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pH-sensitive amide proton transfer (APT) effect dominates the magnetization transfer asymmetry contrast during acute ischemia — Quantification of multi-pool contribution to in vivo CEST MRI

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Abstract

Purpose—To determine the origins of in vivo magnetization transfer (MT) asymmetry (MTR_{asym}) contrast during acute ischemic stroke, particularly in the diffusion lesion, perfusion lesion and their mismatch using a middle cerebral artery occlusion (MCAO) rat model of acute stroke.

Methods—Adult male Wistar rats underwent multi-parametric MRI of diffusion, perfusion, T_1 and amide proton transfer (APT) imaging at 4.7 Tesla following MCAO procedure. A multi-pool Lorentzian model, including nuclear overhauser (NOE), MT, direct water saturation, amine and amide chemical exchange saturation transfer (CEST) effects, was applied for Z-spectrum fitting to determine the sources of in vivo MTR_{asym} following acute stroke.

Results—We showed that changes in amine CEST (2 ppm) and APT (3.5 ppm) effects, particularly, APT MRI effect, dominate the commonly-used MTR_{asym} analysis and hence confer pH sensitivity to APT imaging of acute stroke. Also, NOE and MT show small changes that counteracted each other, contributing less than 0.3% to MTR_{asym} at 3.5 ppm. Moreover, we showed that diffusion lesion had worsened acidosis from perfusion/diffusion lesion mismatch ($P < 0.05$).

Conclusion—The study complements recent in vivo quantitative CEST work to shed light on the sensitivity and specificity of endogenous APT MRI to tissue acidosis.

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Introduction

Tissue pH alters upon metabolic disruption following acute stroke, making pH a valuable surrogate metabolic biomarker for characterization of ischemic tissue (1). Amide proton transfer (APT) MRI is sensitive to tissue acidosis following acute stroke by probing pH-dependent chemical exchange saturation transfer (CEST) effect between the endogenous protein/peptide amide protons and bulk water (2). It has been shown that APT MRI augments the routine perfusion and diffusion MRI for demarcation of the perfusion/diffusion lesion mismatch into benign oligemia and metabolic penumbra, aiding prediction of the stroke outcome (3–7).

Because magnetization transfer (MT) asymmetry (MTR_{asym}) analysis provides an efficient means to correct the direct water saturation, MTR_{asym} has been commonly used in quantifying in vivo APT effect. However, it has been recognized that MTR_{asym} is susceptible to concomitant RF saturation effects, particularly the slightly asymmetric semisolid MT and nuclear overhauser effect (NOE), that may somewhat limit its specificity to changes in pH and amide proton concentration (8–17). To address this, alternative quantitative measures have been investigated. For example, it has been shown that the regression of MTR_{asym} against MT and R_{1w} helps mitigate non-pH heterogeneity within the brain, therefore improving pH specificity for automated ischemic lesion segmentation (5). In addition, the APT effect can be derived from the difference between a Z-spectrum and Lorentzian line fitting that avoids the need of using upfield reference scans (18,19). Also, the spillover and MT can be estimated with a three-way subtraction, allowing less contaminated APT quantification (20). It is also possible to take the signal difference between the peak of interest and the mean of the two neighboring offsets to determine APT and NOE effects independently (10,21). However, this 3-point approach assumes a simplistic linear baseline, which is limited to high-field applications (22). Moreover, an inverse Z-spectrum analysis was introduced to help quantitate direct saturation and MT effects, revealing good contrast between the ischemic lesion and normal tissue (23,24).

Our study aimed to determine the origins of in vivo MTR_{asym} contrast during acute ischemic stroke, particularly in the diffusion lesion, perfusion lesion and their mismatch using a well-established middle cerebral artery occlusion (MCAO) rat model of acute stroke. Five potential sources to MTR_{asym} contrast, including NOE, MT, direct saturation, amine and amide CEST effects were numerically solved from Z-spectrum using a multi-pool Lorentzian function (10). Our study showed that although MTR_{asym} analysis has concomitant contributions from multiple origins, its change at 3.5 ppm is dominated by pH-sensitive APT contrast. This finding is helpful to elucidate the pH specificity of the commonly used MTR_{asym} , support simplified and expedient pH-sensitive image postprocessing, and ultimately, facilitate translating pH MRI to the acute stroke setting.

Methods

Animal Stroke Model

All experiments have been approved by the Institutional Animal Care and Use Committee. Adult male Wistar rats (n=12) were anesthetized initially with 5% isoflurane-air mixture and

then maintained under 1.5–2.0% isoflurane-air mixture during the study. The heart rate and oxygen content of blood (SpO₂) were monitored (Nonin Pulse Oximeter 8600, Plymouth, MN, USA), and body temperature was maintained by a circulating warm water jacket positioned around the torso. Acute ischemia was induced following the standard intraluminal MCAO procedure. Briefly, after exposure of the right carotid bifurcation and suturing of the common carotid and distal external carotid arteries, a silicone-coated 4-0 nylon suture was advanced to block the middle cerebral artery. Animals underwent MRI scans 60 min after MCAO operation. Two rats displayed a small focal lesion in the hypothalamic region, indicating occlusion of only the hypothalamic artery (HTA) not the MCA, and were excluded from the analysis due to failed MCA occlusion.

MRI

MRI scans were collected from a 4.7 Tesla small-bore scanner (Bruker Biospec, Billerica, MA) with a dual radio-frequency (RF) coil setup (70 mm volume transmitter with a 20 mm actively-decoupled surface receiver). Multi-slice MRI (5 slices, slice thickness/gap=1.8/0.2 mm, FOV=20×20 mm², matrix size=48×48) were acquired with a single-shot echo-planar imaging (EPI). After high-order gradient shimming, the water frequency was reset to the reference frequency. Diffusion-weighted MRI was obtained with two b-values of 250 and 1000 s/mm² (repetition time (TR) = 3250 ms, echo time (TE) = 42 ms, 16 averages, scan time = 1.5 min). T₁-weighted images were acquired using inversion recovery EPI, with seven inversion delays ranging from 250 to 3000 ms (TR/TE = 6500/20 ms, 4 averages; scan time = 3 min). In addition, continuous arterial spin labeling (CASL) perfusion MRI was acquired (TR/TE = 5400/20 ms, time of saturation (TS) = 3400 ms, 32 averages, saturation power of B₁ = 4.7 μT, labeling distance of 15 mm, and scan time = 2 min). Also, Z-spectra were obtained from –6 to 6 ppm with intervals of 0.25 ppm under two RF irradiation amplitudes (B₁) of 1.5 and 0.75 μT (TR/TS_{primary}/TS_{secondary}/TE = 6000/2500/500/10 ms, number of averages = 4, scan time = 13.3 min) (13). Moreover, water saturation shift referencing (WASSR) map was collected with B₁ = 0.5 μT (frequency offsets between ±0.5 ppm with intervals of 0.05 ppm).

Image Analysis

Images were analyzed in MATLAB (MathWorks, Natick, MA, USA). Parametric T₁w map was obtained by fitting of the signal intensity with respect to the inversion time ($I(i) = I_0[1 - (1 - \eta)e^{-TI_i/T_1w}]$) per pixel, where η is the inversion efficiency and TI_i is the i^{th} inversion time. The apparent diffusion coefficient (ADC) map was calculated as $ADC = \frac{\ln(I(b_1)/I(b_2))}{\Delta b}$, where $b_{1,2}$ are two diffusion b-values with Δb being their difference. Cerebral blood flow (CBF) was derived as $CBF = \frac{\lambda(I_{ref} - I_{label})}{2 \cdot I_{ref} \cdot T_{1sat}}$. Perfusion and diffusion lesions were manually segmented, and mirrored to the contralateral normal hemisphere.

The Z-spectrum was normalized by the image without RF irradiation and slight B₀ inhomogeneity was corrected using the WASSR approach (25). The conventional MTR_{asym} was calculated as,

$$\text{MTR}_{\text{asym}} = \frac{S_z(-\Delta\omega) - S_z(\Delta\omega)}{S_0} \quad (1)$$

where, S_z and S_0 are images with and without RF irradiation respectively. T_1 -normalized asymmetry analysis was performed by $\text{MTR}_{\text{asym}}/T_{1w}$. In addition, the Z-spectrum was flipped as $Z(\omega) = 1 - S_z/S_0$ and fitted using a multi-pool Lorentzian model of (26),

$$Z(\omega) = \sum_{i=1}^5 L_i(\omega) \quad (2)$$

where, L_i is the Lorentzian spectrum of the i^{th} pool. Saturation transfer effects of NOE, MT, direct water saturation, amine and amide CEST effects were included, with their chemical shifts at -3.2 , -1.5 , 0 , 2.0 and 3.5 ppm, respectively (27),

$$L(\omega) = \frac{A}{1 + 4 \cdot \left(\frac{\omega - \omega_0}{lw} \right)^2} \quad (3)$$

where, ω is the frequency offset related to water resonance. A , ω_0 and lw are the amplitude, center frequency offset and line width of the i^{th} saturation transfer (ST) effect, respectively. Nonlinear constrained fitting routine of “lsqcurvefit” was performed with fitting constraints allowing peak amplitude and line width to vary from 1/5 to 5 times of the initial values and peak frequency offsets ± 0.2 ppm of each chemical shift. Initial guesses were determined from the fitted parameters of the entire brain (28). The ST effects were normalized for T_{1w} relaxation effect (i.e., ST/T_{1w}), and multi-pool contribution to the MTR_{asym} at 3.5 ppm was quantified pixel-by-pixel (23,29). Note that a Lorentzian model has been demonstrated appropriate to fit the MT pool for a relatively small range of chemical shift under moderate RF power levels (30,31).

Statistical Analysis

Two-tailed Student's t-test was performed between paired measurements in the ipsilateral ischemic and contralateral normal regions. The statistical significance of the measurement difference across ROIs was tested using one-way analysis of variance (ANOVA) with Bonferroni correction ($P < 0.05$). Results are presented as mean $\pm$ standard deviation (SD).

Results

Figure 1 shows diffusion (Fig. 1a) and perfusion (Fig. 1b) images from a representative acute ischemic stroke rat with both the ipsilateral ischemic lesion (dashed outline) and the contralateral normal region (solid outline) ROIs outlined, which were overlaid on the corresponding T_1 map (Fig. 1c). Diffusion MRI reveals ischemic lesion in the striatum while perfusion MRI captures ischemia in the territory supplied by hypothalamic, anterior

choroidal artery and middle cerebral arteries. There is clear perfusion/diffusion lesion mismatch in the cortex. Note that although T_1 map shows clear heterogeneity between brain white and gray matter, the change due to ischemia is relatively small, suggesting that T_1 is not very sensitive to acute stroke.

Flipped Z-spectra (i.e., $Z(\omega) = 1 - S_z/S_0$) from regions of diffusion lesion, perfusion lesion, and their mismatch were shown in Figures 2a–c. The spectra were numerically fitted using a multi-pool Lorentzian function. High $R^2 > 0.99$ was observed for all regions, suggesting satisfactory fitting. Compared to those in contralateral normal ROIs, NOE asymmetry effect at 3.5 ppm increases in the ischemic lesions (Figures 2d–f). In comparison, MT, direct saturation, amine and amide CEST effects at 2 and 3.5 ppm, respectively, decrease from that of the contralateral reference regions. Notably, the magnitude of the change (i.e., normal - ischemic) was more pronounced in the diffusion and perfusion lesions than that in perfusion/diffusion lesion mismatch. This is because diffusion lesion suffers more aggravated tissue damage than the perfusion/diffusion mismatch, which roughly approximates the ischemic penumbra.

Figure 3 displays the diffusion (Fig. 3a), perfusion (Fig. 3b), and the commonly used pH-weighted MTR_{asym} image (Fig. 3e). The pixel-wise MT (Fig. 3c) and NOE (Fig. 3d), amine (Fig. 3g) and amide (Fig. 3h) CEST images were determined from multi-pool Lorentzian fitting. Interestingly, MT and NOE counteracted each other, and together (Fig. 3f) showing little changes in the ischemic region. Notably, the ischemic region shows decrease in both amine and amide CEST effects at 2 and 3.5 ppm, respectively, together contributing to MTR_{asym} reduction in the ipsilateral ischemic region.

The fitted amplitude, frequency offset and linewidth maps of each pool were shown in the Supporting Figure S1. Significant contrast between ADC lesion and ADC/CBF lesion mismatch was seen in the APT amplitude map ($P < 0.05$, Figure S1a). There was small yet significant frequency offset shift of amine pool, from 2.01 ± 0.04 ppm in ADC/CBF lesion mismatch to 1.96 ± 0.02 ppm in CBF lesion and 1.95 ± 0.02 ppm in ADC lesion (both $P < 0.05$, Figure S1b). In addition, the fitted linewidth of amine and amide pools in the ADC and CBF lesion regions were significantly smaller than that in ADC/CBF lesion mismatch yet their difference was very subtle (2.12 ± 0.08 , 2.17 ± 0.08 and 2.31 ± 0.08 ppm for the amine pool, and 1.33 ± 0.07 , 1.36 ± 0.05 and 1.42 ± 0.03 ppm for the amide pool in the ADC lesion, CBF lesion, and ADC/CBF lesion mismatch regions, respectively). Other maps were generally homogenous within the three lesion regions.

Figure 4 compares MTR_{asym} and each of the decoupled CEST effects from the ischemic lesions (diffusion, perfusion and their lesion mismatch) and their contralateral control regions. MTR_{asym} decreased significantly in the ischemic lesions from the contralateral normal regions. In addition, perfusion/diffusion lesion mismatch showed significantly less MTR_{asym} decrease than both perfusion and diffusion lesions, suggesting milder pH drop. Although ischemic lesions also show significant change in NOE and MT from those of the contralateral normal tissue, there was little difference among the lesions (i.e., diffusion lesion, perfusion lesion and their mismatch). Importantly, both amine and amide CEST effects show not only significant drop in the ischemic lesion from the contralateral normal

regions, but also significant difference in the perfusion/diffusion lesion mismatch from diffusion and perfusion lesions. This suggests that CEST effects at 2 and 3.5 ppm captures heterogeneous ischemic tissue acidosis. In addition, the magnitude of CEST at 3.5 ppm is noticeably higher than that at 2 ppm, showing that amide CEST effect dominates MTR_{asym} contrast during acute stroke. Similar results were observed under the radiation RF power of $0.75 \mu\text{T}$ (figure not included), except that amine CEST effect did not exhibit significant intralesional difference (i.e., diffusion lesion, perfusion lesion and their mismatch).

Change of T_1 values was found to gradually increase from the perfusion/diffusion lesion mismatch (0.03 ± 0.02 s), perfusion lesion (0.08 ± 0.03 s) to diffusion lesion (0.10 ± 0.03 s). We calculated the change in MTR_{asym} and each decoupled CEST effect between the ischemic lesions and their contralateral normal regions (Table 1). The magnitude of MTR_{asym} change consistently worsened from the perfusion/diffusion lesion mismatch, perfusion lesion to diffusion lesion. Although the change in NOE and MT from the contralateral normal tissue is significant, there was little intralesional difference. This suggests that both NOE and MT are not very sensitive to acidosis, which is significantly different between the diffusion lesion (infarction core) and perfusion/diffusion lesion mismatch (penumbra). Importantly, both amine and amide CEST effects show the same trend of worsened decrease as MTR_{asym} . In addition, the magnitude of amide CEST effect change was approximately two to three times of that from amine CEST effect in lesion regions, suggesting that APT effect dominates the commonly used pH-weighted MTR_{asym} during acute ischemic stroke.

Discussion

Our study decoupled multi-pool contributions to the commonly used pH-weighted MTR_{asym} analysis in acute stroke, and showed that the change in MTR_{asym} contrast is predominantly attributable to amine (2 ppm) and amide (3.5 ppm) CEST effects. As both amine and amide effects are predominantly base-catalyzed chemical exchange, so is the overall signal (i.e., MTR_{asym}) (21). In addition, the magnitude of change in APT effect was about 2–3 fold of that of amine CEST effect to MTR_{asym} at 3.5 ppm, suggesting that the commonly used MTR_{asym} at 3.5 ppm is dominated by APT effect. Because graded acidification has been well documented between ischemic core and penumbral tissue (32), our observation of little NOE and MT changes within the ischemic lesions shows that they are not very pH sensitive, consistent with previous findings (10). This provides supporting evidence to the magnetization transfer and relaxation-normalized APT (MRAPT) analysis that correction of baseline pH-independent MT asymmetry shift improves pH specificity across the brain.

During permanent ischemia, lactate buildup has been associated with tissue acidification, and hence the term lactic acidosis (33). Impaired oxygen metabolism and concomitant pH changes are crucial hallmarks during the progression of the ischemic insult. Our study showed that the commonly used MTR_{asym} progressively worsened from the perfusion/diffusion lesion mismatch, perfusion lesion and diffusion lesion. Because diffusion lesion resides within and accounts for about 80% of the perfusion lesion volume, it is not surprising that there was a trend yet insignificant change in amine and amide CEST effects between diffusion and perfusion lesions. Fortunately, the significant difference in amine and amide

CEST effects between perfusion/diffusion lesion mismatch, an approximation of ischemic penumbra and infarction core of the diffusion lesion corroborated the claim that change in MTR_{asym} is dominated by pH (3,34).

There has been tremendous interest to derive absolute tissue pH from pH-weighted imaging (35). One of the challenges is to determine how the concomitant RF irradiation effects, particularly, MT and NOE, impact the accuracy of in vivo pH mapping. To address this question, our study elucidated the origins of the commonly used MTR_{asym} contrast during acute ischemia. Recently, Zhang et al. reported a new NOE-mediated MT signal at around -1.6 ppm that shows strong contrast after acute ischemia (36). The -1.6 ppm CEST signal, however, was not apparent in our results, likely because our study used a relatively low magnetic field (4.7 vs. 7 Tesla). The small NOE difference between ischemic and normal regions may be related to changes in the aliphatic signal during severe energy failure (37) and/or an increase in neuronal lipid droplets in the infarcted region following stroke (38). MT, on the other hand, has a much broader linewidth that may include contributions from super-faster exchange species of complex/unknown origins, which may increase slightly at acidic pH (39). Fortunately, such contributions have very broad linewidth, and shall not affect the well-resolved amine, amide and NOE contributions. Interestingly, NOE and MT showed opposite trends of change, somewhat neutralizing each other. Notably, the concomitant MT and NOE effects were lower at 0.75 than those at 1.5 μT , suggesting even less contamination. A small difference in fitted water amplitude was observed between normal and ischemic regions. Because of the normalization of Z-spectrum with S_0 , this small water amplitude change is likely not related to subtle water content change following acute permanent MCAO. Instead, this likely suggests that there is a slight difference in measured water signal partition, between free water state and that interacts with MT/NOE/amine and amide.

The amide-water exchange rate was reported to approximately decrease 67% in the core region using cardiac arrest model of global ischemia (2). In our study, however, less APT reduction was observed, which may be due to the slightly different severity of acidosis between focal and global ischemia models. In addition, our study showed approximately 37% decrease of APT signal in the diffusion lesion, corresponding to about 0.2 unit of pH drop with respect to the contralateral normal region (2). This is slight less but in good agreement with that (-0.29) determined by Peek et al using autoradiography (40). Our study confirmed that APT MRI effect accounts for at least 70% of MTR_{asym} change, for both 0.75 and 1.5 μT . For longer stroke onset time, the development of edema may alter relaxation and amide proton pool size ratio noticeably, and its influence on MTR_{asym} can be taken into account given the strong relationship between T_1 and water content (41), assuming little change in the total amide proton content. In addition, a systematic calibration of pH MRI requires gold standard measurements such as ^{31}P MRS, which shall be investigated in the future. Different from acute ischemia where changes in concomitant effects and relaxation are relatively small and thus MTR_{asym} is highly pH-sensitive, the origins of MTR_{asym} in tumor may be complex with confounding factors to consider, including labile proton concentration, pH, edema, relaxation as well as potentially new sources of CEST effects, which are beyond the scope of our work. For example, significant reduction of NOE at -1.6 ppm originating from choline phospholipids was observed in brain tumor compared with

normal tissue (42,43). Nevertheless, multi-pool quantitative CEST analysis is critical for elucidation of the contrast mechanism and origins of in vivo CEST contrast. It is worthwhile to point out that an endogenous ratiometric pH imaging approach has been demonstrated, using CEST effects of amine and amide that resonates at 2 (44) or 2.75 ppm (45). Although potentially more accurate than MTR_{asym} -based analysis, this ratiometric approach requires relatively densely sampled Z-spectrum/spectra, and may be somewhat challenging at sub-high/low field for capturing evolving acute stroke. Nevertheless, the amine CEST effect should be explored in conjunction with APT MRI to improve the accuracy of pH mapping in the future.

Conclusion

Our study confirmed that the commonly used MTR_{asym} is dominated by pH-sensitive amine and amide proton CEST effects, particularly that at 3.5 ppm. We also found that there are small changes in MT and NOE effects that are not pH-dependent. As such, the commonly used MTR_{asym} index is pH sensitive, and its pH specificity can be enhanced by accounting for MT and NOE heterogeneity across the brain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Maps of (a) apparent diffusion coefficient (ADC) and (b) cerebral blood flow (CBF) in a representative acute ischemic stroke rat, with lesion (dot) and contralateral control (solid) regions overlaid on (c) the respective T_1 map.

Figure 2.

Flipped Z-spectra and resolved nuclear overhauser (NOE), magnetization transfer (MT), direct water saturation (DWS), saturation transfer effect at 2 ppm, and amide proton transfer (APT) obtained from a multi-pool Lorentzian model in (a) ADC lesion, (b) CBF lesion and (c) ADC/CBF lesion mismatch as well as their respective contralateral control regions, with corresponding asymmetry analysis illustrated in (d-f), respectively.

Figure 3.

Maps of (a) apparent diffusion coefficient (ADC), (b) cerebral blood flow (CBF), and T_1 -normalized (c) magnetization transfer (MT), (d) nuclear overhauser (NOE), (e) conventional asymmetry analysis (MTR_{asym}), (f) sum of NOE and MT, (g) saturation transfer effect at 2 ppm, and (h) amide proton transfer (APT) resolved from a multi-pool Lorentzian model in a representative acute ischemic stroke rat. The unit of CEST effect is s^{-1} .

Figure 4.

T₁-normalized (a) routine asymmetry analysis (MTR_{asym}), (b) sum of nuclear overhauser (NOE) and magnetization transfer (MT), (c) NOE, (d) MT, (e) saturation transfer effect at 2 ppm, and (f) amide proton transfer (APT) in ischemic stroke lesion and contralateral normal regions.

Table 1

Alteration of T₁-normalized saturation transfer effects in ipsilateral diffusion lesion (ADC lesion), perfusion lesion (CBF lesion) and ADC/CBF lesion mismatch with respect to their contralateral normal regions under radiation RF power (B₁) levels of 1.5 and 0.75 μ T.

		MTR _{asym}	NOE	MT	2 ppm	APT
1.5 μT	ADC lesion	-1.14 $\pm$ 0.35	0.62 $\pm$ 0.22	-0.37 $\pm$ 0.18	-0.37 $\pm$ 0.11	-0.96 $\pm$ 0.21
	CBF lesion	-0.95 $\pm$ 0.26	0.63 $\pm$ 0.15	-0.37 $\pm$ 0.16	-0.34 $\pm$ 0.08	-0.83 $\pm$ 0.18
	ADC/CBF mismatch	-0.29 $\pm$ 0.24	0.56 $\pm$ 0.20	-0.29 $\pm$ 0.20	-0.21 $\pm$ 0.06	-0.38 $\pm$ 0.17
0.75 μT	ADC lesion	-0.98 $\pm$ 0.30	0.16 $\pm$ 0.22	-0.09 $\pm$ 0.22	-0.34 $\pm$ 0.13	-0.70 $\pm$ 0.21
	CBF lesion	-0.84 $\pm$ 0.22	0.23 $\pm$ 0.15	-0.15 $\pm$ 0.17	-0.29 $\pm$ 0.10	-0.62 $\pm$ 0.14
	ADC/CBF mismatch	-0.28 $\pm$ 0.17	0.38 $\pm$ 0.27	-0.23 $\pm$ 0.20	-0.13 $\pm$ 0.15	-0.31 $\pm$ 0.07

ADC – apparent diffusion coefficient, CBF – cerebral blood flow, MTR_{asym} – magnetization transfer asymmetry analysis, NOE – nuclear overhauser, MT – magnetization transfer, APT – amide proton transfer. The unit is % /s.