTR_{asym} (Fig. 2). Because creatine has no up-field CEST effect, MTR_{asym} provides reliable CEST effect calculation with minimal confounding contribution. The three-offset method assumes that the direct saturation effect between the two boundary frequency offsets is approximately linear (blue dotted lines, Fig. 2a), which deviates from the non-linear direct saturation effect estimated using Eq. 3 (dashed line). This discrepancy is minimal for a narrow Cr CEST peak with a flat baseline in Z (red dotted lines, Figure 2a). The boundary offsets of creatine peak were found to be +1.2 ppm and +2.5 ppm. Fig. 2b shows that Cr CEST image from the three-offset method had generally smaller CEST effect than MTR_{asym} approach, with an underestimation of $55.5 \pm 23.4\%$ ($B_1 = 0.75 \mu\text{T}$). In comparison, the Cr CEST map from the DISC-CEST approach was closer to MTR_{asym} , with their difference being $18.7 \pm 2.8\%$. Over all B_1 levels, the DISC-CEST approach showed a noticeably higher correlation with MTR_{asym} than the original three-offset method (Fig. 2c). Despite the two approach had similar correlation coefficients with MTR_{asym} for each B_1 (p-value n.s.), the DISC-CEST measurement showed less deviation from the identity line than the three-offset method, especially with increased B_1 where spillover becomes stronger. Similar findings were observed in creatine-gel phantoms with varied spillover and MT effects (Fig. S1).

In Vivo Studies

Normal Rats—We evaluated the proposed DISC-CEST method in comparison with MTR_{asym} and the three-offset method in normal rat brains. Fig. 3a shows the averaged Z , Z' and Z from the ROIs of WM and GM. Distinct APT peaks at +3.5 ppm and NOE peaks at -3.5 ppm were observed in Z and Z . The boundary offsets of each peak were determined based on the first derivative test described above and were found to be +2.8 ppm and +4.5 ppm for APT and -2.3 ppm and -5 ppm for NOE. To allow direct comparison between the three-offset and the DISC-CEST methods, we used the same set of $\delta_{1,2}$ for both methods. Fig. 3b compares the MTR_{asym} , APT* and NOE* effects estimated from the three-offset method, and APT' and NOE' effects from the DISC-CEST approach, between WM and GM of normal brains ($N = 6$). For each measurement, the mean and standard deviation within the ROIs from individual animals and their signal-to-noise ratios were shown in Fig. S2. Despite apparent contrast between WM and GM, the MTR_{asym} maps were generally negative, overwhelmed by confounding upfield NOE and asymmetric MT effects. Both the three-offset and DISC-CEST analyses revealed significantly stronger APT effect and smaller NOE effect in GM than WM. In addition, the newly proposed approach provided significantly stronger APT' and NOE' than the three-offset approach (i.e., APT* and NOE*).

Glioma Rats—We further compared the original three-offset and DISC-CEST methods, and MTR_{asym} in a rat glioma model. Fig. 4 shows representative Z , Z' and Z from ROIs of the contralateral normal brain tissue and the tumor. In addition to the bulk water peak and underlying broad MT effect, apparent APT and NOE peaks were present in both Z and Z . The tumor region shows relatively stronger APT effect than the normal tissue. This contrast became more apparent in Z wherein the direct saturation was supposed to be corrected. Interestingly, no apparent difference was observed for the NOE effect between the normal

tissue and tumor. For the three-offset and DISC-CEST analyses, the same boundary offsets of APT or NOE peaks as in the normal WM and GM were selected. Fig. 5 shows relaxation maps, MTR_{asym} maps and APT*, APT', NOE*, NOE' maps from two typical slices of a representative rat brain with glioma. T_1 maps show a clear contrast between the tumor and normal brain tissue. T_2 map shows a relatively lower T_2 in the tumor core with elevated T_2 in the tumor rim. The MTR_{asym} maps display a positive contrast in the tumor, but the MTR_{asym} values were negative in the normal tissue due to contamination from upfield NOE effect and asymmetric MT effect. On the other hand, the cerebrospinal fluid (CSF) appeared hyperintense in MTR_{asym} maps (black arrow, Fig. 5), at the similar level to MTR_{asym} in the cancerous tissue. In comparison, the tumor appears hyperintense in both APT* and APT' maps with a substantially stronger contrast observed in the APT' maps. Meanwhile, no obvious difference between the tumor and normal regions was found in NOE* or NOE' maps. The results from all eight tumor rats were summarized in Table 1. The APT' and NOE' effects were significantly higher than the APT* and NOE* ($p < 0.001$, paired t-tests). More importantly, a significant increase in the APT contrast between normal tissue and tumor was found, being APT* $1.04 \pm 0.6\%$ and APT' $1.26 \pm 0.6\%$ ($p < 0.001$, paired t-tests).

Discussion

Our study compensated nonlinear direct saturation effect from relaxation measurements, correction of which improves in vivo CEST quantification, augmenting both the conventional MTR_{asym} approach and the original three-offset method. Notably, the original three-offset is applicable for cases of high B_0 field, weak saturation power levels and frequency offsets far from the water resonance, where the direct saturation effect over the frequency range can be approximated by a linear function. Otherwise, it risks substantial underestimation of CEST effect. We showed that the proposed DISC-CEST method overcomes these limitations as our results demonstrated the improved three-offset determination of CEST effects at 4.7 Tesla for frequency offset as small as 1.9 ppm and saturation power as high as $1.5 \mu\text{T}$. After direct saturation correction, the CEST peak sits on a reasonably linear baseline, which is likely attributable to broad MT effect. As such, the CEST effect can be well delineated from the baseline using a generalized three-offset analysis. As the linear approximation holds for a much broader frequency range than in the uncorrected Z, the boundary offset shifts, or $\delta_{1,2}$, can be slightly larger to minimize residual CEST effect from the peak.

In the normal brain, both the three-offset and DISC-CEST analyses showed stronger APT effect in GM and larger NOE effect in WM, consistent with previous findings (31,50). However, the difference between the APT* and NOE* from the original three-offset method was substantially lower than MTR_{asym} ($p < 0.005$), indicating non-negligible contribution from macromolecular MT effect. Interestingly, the DISC-CEST approach yielded significantly stronger APT' and NOE' values, with their difference closer to MTR_{asym} than that between APT* and NOE*. It is worthwhile to point out that the MT effect induces substantial signal drop over a wide frequency range and is asymmetric around the water peak. It can be estimated by fitting wide-offset data with a super-Lorentzian lineshape (26,50). Alternatively, the macromolecular MT effect within a small frequency range around the water resonance can be fitted by a Lorentzian line shape without the need of acquiring

wide-offset data (51). However, since the MT is much broader than other major CEST peaks, it could be approximated as a linear function or even a constant over a small frequency range. As such, the residual MT effect would have a less impact on the DISC-CEST approach than on MTR_{asym} . In this study, we obtain Z-spectrum from -6 to 6 ppm to demonstrate the concept and to determine the boundaries objectively. With predetermined boundaries, the DISC-CEST method allows reliable in vivo APT quantification without acquiring the whole Z-spectrum. In theory, it minimally requires three offsets provided homogeneous B_0 field, which drastically reduces the scan time and shows promise for clinical use. Meanwhile, the B_0 field inhomogeneity in vivo typically varies within 50H after high-order shimming. Therefore, acquisition of more offsets can provide more robust correction of field inhomogeneity.

Because tumor has significant T_1 and T_2 changes from normal tissue (Fig. 5 and Table 1), it experiences different direct saturation effects, correction of which is necessary. Using the three-offset or DISC-CEST analyses, significantly higher APT effect was found in the tumors, similar to previous findings from APT imaging of glioma (20,22,50). The APT changes have been suggested to arise from elevated intracellular mobile proteins/peptides concentration (52). More importantly, the APT' maps show significantly stronger contrast between the normal and tumor tissues than APT* map. Therefore, the proposed DISC-CEST method may serve as a promising means to quantify in vivo APT change, which can be potentially used for tumor detection, tracking tumor growth and monitoring APT changes in response to treatments. It can also be applied to APT imaging of ischemic tissues to determine pH changes. Our study found no apparent NOE contrast between the normal tissue and tumor, in line with the previous studies using conventional CEST imaging protocol at similar irradiation powers without further T_1 normalization (13,50,53,54). The NOE quantification has been difficult due to its broad linewidth, which will lead to significant error if the non-linear direct saturation and macromolecular MT contributions are not well eliminated. Previous NOE measurements with similar saturation powers under the same field strength vary from 8% to 10% in normal brain (50,54). In this study, we found the NOE' values are around 4%, which are approximately two times of the NOE* values (2%) but still lower than the literature values. Note the NOE for aliphatic and olefinic protons ranges from 0 to 5 ppm up-field from the water (38,55). Therefore, the underestimation in our NOE' measurement likely arises from residual NOE effects in the boundary offsets chosen here, especially -2.3 ppm. Nevertheless, NOE' measurement is sensitive in detecting NOE difference between WM and GM. Further improvement may be possible by removing the broad MT effect from the direct saturation-corrected Z-spectrum using numerical fitting of a theoretical MT model. Notably, the DISC CEST MRI decouples APT from NOE, which is advantageous when both alter at the same time.

Conclusion

Our study evaluated a relaxation-based direction saturation-corrected CEST analysis that provides improved in vivo CEST determination, complementing the commonly used MTR asymmetry analysis and simplistic three-point calculation. The proposed approach enables more reliable in vivo CEST quantification such as APT and NOE mapping in brain tumor, which is promising for clinical translation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

a) Experimental Z-spectra (Z , solid lines), analytic estimation of RF direct saturation effect (Z' , dashed lines) and direct saturation corrected Z-spectrum (Z , dotted lines) of a multi-compartment creatine-gel phantom with creatine concentration varied from 10 mM to 50 mM ($B_1 = 0.75 \mu\text{T}$). The direct saturation-corrected Z-spectrum was obtained by subtracting the experimental Z-spectrum from the Z-spectrum analytically derived from relaxation measurements ($Z = Z' - Z$). **b)** Comparison of Z (solid lines), Z' (dashed lines) and Z (dotted lines) from one compartment of the phantom (50 mM) at varied saturation power levels ($B_1 = 0.5, 0.75, 1, 1.25$ and $1.5 \mu\text{T}$).

Figure 2.

a) Illustration of MTR_{asy} obtained using two-offset analysis (black circles) and three-offset method on Z (blue dots) and DISC-CEST analysis on Z (red dots). **b)** Comparison of MTR_{asy} map from two-offset analysis, CEST maps from three-offset method and DISC-CEST analysis of the multi-compartment creatine phantom. **c)** Correlation analysis between MTR_{asy} and three-offset method on Z or DISC-CEST on Z of the multi-compartment creatine phantom at varied saturation power ($B_1=0.5/0.75/1/1.25/1.5 \mu$ T). The identity line is shown as a gray dash line.

Figure 3.

a) Comparison of the experimental Z-spectra (Z , solid lines), analytic estimation of direct saturation effect (Z' , dashed lines) and direct saturation-corrected Z-spectrum (Z , dotted lines) of white matter (WM) and gray matter (GM) from a normal rat brain. **b)** The regions of interest (ROIs) at WM and GM were overlaid on T_1 map and maps of MTR_{asym} , APT^* and NOE^* effects estimated from three-offset method on Z , APT' and NOE' effects from DISC-CEST method on Z were shown.

Figure 4.

Comparison of MTR_{asy}, APT* and NOE* effects, APT' and NOE' effects from DISC-CEST method on Z between WM and GM of normal brains (N=6). Mean and standard deviation were shown. Mann-Whitney tests were performed with ***p<0.001, *p<0.05 significantly different between WM and GM or between the measurements from three-offset and DISC-CEST methods.

Figure 5.

a) The regions of interest (ROIs) of the tumor and normal tissue were defined on T1 map. **b)** Comparison of Z, Z' and Z spectra between a D74 glioma tumor and contralateral normal tissue from a representative rat.

Figure 6.

Multi-parametric MR maps of a representative rat with a D74 glioma tumor, including quantitative relaxation maps of T_1 , T_2 , MTR_{asym} , APT^* and NOE^* maps estimated from three-offset method, APT' and NOE' maps from DISC-CEST method.

Table 1

Comparison of relaxation times, MTR_{asym} and CEST effects estimated using three-offset analysis (APT^* and NOE^*) and DISC-CEST approach (APT' and NOE') in normal and tumor regions of all tumor rats ($N = 8$). Mean $\pm$ standard deviation were shown.

	Normal	Tumor
T_1 (s)	1.54 ± 0.13	$1.97 \pm 0.10^{***}$
T_2 (ms)	56.7 ± 2.1	$53.4 \pm 2.4^*$
MTR_{asym} (%)	-3.79 ± 1.61	$-1.25 \pm 1.33^{**}$
APT^* (%)	1.28 ± 0.58	$2.32 \pm 0.66^{**}$
APT' (%)	$1.99 \pm 0.56^\dagger$	$3.25 \pm 0.61^{***\dagger}$
NOE^* (%)	1.80 ± 0.14	1.71 ± 0.69
NOE' (%)	$3.92 \pm 0.25^{\dagger\dagger\dagger}$	$4.40 \pm 0.75^{\dagger\dagger}$

Mann-Whitney tests were performed with $***p < 0.001$, $**p < 0.01$, $*p < 0.05$ indicating significant difference between normal and tumor tissues, and $\dagger\dagger\dagger p < 0.001$, $\dagger p < 0.05$ indicating significant difference between the three-offset and DISC-CEST analyses (APT^* vs. APT' , NOE^* vs. NOE').