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Chemical exchange saturation transfer MRI using intermolecular double-quantum coherences with multiple refocusing pulses*

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Abstract

Chemical exchange saturation transfer (CEST) provides a new type of image contrast in MRI. Due to the intrinsically low CEST effect, new and improved experimental techniques are required to achieve reliable and quantitative CEST images. In the present work, we proposed a novel and more sensitive CEST acquisition approach, based on the intermolecular double-quantum coherence with a module of multiple refocusing pulses (iDQC-MRP). Experiments were performed on creatine and egg white phantoms using a Varian 7 T animal MRI scanner. The iDQC-MRP CEST technique showed a substantial enhancement in CEST and nuclear Overhauser enhancement (NOE) signal intensities, compared to the standard single-quantum coherence approach. In addition, the iDQC-MRP approach increased the signal-to-noise ratio of acquired saturation images, compared to the conventional iDQC approach. The new iDQC-MRP CEST sequence provides a promising way for exploiting *in vivo* CEST and NOE imaging applications.

Keywords

CEST; MRI; Intermolecular double-quantum coherence; Image contrast; Nuclear Overhauser enhancement

1. INTRODUCTION

Chemical exchange saturation transfer (CEST) MRI, a new type of magnetization transfer imaging, has recently emerged as a powerful molecular imaging approach. The technique employs the transfer of saturation from a low-concentration exogenous or endogenous pool

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of exchangeable solute protons (such as NH and OH) to the bulk water proton pool [1–3]. In the conventional CEST imaging technique, radiofrequency (RF) pulses at a specific frequency offset and an appropriate power level are used to saturate the exchangeable protons, and the saturation is then transferred into the bulk water pool, leading to a reduced bulk water magnetization. CEST imaging may provide a sensitive way to indirectly image various low-concentration solutes in tissue.

Currently, most CEST studies involve the development of diamagnetic and paramagnetic contrast agents in phantoms or animal models [4–7], and several endogenous CEST approaches have been reported for humans. These include the urea detection in the kidney [8], amide proton transfer (APT) imaging of mobile proteins and peptides in tumors [9–13], imaging of glycosaminoglycans in cartilage [14–16], and imaging of glutamates [17]. CEST imaging can also be used as a sensitive indicator of tissue pH [18], which has shown a great potential in stroke studies, where pH decreases [19–21]. Once fully developed, CEST MRI would become an important supplement to the clinical MRI studies for a broad range of human diseases.

In the past decades, intermolecular multiple-quantum coherences (iMQCs) in solution and soft tissues have been used in NMR and MRI studies. Owing to the special characteristics, such as exclusive relaxation and diffusion properties and controllable dipolar correlation distances [22–25], iMQCs have been applied in both *in vitro* and *in vivo* MRI. However, compared to the conventional single-quantum coherence (SQC), the iMQC signals are generally weak [26,27]. The intrinsically low signal-to-noise ratio (SNR) is a major obstacle for the widespread application of iMQC MRI.

It has been proven recently that the Carr-Purcell-Meiboom-Gill (CPMG) sequence can give a higher refocusing signal intensity than a spin-echo sequence in the same total duration even without an external gradient [28,29]. This is because that the CPMG sequence can well suppress the effects of imperfect hard pulses and field inhomogeneity [30–33]. Inspired by the idea of CPMG sequence, in this paper we utilized multiple refocusing pulses (MRP) to replace the single refocusing pulse in the detection period to increase the intermolecular double-quantum coherence (iDQC) signal intensity (thus, the SNR of acquired iDQC images).

The CEST effect is intrinsically small, and new and improved experimental techniques are required to achieve reliable and quantitative CEST images. Recently it was shown that the CEST-MRI effect can be enhanced in model systems by the use of iMQC [34–37]. In this paper, we will evaluate the feasibility and advantages of an improved CEST-MRI sequence that combines the iDQC CEST detection scheme with MRP, named iDQC-MRP CEST, compared to the conventional SQC and iDQC CEST methods.

2. MATERIALS AND METHODS

2.1. Pulse sequence design

The pulse sequences used in this study are depicted in Fig. 1. Fig. 1a is the SQC CEST sequence, and Fig. 1b is the conventional iDQC CEST imaging sequence. Fig. 1c is the

iDQC-MRP CEST sequence, in which a nonselective π pulse is inserted in the middle of the evolution period τ to refocus the chemical shifts and magnetic field inhomogeneities while retaining residual intermolecular dipolar interactions [38], and multiple refocusing pulses with equal time spaces in a total time duration are added to increase the iDQC buildup and fully refocus the magnetization in the imaging period [28,29]. In addition, the cases without the saturation pulses were used to show the advantages of multiple refocusing pulses in iDQC CEST imaging.

The coherence selection in the iDQC sequence is accomplished by the application of two gradients with an area ratio of 1:2. To achieve pure iDQC signal, a four-step phase cycling with the phases $(x, -x, y, -y)$ for the first RF pulse and $(x, x, -x, -x)$ for the receiver was employed.

2.2. iDQC CEST signal intensity

A two-site exchange system is the most popular model for studying the chemical exchange process [39]. It can be described symbolically by $S \rightleftharpoons W$, where S is a small pool for water-exchangeable solute protons, and W is a large pool for bulk water protons. When an off-resonance saturation pulse (off-resonance frequency ω relative to water) is applied to pool S , the remaining bulk water magnetization after saturation transfer can be approximately expressed as

$$M_z(+\omega) = \eta M_0, \quad (1)$$

where η is the saturation efficiency, depending on the exchange and relaxation parameters, as well as some possible experimental parameters, such as the B_1 magnitude and duration, and M_0 represents the equilibrium magnetization of water protons per unit volume.

According to previous studies [23,40,41], at the echo time the acquired iDQC signal from the sequences shown in Fig. 1b and c can be expressed as

$$M_{z,iDQC} = i\eta M_0 \left(\frac{3\sqrt{3}}{2} \right) \left(\frac{\tau_d}{t_2 \Delta_s} \right) J_2 \left(\frac{-t_2 \Delta_s}{\tau_d} \right) e^{-t_2/T_2}, \quad (2)$$

where J_2 is the second order Bessel function, $t_2 = +TE$, T_2 is the transverse relaxation time of conventional SQC, $\tau_d = (\gamma \mu_0 M_0) - 1$ (γ is the gyromagnetic ratio and μ_0 is the vacuum

magnetic permeability), and $\Delta_s = \frac{3(\hat{s} \cdot \hat{z})^2 - 1}{2}$ ($\hat{s}$ is the unit vector along the direction of coherence selection gradients and $\hat{z}$ is the unit vector along the Z direction). When the gradients are oriented along the Z direction, i.e., $\hat{s} = \hat{z}$, we have $\Delta_s = 1$. Based on the properties of the Bessel functions, the observable iDQC signal can be described as

$$M_{z,iDQC} = i \frac{3\sqrt{3}}{8} \eta^2 M_0^2 \gamma \mu_0 t_2 e^{-t_2/T_2}. \quad (3)$$

Equation (3) shows the iDQC signal intensity using the sequences in Fig. 1b and c, which is different from the SQC signal intensity that is proportional to ηM_0 . Hence, after the saturation transfer, we have $S_{SQC}(+\omega) \propto \eta M_0$ and $S_{iDQC}(+\omega) \propto (\eta M_0)^2$, where $S(+\omega)$ is the signal intensity at the positive saturation offset.

Further assume $S_{SQC}(-\omega) \propto \nu M_0$ and $S_{iDQC}(-\omega) \propto (\nu M_0)^2$, where $S(-\omega)$ is the signal intensity at the negative saturation offset. The factor ν depends on the upfield direct water saturation and conventional magnetization transfer effects (no CEST effect). The magnetization transfer ratio asymmetry (MTR_{asym}) is often used for CEST quantitative analysis. Thus, the SQC CEST signal can be described as

$$CEST_{SQC} = MTR_{asym}^{SQC} = \frac{S_{SQC}(-\omega) - S_{SQC}(+\omega)}{S_{SQC}} = \frac{\nu M_0 - \eta M_0}{M_0} = \nu - \eta. \quad (4)$$

Similarly, the iDQC-MRP CEST signal is

$$\begin{aligned} CEST_{iDQC} &= MTR_{asym}^{iDQC} = \frac{S_{iDQC}(-\omega) - S_{iDQC}(+\omega)}{S_{iDQC}} = \frac{(\nu M_0)^2 - (\eta M_0)^2}{(M_0)^2} = \nu^2 - \eta^2 \\ &= 2\eta MTR_{asym}^{SQC} + \left(MTR_{asym}^{SQC}\right)^2. \end{aligned} \quad (5)$$

Because $\eta = 0.7 \sim 1$ in our studies, the iDQC CEST signal is nearly twice as large as the SQC CEST signal.

2.3. Phantom preparation and MR scanning

Several tissue-like phantoms were prepared using creatine and agar. Six creatine concentrations (5, 10, 15, 20, 25, 30, and 35 mM) at pH = 7.2 were used. One phantom that consisted of two co-axial tubes was made. The inner tube was filled with 2% (w/w) agar gel and 30 mM creatine, and the outer one was filled with 2% (w/w) agar gel. Amine protons of creatine have a chemical shift of 1.8 ppm downfield from water, exhibiting a significant CEST effect. Fresh hen egg was used to demonstrate the APT and the nuclear Overhauser enhancement (NOE) effects.

All data were collected at 298 K on a Varian 7T/160mm animal MRI scanner with a 63/95 mm quadrature birdcage coil and a gradient strength up to 400 mT/m. The main magnetic field was shimmed to minimize the field inhomogeneity artifacts and the RF field (B_1) was calibrated before experiments. The imaging parameters were as follows: $G = 0.2$ T/m, $\delta = 2$ ms, repetition time = 5 s, TE = 12 ms, field of view = 128×128 matrix, and thickness = 2

mm. The RF saturation power was 0.37, 0.65, or 1.17 μT , and the saturation time was 1.5 or 3 s. In the Z-spectrum experiments, the saturation pulse frequency was swept from -3 ppm to $+3$ ppm in an increment of 0.1 ppm for creatine phantoms or from -6 ppm to $+6$ ppm in an increment of 0.5 ppm for egg. The image with RF irradiation applied at the frequency offset of 50 ppm was acquired as a reference.

3. RESULTS AND DISCUSSION

3.1. iDQC-MRP signal characteristics

Fig. 2 shows the variations of SQC and iDQC signal intensities with time for the three samples (water, agar, and egg white) under the sequences in Fig. 1 without the saturation pulses. Unlike the conventional SQC signal which dropped monotonically due to the transverse relaxation, the iDQC signals formed under the distant dipolar field rose initially, reached the maximum at a certain time, and then decayed with increasing time [23,42,43]. For the water sample (Fig. 2a and b), the maximum iDQC signal intensity was about 33% of the SQC signal intensity [37]. For the agar and egg white phantoms (Fig. 2c and d), weaker signals (a few percent of the SQC signal intensity) were obtained because the faster relaxation and other dynamic processes blurred the modulation of the distant dipolar field [44]. There was a competition between the signal growing (distant dipolar field effect) and signal decaying (relaxation, etc.). For the three samples, the maximum signals were obtained at different time. The optimal total duration time of the MRP was ~ 200 ms for the water sample and 40 $\sim$ 80 ms for agar and egg white.

According to the results, the iDQC-MRP sequence produced the strongest iDQC signals for all samples when the number of refocusing pulses $n = 4$, which was further used for CEST-MRI experiments in this study. Therefore, the iDQC-MRP CEST method can increase the SNRs of acquired saturation images, compared to the conventional iDQC approach, showing considerable potential in CEST applications *in vivo*.

3.2. SQC CEST, conventional iDQC CEST, and iDQC-MRP CEST

Fig. 3 compares the Z-spectra and MTR_{asym} spectra of a creatine phantom (30 mM) acquired with the SQC, conventional iDQC, and iDQC-MRP imaging sequences using the same parameters. According to the results, the three CEST Z-spectra were both symmetric around the water signal (assigned as 0 ppm), except at 1.8 ppm downfield from water. Comparing the regions at ± 1.8 ppm, one can see noticeable drops in the Z-spectra on the positive offset side and clear difference in signal intensities at ± 1.8 ppm. The Z-spectra and MTR_{asym} spectra show that the CEST signals achieved with the conventional iDQC and iDQC-MRP sequences are larger than that achieved with the SQC sequence.

Fig. 4 compares the imaging results on a creatine phantom that was made up of two co-axial tubes. The images with the saturation irradiation at 1.8 ppm downfield from the water resonance had the CEST effect, while the images with the saturation irradiation at -1.8 ppm upfield from the water resonance did not have the CEST effect, leading to the difference in signal intensities between these images. The SQC and iDQC images have different intrinsic signal intensities, and the SQC signal intensities measured were much stronger than the

conventional iDQC and iDQC-MRP signal intensities. Thus, the absolute difference in signal intensities was larger with the SQC sequence than with the conventional iDQC and iDQC-MRP sequences. However, the CEST signal was stronger with the iDQC-MRP sequence (0.187) than with the SQC sequence (0.114), as shown in the $MTR_{asym}(1.8\text{ppm})$ images. Although the CEST signals were similar for the conventional iDQC and iDQC-MRP sequences, the SNR of the acquired image was larger with the iDQC-MRP sequence (134, compared to 77.9 for the conventional iDQC, as measured from the $S_{sat}(+1.8\text{ppm})$ images), as described in Section 3.1.

3.3. Varied concentration study

Experiments were performed on the creatine solution (in agar) phantoms with six concentrations (5, 10, 15, 20, 25, 30, and 35 mM) at pH = 7.2. Fig. 5a shows the acquired saturation images, difference images, and CEST signal (MTR_{asym}) using the SQC and iDQC-MRP imaging sequences. Fig. 5b shows the dependence of the quantified CEST signal on the creatine concentration. When the conventional SQC was used, the CEST signal increased linearly with the concentration of creatine, as reported before [45]. When the iDQC-MRP was used, the relationship between the CEST signal and the concentration of creatine was seemingly non-linear, as predicted in Eq. (5). Approximately, the iDQC-MRP CEST signal intensity was nearly twice as large as the SQC CEST signal intensity in the concentration range studied. Therefore, iDQC-based CEST imaging could be more sensitive to the low-concentration solute in the CEST detection. This method can develop a new technique to measure concentration and distribution of creatine in muscle during exercise [46].

3.4. Hen egg study

Recently, several CEST-MRI studies at higher magnetic fields reported the presence of both the CEST and NOE effects *in vitro* and *in vivo* [47–50]. Interestingly, a fresh hen egg happens to present a big cytoplasmic compartment (egg white) that contains a high protein concentration, in which the APT and NOE effects can be detected. Fig. 6 shows a comparison of the z-spectra and MTR_{asym} spectra of egg white at 7 T with three B_1 values of 0.37, 0.65, and 1.17 μT (saturation pulse length 3 s). It can be seen that all Z-spectra had one broad signal dip at roughly 3.5 ppm downfield from the water resonance due to the APT effect and another broad signal dip at roughly –3.5 ppm upfield from the water resonance due to the NOE effect. The APT and NOE effects were power dependent. Moreover, at the same B_1 level, both the APT and NOE effects were significantly higher by the iDQC-MRP method than by the SQC method.

The further MTR asymmetry analysis showed that at the two lower saturation powers (0.37 and 0.65 μT), the downfield APT signals were lower than the upfield NOE signals, and the MTR_{asym} values were thus negative or close to zero for most frequency offsets due to the NOE. This observation was seemingly different from the results observed at 4.7 T [48]. However, at the relatively higher saturation power (1.17 μT), the downfield APT signals became higher than the upfield NOE signals. According to the MTR asymmetry analysis, APT_{SQC} was about 7.0% and APT_{iDQC} was 11.0%.

Finally, we would like to quantify the APT and NOE effects using the three-offset measurement approach [47], because the conventional asymmetry analysis seemed problematic. For the APT, we used the label image acquired at 3.5 ppm and two boundary images acquired at 5 and 2 ppm:

$$APT^* = \{[S_{sat}(5\text{ppm}) + S_{sat}(2\text{ppm})]/2 - S_{sat}(3.5\text{ppm})\}/S_0. \quad (6)$$

Similarly, the NOE can approximately be obtained from the three images acquired at -5, -2, and -3.5 ppm:

$$NOE^* = \{[S_{sat}(-5\text{ppm}) + S_{sat}(-2\text{ppm})]/2 - S_{sat}(-3.5\text{ppm})\}/S_0. \quad (7)$$

According to these equations, $APT^*_{SQC} = 8.3\%$, $APT^*_{iDQC} = 13.7\%$, $NOE^*_{SQC} = 3.9\%$, $NOE^*_{iDQC} = 7.8\%$. The results show that both the APT and NOE effects measured by the SQC approach were much weaker than those measured by the iDQC method.

4. CONCLUSIONS

CEST imaging is a unique molecular MRI technique by which low concentration solutes can be visualized indirectly through the bulk water signal. In this study, we have developed a novel and more sensitive CEST acquisition approach that combines an iDQC sequence with multiple refocusing pulses. We have systematically analyzed the signal characteristics of this iDQC-MRP CEST imaging sequence on several phantoms by comparing them with the SQC and conventional iDQC signal characteristics obtained from the standard spin-echo imaging. The new iDQC-MRP CEST imaging technique shows the substantial enhancement in the CEST signal, compared to the SQC approach. Potentially, it may also provide the CEST images with fewer artifacts and increased SNRs, compared to the conventional iDQC approach. Finally, the method can be incorporated with any imaging sequence, such as the gradient-echo and the fast spin-echo sequences, to reduce the imaging time. Future work will have to realize their potential in the *in vivo* settings.

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Fig. 1.

CEST imaging pulse sequences used in this study. (a) SQC CEST. $\tau = 0$ ms, $TE = 12$ ms (minimum). (b) Conventional iDQC CEST. (c) iDQC-MRP CEST. In (b) and (c), a pair of asymmetric z -gradients is used for the coherence selection. The iDQC-MRP sequence (c) inserts n equidistant π refocusing pulses into the detection period. $\tau = 40$ ms (creatine in agar) or 70 ms (egg white), $TE = 12$ ms.

Fig. 2.

Variations of SQC and iDQC signal intensities as a function of time for the samples of water, agar, and egg white, using the sequences in Fig. 1 without the saturation pulses. The signal intensities were normalized to the maximum SQC signal. The iDQC-MRP signal intensities generated by $n = 2, 4$, and 6 are stronger than those generated by $n = 1, 3, 5$.

Fig. 3.

Z-spectra and MTR_{asym} spectra measured with SQC, conventional iDQC, and iDQC-MRP CEST ($n = 4$) on a phantom filled with 2% (w/w) agar gel and 30 mM creatine. The RF saturation power was $1.17 \mu\text{T}$, and the saturation time was 1.5 s.

Fig. 4.

SQC and iDQC-MRP CEST ($n = 4$) images of a co-axial tube phantom. The inner tube was filled with 2% (w/w) agar gel and 30 mM creatine, and the outer one was filled with 2% (w/w) agar gel. The RF saturation power was $1.17 \mu\text{T}$, and the saturation time was 1.5 s. The difference images were defined as $S_{\text{sat}}(-1.8\text{ppm}) - S_{\text{sat}}(+1.8\text{ppm})$.

Fig. 5.

(a) SQC and iDQC-MRP CEST ($n = 4$) images of a phantom containing different creatine concentrations. (b) Dependence of creatine CEST signal ($MTR_{asy m}$) on concentration. The RF saturation power was $1.17 \mu\text{T}$, and the saturation time was 3 s. For the same concentration, the iDQC-MRP CEST signal was significantly increased, compared to the SQC CEST signal.

Fig. 6.

(a, b) Average Z-spectra and MTR_{asymp} spectra of the egg white measured at three different RF saturation powers (0.37, 0.65, and 1.17 μT) with (a) SQC and (b) iDQC-MRP CEST ($n = 4$). (c) MTR_{asymp} spectra of the egg white at 1.17 μT with two different imaging methods.