Preliminary Phantom Experiments to Map Amino Acids and Neurotransmitters Using MRI

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The objective of this study was to evaluate the chemical exchange saturation transfer (CEST) effect of amino acids and neurotransmitters, which exist in the human brain, depending on the concentration, pH, and amplitude of the saturation radiofrequency field. Phantoms were developed with asparagine (Asn), γ-aminobutyric acid (GABA), glutamate (Glu), glycine (Gly), and myoinositol (MI). Each chemical had three different concentrations of 10, 30, and 50 mM and three different pH values of 5.6, 6.2, and 7.4. Full Z-spectrum CEST images for each phantom were acquired with a continuous-wave radiofrequency (RF) saturation pulse with two different B1 amplitudes of 2 μT and 4 μT using an animal 9.4T MRI system. A voxel-based CEST asymmetry was mapped to evaluate exchangeable protons based on amide (−NH), amine (−NH₂), and hydroxyl (−OH) groups for the five target molecules. For all target molecules, the CEST effect was increased with increasing concentration and B1 amplitude; however, the CEST effect with varying pH displayed a different trend depending on the characteristics of the molecule. On CEST asymmetric maps, Glu and MI were well visualized around 3.0 and 0.9 ppm, respectively, and were well separated macroscopically at a pH of 7.4. The exchange rates of Asn, Glu, BABA, and Gly usually decreased with increasing pH. The CEST effect was dependent on the concentration, acidity of the target molecules, and B1 amplitude of the saturation RF pulse. The CEST effect for Asn can be observed in a 9.4T MRI system. The results of this study are based on applying the CEST technique in patients with neurodegenerative diseases when proteins in the brain are increased with disease progression.

Keywords: 9.4T MRI, Chemical exchange saturation transfer, Amino acid, Neurotransmitter, Exchange rate

Introduction

Chemical exchange saturation transfer (CEST) MRI allows for molecular or protein imaging by detecting the signal of the saturated solute protons which are exchanged with the bulk water protons and it makes MRI sensitive to the concentrations of endogenous metabolites and their environments. The endogenous CEST MRI uses the molecules which already exist within the human body. It may be used as imaging biomarkers for disease diagnosis. The current representative endogenous CEST agents within the human body are amide (−NH), amine (−NH₂), and hydroxyl (−OH) groups.

The amide protons exist in the amino acid side chain
or the backbones of proteins. Asparagine (Asn) which has the amide proton is one of the basic components of proteins. Furthermore, amino acids in the Aβ proteins are connected with the amide bond. The amine protons also exist in the amino acid side chain or proteins. Glutamate (Glu), gamma-aminobutyric acid (GABA), and glycine (Gly) have the amine protons. GABA, which is well known inhibitory neurotransmitter in the human central nervous system, plays the principal role in reducing neuronal excitability through the nervous system. Glutamate, which is the carboxylate anion of the glutamic acid, is well known excitatory neurotransmitter that plays the principal role in a neural activation. Both GABA and Glu are important for understanding disease progression in a neurodegenerative disease.3) Glycine, which also has the amine proton, is one of the most abundant amino acids in Aβ proteins.4-6) Glycine is also used for treating several metabolic disorders and to protect a harmful side effect of certain drugs (http://www.webmd.com). The hydroxyl protons are one of the most abundant protons in the amino acids and proteins.7) The myoinositol (MI) metabolite, which has the exchangeable hydroxyl proton, is existed in the human brain.7-9) Previous MR spectroscopy studies showed that MI was increased in the brain of patients with Alzheimer’s disease compared with that in the brain of the health control subjects.9)

Previously, we evaluated the CEST effects of those materials by using a clinical 3T MRI system.10) However, the CEST effects for Asn and MI were not obtainable with the clinical system. It should be important to obtain images of those amino acids and neurotransmitter using any modality in high resolution over the entire brain noninvasively. Those amino acids compose in the proteins, metabolites, and neurotransmitters in the human brain and are altered under several different conditions in the brain.7) The objective of this study, therefore, was to have experiments to map Asn, Glu, GABA, Gly, and MI by means of CEST MRI by using a 9.4T MRI system with variable concentrations and pH values. In addition, we will measure the exchange time of those materials.

### Materials and Methods

#### 1. CEST imaging

To measure the dependence of the CEST effect of each target molecule on the concentration and the pH, each sample was prepared with three different concentrations, 10, 30, and 50 mM, and with three different pH values, 5.6, 6.2, and 7.4. After each phantom was placed in a 5-ml centrifuge tube, five small tubes were then placed in a 100-ml polyethylene (PE) bottle (Fig. 1), and every PE bottle was filled with 2% agarose solution that had been boiled to avoid movement of the inner small tubes during imaging. Detailed explanations for preparation of the phantom can be found in our previous paper.10)

All experiment was performed by using an animal 9.4T MRI system (Agilent Technologies, Santa Clara, CA) with a quadrature volume coil (63/95 mm, 400 MHz Quad Birdcage) for radiofrequency (RF) transmission and a 4-channel coil for reception. This animal MRI system has the ability to generate a saturated continuous-wave (CW)
RF pulse which is usually provided to higher saturation efficiency than a pulsed RF.\textsuperscript{11)} The full Z-spectrum was acquired with the saturation frequency offsets of ± 2396 Hz which is ±6 ppm at 9.4T and the frequency interval of 61 Hz (0.155 ppm at 9.4T) with 79 dynamics. The total duration of the CW RF saturation pulse was 5 s. We also used two B1 amplitudes at 2 μT and 4 μT to evaluate the RF power dependence of the CEST imaging contrast. A single-slice fast spin echo (FSE) imaging sequence was used to acquire the CEST images with an echo train length (ETL) of 16 and a matrix size of 64×64. The other imaging parameters were: Repetition time (TR)=5300 ms, echo time (TE)=5.88 ms, flip angle (FA)=90°, number of average=1, field of view (FOV)=55×55 mm\(^2\), pixel size=0.86×0.86 mm\(^2\), and slice thickness=2 mm. The total scan time was 55 min 48 sec (27 min 54 s for each CEST image of 2 and 4 μT, respectively). In addition, a reference image without the saturation pulse was acquired to normalize the CEST images.

2. Mapping the CEST asymmetry

CEST analysis was performed in MATLAB (Mathworks, Natick, MA, USA). After the dynamic CEST images were normalized by using the reference image, a voxel-based \(B_0\) inhomogeneity correction was performed by using the spline fitting method on a pixel-by-pixel basis to fit the full Z-spectrum to estimate the center frequency of the water resonance. After the water resonance frequency was estimated as the frequency with the lowest signal intensity it was shifted along the direction of the offset axis to 0 Hz at its lowest intensity. A voxel-based CEST asymmetry (CEST\(_{\text{asym}}\)) was calculated by using

\[
\text{CEST}_{\text{asym}} = \frac{S_{\text{sat}}(\Delta \omega) - S_0(\Delta \omega)}{S_0}
\]

Here, \(\Delta \omega\) is the frequency difference from water, \(S_{\text{sat}}\) and \(S_0\) are the signals with and without the saturation pulse, respectively. After we had calculated the CEST asymmetry for half of the frequency range, we selected the specific frequency offsets (\(\Delta \omega\)) for the target protons which were NH, NH\(_2\), and OH. The selected offset frequencies were: 2.91 ppm for Asn, 3.06 ppm for Glu, 2.76 ppm for GABA, 2.76 ppm for Gly, and 0.92 ppm for MI. Finally, a region-of-interest (ROI) was placed at the center of the phantom to obtain the full Z-spectra and the CEST asymmetry curves for each target molecule.

3. Experiments to measure the chemical exchange rate

The determination of the chemical exchange rate is important to CEST MRI because CEST signals are dependent on this exchange rate. Experiments to measure the

| Target molecule | Concentration (mM) | 2 μT pH 5.6 | pH 6.2 | pH 7.4 | 4 μT pH 5.6 | pH 6.2 | pH 7.4 |
|-----------------|-------------------|-------------|--------|--------|-------------|--------|--------|
| Asn             | 10                | 8.94        | 8.07   | 1.92   | 10.78       | 14.33  | 4.74   |
|                 | 30                | 22.26       | 14.91  | 4.38   | 33.98       | 31.19  | 8.70   |
|                 | 50                | 23.29       | 21.32  | 5.86   | 44.77       | 42.13  | 8.67   |
| Glu             | 10                | 10.04       | 10.58  | 6.50   | 13.34       | 14.31  | 12.25  |
|                 | 30                | 22.99       | 18.82  | 11.69  | 31.62       | 30.19  | 24.61  |
|                 | 50                | 32.14       | 32.86  | 16.81  | 45.93       | 48.38  | 34.69  |
| GABA            | 10                | 8.35        | 9.84   | 3.15   | 7.61        | 16.30  | 6.82   |
|                 | 30                | 24.24       | 17.80  | 4.03   | 30.93       | 33.92  | 9.30   |
|                 | 50                | 31.65       | 26.33  | 5.02   | 44.85       | 47.15  | 11.51  |
| Gly             | 10                | 4.12        | 8.96   | 4.58   | 3.28        | 11.04  | 9.93   |
|                 | 30                | 19.93       | 20.52  | 6.14   | 23.60       | 33.70  | 14.64  |
|                 | 50                | 24.00       | 27.07  | 3.94   | 30.28       | 46.89  | 9.62   |
| MI              | 10                | 17.98       | 12.63  | 15.84  | 12.12       | 14.54  | 20.90  |
|                 | 30                | 36.69       | 29.26  | 30.92  | 35.50       | 31.30  | 30.12  |
|                 | 50                | 40.40       | 43.77  | 48.63  | 40.43       | 38.31  | 44.09  |

The CEST asymmetry was measured at 2.91 ppm for asparagine (Asn), 3.06 ppm for glutamate (Glu), 2.76 ppm for both \(\gamma\)-aminobutyric acid (GABA) and glycine (Gly), and 0.92 ppm for myoinositol (MI).
chemical exchange rate were also performed by using the 9.4T MRI system. In order to identify the tendency between the exchange rate and acidity, three phantoms with different pH, 5.6, 6.2, and 7.4, at a concentration of 50 mM were used in the experiment. The B1 amplitude was 3 $\mu$T and the saturation durations [t$_{sat}$] were used with 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 s, with a minimum TR for each scan. An imaging matrix size of 32×32 and an echo train length (ETL) of 32 were used to acquire images. The other parameters were the same as the CEST imaging parameters. To identify the chemical exchange rate, the `lsqcurvefit` function built-in to MATLAB was used to fit the pseudo-first exchange

![Fig. 2. Normalized Z-spectra and CEST asymmetric curves with 10 (red), 30 (green), and 50 mM (blue) concentrations with a B$_1$ amplitude of 2$\mu$T at pH 5.6 for (a) asparagine, (b) glutamate, (c) GABA, (d) glycine, and (e) myoinositol. The left vertical axis is percentage of the CEST asymmetry, and the right one is the normalized signal ratio for the Z-spectrum.](image-url)
rate. Calculation of the exchange rate was performed by using the following equation:\textsuperscript{1,2,13}

\[
PTR = \frac{S_{w} - S_{w}(t_{sat}, \alpha)}{S_{w}} = \frac{k_{sw} \times \alpha \times X_{CA}}{R_{1w} + k_{sw} \times X_{CA} \times [1 - e^{-t_{sat} \times k_{1w} \times X_{CA}}]}
\]

Where $S_{w}$ and $S_{w}(t_{sat}, \alpha)$ are the water signals without and with saturation, respectively. $t_{sat}$ and $\alpha$ are the saturation duration and saturation efficiency, respectively. $R_{1w}$ is the longitudinal relaxation rate (1/T\textsubscript{1}) for water ($R_{1w} = 0.248$ s\textsuperscript{-1}), $X_{CA}$ is the fractional concentration of exchangeable protons of the CEST agent, and the term $k_{1} = k_{sw} \times X_{CA}$ can be expressed as the pseudo first-order exchange rate constant $k_{1}$. We fitted Eq. [1] to obtain $k_{1}$ with assumption of $R_{1w} = 0.248$ s\textsuperscript{-1} and $\alpha = 1$.

### Results

Z-spectra and CEST asymmetric curves for five target molecules were analyzed to distinguish target molecules. The CEST asymmetric maps were expressed on a color map. Table 1 summarizes all experimental results to measure the CEST asymmetry (%) for five target samples obtained from the 9.4T MRI system.

1. Concentration effect for the molecules on the CEST asymmetry

The normalized Z-spectra and CEST asymmetric curves were shown in Fig. 2 at pH 5.6 and a B\textsubscript{1} amplitude of 2 μT for different concentrations for five target molecules. The CEST effect was clearly observed at different concentrations. The CEST effect of every target molecule was increased with increasing concentrations. Furthermore, the CEST effects for Asn and MI were very large. Therefore, a high field MRI has an advantage to the CEST experiment. Fig. 3 shows the CEST asymmetric (CEST\textsubscript{asym}) maps with different concentrations for the five targeted molecules (Asn=2.91 ppm, Glu=3.06 ppm, GABA and Gly=2.76 ppm, and MI=0.92 ppm). The image scale of the asymmetric maps is 0–40%.

![Fig. 3. CEST asymmetric maps with 10, 30, and 50 mM concentrations for the five targeted molecules with the B\textsubscript{1} amplitude of 2 μT and at pH 5.6. The layout of the phantom was shown in Fig. 1. The asymmetric maps were calculated at the specific frequencies which have the highest CEST asymmetries for the five target molecules (Asn=2.91 ppm, Glu=3.06 ppm, GABA and Gly=2.76 ppm, and MI=0.92 ppm). The image scale of the asymmetric maps is 0–40%.](image-url)
concentrations for the five target molecules.

2. Acidity effect of the molecules on the CEST asymmetry

Fig. 4 show the normalized Z-spectra and CEST asymmetric curves with different pH values for the five target molecules at a concentration of 50 mM and a B₁ amplitude of 2 μT. The CEST effect of Asn, Glu, and Gly was weakly observed at pH 7.4. However, it was clearly observed at pH 5.6 and 6.2. For GABA, the largest CEST effect was observed at pH 5.6. For MI, the difference in the CEST effect was not

![Diagram](image-url)
shown with varying pH. Fig. 5 shows the CEST asym maps with different pH values for the five target molecules.

3. B1 amplitude effect of the saturation RF pulse on the CEST asymmetry

Fig. 6 shows the normalized Z-spectra and CEST asymmetric curves with different B1 amplitude values for the five target molecules at a concentration of 50 mM and pH 5.6. The CEST effect for Asn, Glu, GABA, and Gly increased with increasing B1 amplitude. For MI, reduction in the MR signal at 0.9 ppm was greater at 4 μT than at 2 μT on the Z-spectrum. However, on the CEST asymmetric curve, asymmetry showed the opposite results. Fig. 7 shows the CEST asym maps with different B1 amplitudes for the five target molecules.

4. Experiments of the chemical exchange rate

Fig. 8 shows the fitting results of the exchange rate experiments with different pH values for the five target molecules at a concentration of 50 mM. Table 2 lists the values of $k_1 = k_{sw} X_{sw}$ and the squared 2-norm of the residual (resnorm). The pseudo-first exchange rates ($k_1$) for Asn, Glu, and Gly were obviously increased with decreasing acidity. For GABA, the largest result was observed at pH 6.2. For MI, a significant difference was not shown with varying pH, however, the largest result was observed at pH 7.4.

Discussion

1. CEST asymmetry was increased with increasing the concentration of the molecules

The CEST peak shows greater asymmetry with a greater
signal loss which can be obtained by increasing numbers of exchange protons between pure water and saturated solute. Higher concentrations of the target molecule increase the number of exchangeable solute protons. Therefore, the first requirement to obtain reasonable CEST asymmetry peak in the human brain should exist at a sufficiently high concentration of the molecules with many exchangeable protons for detection.

The specific frequencies which have the highest CEST asymmetry were 2.91 ppm for Asn, 3.06 ppm for Glu, 2.76 ppm for GABA and Gly, and 0.92 ppm for MI. Our result was similar to that of the previous 7T MRI studies for Glu.
and MI.\textsuperscript{9} The CEST signal for Asn can be detected by amine protons rather than amide protons because the highest CEST\textsubscript{asym} peaks were observed at 2.91 ppm at 9.4T shown in this study and at 3.0 ppm at 3T shown in our previous study.\textsuperscript{10} The measurement of CEST asymmetry at 9.4T may be more precise than that at 3T. Previous our study showed that GABA and Glu molecules cannot separate each other with CEST signals at 3T MRI.\textsuperscript{10} However, this study showed that those molecules can be distinguished from each other. Previous our study also showed that the CEST asymmetry mapping of MI was not easy with 3T MRI because the CEST peak of MI appeared at 0.9 ppm which is too close to water peak. However, the CEST asymmetry peak of MI was much clearer at 9.4T. Therefore, a high magnetic field MRI, such as 9.4T, is beneficial to map MI.

2. CEST asymmetry was varied with varying the acidity of the molecules

The CEST asymmetry of the target molecules in this study was decreased with increasing pH at 9.4T MRI. The CEST asymmetry values for GABA, Glu, and Gly which contain amine groups were lowest with the highest pH which was consistent with the previous amine group studies,\textsuperscript{15} which showed the decreased CEST asymmetry values with increasing pH for the amine protons. Usually, the amide group has a slow exchange rate,\textsuperscript{14} and the CEST effect for the amide protons increases as pH increases because the exchange rate increases in a low pH.\textsuperscript{16,17} We showed the pseudo-exchange rates of Glu and Gly were decreased with increasing the acidity. The decreased exchange rate at pH=7.4 can be affected in decreasing the CEST asymmetry for the amine proton. The CEST effect is strongly dependent on pH which is an important factor in the CEST technique. The exchange rate and acidity are complexly related, and defined by the following equation:\textsuperscript{12}

\begin{equation}
    k_w = k_0 + k_a \times 10^{n_H} + k_b \times 10^{p_H} + k_c,
\end{equation}

where $k_w$ is the single-proton solute-water exchange rate, $k_0$ is the spontaneous exchange, $k_a$ is the acid-catalyzed exchange, $k_b$ is the base-catalyzed exchange, $k_c$ is the ionization constant of water. When the concentration of the molecules remained the same, the number of exchangeable protons also remained the same. When the pH was varied, the exchange rate changed, and therefore the CEST effect was affected by pH. Previous research has demonstrated that the CEST effect of amide and amine groups has a complex relationship with the chemical exchange rate and acidity.\textsuperscript{16,17}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig7.png}
\caption{The CEST asymmetric maps with B\textsubscript{1} amplitudes of 2 and 4 \textmu T of the saturation RF pulse at pH 5.6 and the concentration of 50 mM. The layout of the phantom was shown in Fig. 1. The asymmetric maps were calculated at the specific frequencies which have the highest CEST asymmetries for the five target molecules (Asn=2.91 ppm, Glu=3.06 ppm, GABA and Gly=2.76 ppm, and MI=0.92 ppm). The image scale of the asymmetric maps is 0~40%.}
\end{figure}
3. CEST asymmetry was increased as the $B_1$ amplitude of the saturation RF pulse was increased

A previous study showed the relationship between CEST asymmetry and $B_1$ amplitude. A previous study showed the relationship between CEST asymmetry and $B_1$ amplitude. A previous study showed the relationship between CEST asymmetry and $B_1$ amplitude. A previous study showed the relationship between CEST asymmetry and $B_1$ amplitude. A previous study showed the relationship between CEST asymmetry and $B_1$ amplitude.

Saturation efficiency, known as $\alpha$, which express the relationship, is increased with increasing $B_1$ power in a certain range and is reached a steady-state with a further increased $B_1$ power. The proton transfer rate (PTR) which represents the CEST asymmetry in the two-poll exchange model is proportional to the saturation efficiency shown in the following equations:

Fig. 8. The pseudo-first exchange rate experiments with different pH values at 9.4T at the $B_1$ amplitude of 2.35 $\mu$T and the concentration of 50 mM for (a) asparagine, (b) glutamate, (c) GABA, (d) glycine, and (e) myoinositol. Data points with different saturation durations are shown with a circle (pH 5.6), a cross (pH 6.2), and a triangle (pH 7.4). Results were plotted with a solid line at pH 5.6, a dashed line at pH 6.2, and a dotted line at pH 7.4.
where \[ C_s \] is the fractional concentration, \( k_{sw} \) is the exchange rate, \( T_1w \) is the longitudinal relaxation rate of water, and \( t_{sat} \) is the saturation time. According to Eqs. \([3]\) and \([4]\), the B1 amplitude is related to \( \alpha \). As the exchange rate is constant (\( k_{sw} \) is 1), the CEST effect in turn increases with increasing \( \alpha \). From our results, it can easily be seen that there is a tendency for the CEST effect to increase as the B1 amplitude increases. A previous study showed that the saturation efficiency is deceased with the fast exchanging rate.\(^{18}\)

CEST MRI was performed with a continuous wave (CW) RF pulse, which has several advantages. The saturation preparation depends only on the B1 amplitude and saturation duration, and maximal proton transfer enhancement can easily be achieved using sufficiently long saturation durations. However, a sufficiently long RF saturation pulse cannot be generated on most clinical scanners due to hardware limitations. Moreover, the typical applied RF power and long irradiation times can exceed the specific absorption rate (SAR) safety limits and cannot be used for in vivo imaging. Several studies have shown that Gaussian-shaped pulses provide better frequency selectivity and higher CEST contrast than Sinc- or rectangular-shaped pulses.\(^{19}\) Other studies have demonstrated optimized parameters for the RF pulse train,\(^{20,21}\) however, the golden standard for the optimum RF pulse train has not yet been reached.

### Table 2. Summary of the pseudo-first exchange rate of \( k_1 = k_{sw} \times X_{CA} \) [s\(^{-1}\)] for the five target molecules at 9.4T.

| Target molecule | pH  5.6 | pH 6.2 | pH 7.4 | k\(_1\) [s\(^{-1}\)] | Resnorm |
|-----------------|--------|--------|--------|----------------|---------|
| Asn             | 0.1277 | 0.0550 | 0.0262 | 0.0017         |         |
| Glu             | 0.1497 | 0.1155 | 0.0994 | 0.0045         |         |
| GABA            | 0.1364 | 0.1615 | 0.0156 | 0.0028         |         |
| Gly             | 0.1468 | 0.0613 | 0.0000 | 0.0005         |         |
| MI              | 0.2332 | 0.2302 | 0.2637 | 0.0087         |         |

Resnorm: the value of the squared 2-norm of the residual.
The \( k_1 \) value was measured at 2.91 ppm for asparagine (Asn), 3.06 ppm for glutamate (Glu), 2.76 ppm for both gamma-aminobutyric acid (GABA) and glycine (Gly), and 0.92 ppm for myo-inositol (MI).

```latex
\begin{equation}
PTR = C_s \star \alpha \star K_{sw} \star T_{1w} (1 - e^{-\omega / T_{1w}})
\end{equation}
```

where \( C_s \) is the fractional concentration, \( k_{sw} \) is the exchange rate, \( T_{1w} \) is the longitudinal relaxation rate of water, and \( t_{sat} \) is the saturation time. According to Eqs. \([3]\) and \([4]\), the B1 amplitude is related to \( \alpha \). As the exchange rate is constant (\( k_{sw} \) is 1), the CEST effect in turn increases with increasing the B1 amplitude. From our results, it can easily be seen that there is a tendency for the CEST effect to increase as the B1 amplitude increases. A previous study showed that the saturation efficiency is deceased with the fast exchanging rate.\(^{18}\)

### 4. Experiments of the chemical exchange rate

The exchange rates for Asn, Glu, BABA, and Gly were usually decreased with increasing pH, but that of the OH proton in MI was highest at highest at pH 7.4. In the present study, we estimated the pseudo-first exchange rate (\( k_{sw} \times X_{CA} \)) to measure the chemical exchange rate of the molecules. This experiment is based on the fact that 1) the CEST effect should reach a steady-state as the saturation duration increases\(^{12}\) and 2) CEST asymmetry can be reached at a saturation duration of almost 5 s. To fit \( k_{sw} \times X_{CA} \), we assumed that the \( \alpha \) constant was 1. However, \( \alpha \) can affect the fitting results, has a complex relationship with the exchange rate, and is hard to calculate the exact \( \alpha \) value from the experiment.\(^{12}\) To measure the exchange rate, the saturation duration is lengthened to several seconds that we used up to 10 s in this experiment. Understanding the chemical exchange rate is important in the identification and explanation of the CEST effect. To verify the exchange rate measured in this study, we should be confirmed our result by using comparisons with another reliable method such as water exchange sequence (WEX) spectroscopy.\(^{12}\)

### Conclusion

This study was performed to evaluate amino acids and neurotransmitter based on CEST imaging technique using the 9.4T MRI system. The CEST effect depended on the concentration and the acidity of the target molecules and on the B1 amplitude of the saturation RF pulse. The CEST effect for Asn can be observed at 9.4T MRI system, which was limited at a clinical 3T MRI. The exchange rates for Asn, Glu, BABA, and Gly were usually decreased with...
increasing pH. The result of this study can be used as the baseline of applying the CEST technique in patients with neurodegenerative diseases when proteins in the brain are increased with disease progression.

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Conflicts of Interest

The authors declare that they have no conflicts of interest, except that Ha-Kyu Jeong is an employee of Philips Korea.

Availability of Data and Materials

All relevant data are within the paper and its Supporting Information files.

Ethics Approval and Consent to Participate

The study was approved by the institutional review board (IRB approval number; 2015-02-006-001).

References

1. Guivel-Scharen V, Sinnwell T, Wolff SD, Balaban RS. Detection of proton chemical exchange between metabolites and water in biological tissues. J Magn Reson 1998;133: 36-45.
2. Dagher AP, Aletras A, Choyke P, Balaban RS. Imaging of urea using chemical exchange-dependent saturation transfer at 1.5T. J Magn Reson Imaging 2000;12: 745-748.
3. Cai K, Haris M, Singh A, et al. Magnetic resonance imaging of glutamate. Nat Med 2012;18: 302-306.
4. Ahmed M, Davis J, Aucoin D, et al. Structural conversion of neurotoxic amyloid-beta(1-42) oligomers to fibrils. Nat Struct Mol Biol 2010;17: 561-567.
5. Rauscher S, Baud S, Miao M, Keeley FW, Pomes R. Proline and glycine control protein self-organization into elastomeric or amyloid fibrils. Structure 2006;14: 1667-1676.
6. Harmeyer A, Wozny C, Rost BR, et al. Role of amyloid-beta glycine 33 in oligomerization, toxicity, and neuronal plasticity. J Neurosci 2009;29: 7582-7590.
7. Kogan F, Hariharan H, Reddy R. Chemical Exchange Saturation Transfer (CEST) Imaging: Description of Technique and Potential Clinical Applications. Curr Radiol Rep 2013;1: 102-114.
8. Haris M, Singh A, Cai K, et al. MICEST: a potential tool for non-invasive detection of molecular changes in Alzheimer’s disease. J Neurosci Methods 2013;212: 87-93.
9. Haris M, Cai K, Singh A, Hariharan H, Reddy R. In vivo mapping of brain myo-inositol. Neuroimage 2011;54: 2079-2085.
10. Oh JH, Kim HG, Woo DC, Jeong HK, Lee SY, Jahng GH. Chemical-Exchange-Saturation-Transfer Magnetic Resonance Imaging to Map Gamma-Aminobutyric Acid, Glutamate, Myo-inositol, Glycine, and Asparagine: Phantom Experiments. J Korean Phys Soc 2017;70: 545-553.
11. McVicar N, Li AX, Meakin SO, Bartha R. Imaging chemical exchange saturation transfer (CEST) effects following tumor-selective acidification using lonidamine. NMR Biomed 2015;28: 566-575.
12. McMahon MT, Gilad AA, Zhou J, Sun PZ, Bulte JW, van Zijl PC. Quantifying exchange rates in chemical exchange saturation transfer agents using the saturation time and saturation power dependencies of the magnetization transfer effect on the magnetic resonance imaging signal (QUEST and QUESP): Ph calibration for poly-L-lysine and a starburst dendrimer. Magn Reson Med 2006;55: 836-847.
13. Jahng GH, Oh JH. Physical Modeling of Chemical Exchange Saturation Transfer Imaging. Progress in Medical Physics 2017;28: 135-143.
14. Zaiss M, Bachert P. Chemical exchange saturation transfer (CEST) and MR Z-spectroscopy in vivo: a review of theoretical approaches and methods. Phys Med Biol 2013; 58: R221-269.
15. McVicar N, Li AX, Goncalves DF, et al. Quantitative tissue
pH measurement during cerebral ischemia using amine
and amide concentration-independent detection (AACID)
with MRI. J Cereb Blood Flow Metab 2014;34: 690-698.
16. Zong X, Wang P, Kim SG, Jin T. Sensitivity and source
of amine-proton exchange and amide-proton transfer
magnetic resonance imaging in cerebral ischemia. Magn
Reson Med 2014;71: 118-132.
17. Ward KM, Balaban RS. Determination of pH using water
protons and chemical exchange dependent saturation
transfer (CEST). Magn Reson Med 2000;44: 799-802.
18. van Zijl PC, Yadav NN. Chemical exchange saturation
transfer (CEST): what is in a name and what isn't? Magn
Reson Med 2011;65: 927-948.
19. Schmitt B, Zaiss M, Zhou J, Bachert P. Optimization of
pulse train presaturation for CEST imaging in clinical
scanners. Magn Reson Med 2011;65: 1620-1629.
20. Zu Z, Li K, Janve VA, Does MD, Gochberg DF. Optimizing
pulsed-chemical exchange saturation transfer imaging
sequences. Magn Reson Med 2011;66: 1100-1108.
21. Tee YK, Khrapitchev AA, Sibson NR, Payne SJ, Chappell
MA. Evaluating the use of a continuous approximation for
model-based quantification of pulsed chemical exchange
saturation transfer (CEST). J Magn Reson 2012;222: 88-95.