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Flip-angle based ratiometric approach for pulsed CEST-MRI pH imaging

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ABSTRACT

Several molecules have been exploited for developing MRI pH sensors based on the chemical exchange saturation transfer (CEST) technique. A ratiometric approach, based on the saturation of two exchanging pools at the same saturation power, or by varying the saturation power levels on the same pool, is usually needed to rule out the concentration term from the pH measurement. However, all these methods have been demonstrated by using a continuous wave saturation scheme that limits its translation to clinical scanners. This study shows a new ratiometric CEST-MRI pH-mapping approach based on a pulsed CEST saturation scheme for a radiographic contrast agent (iodixanol) possessing a single chemical exchange site. This approach is based on the ratio of the CEST contrast effects at two different flip angles combinations (180°/360° and 180°/720°), keeping constant the mean irradiation RF power ($B_{avg\ power}$). The proposed ratiometric approach index is concentration independent and it showed good pH sensitivity and accuracy in the physiological range between 6.0 and 7.4.

1. Introduction

Chemical Exchange Saturation Transfer (CEST) is an innovative MRI contrast mechanism that can detect molecules with exchangeable protons upon saturation with selective radiofrequency pulses [1–3]. Exchanging proton pools include endogenous protons (amide, hydroxyls), as well as exogenous ones belonging to added diamagnetic or paramagnetic agents [4–13]. Several applications have been reported, including the assessment of ischemic acidosis [14], tumor detection [4,15,16], cell tracking [17–19], proteins structural properties [20–22], metabolites [23,24], redox potential [25,26], gene expression [27,28] and enzymatic activity [29]. In particular, great attention has been dedicated to design agents able to map tissue pH [30–33]. In this context, a good example is represented by iopamidol, a clinical approved X-ray contrast agent possessing two types of amide protons whose different exchange rate has been exploited to set up a ratiometric approach for imaging tissue pH [34–37]. Similar results have been obtained with the related iopromide agent [38] or with imidazole-based pH sensors [39]. The above method relies on the presence of two exchangeable pools in order to exploit the ratiometric approach for a concentration independent measure of pH [40]. Recently, another X-ray agent containing only one mobile amide proton pool, iobitridol, was used to image tumor pH *in vivo* by ratioing the CEST effects resulting from the application of radiofrequency (RF) pulses of different power [41]. In general, the reported CEST studies relied on the application of a continuous wave (CW) irradiation scheme, consisting of a long off-resonance rectangular RF irradiation pulse. A major drawback of this irradiation scheme is represented by the high specific absorption rate (SAR) that limit the translation of the preclinical procedures to commercial human MRI scanners. Conversely, the pulsed-CEST imaging scheme addresses the hardware and SAR concerns by exploiting repetitive short RF pulses as irradiation scheme [42–49]. This saturation scheme is commonly applied at clinical level for amide proton transfer imaging [42,50–52]. Recent studies have shown that pulsed CEST contrast comprises both saturation and rotation effects (arising from an oscillating component). Consequently, the repeated rotation of the spin magnetization provides a complementary contribution to the decrease of the bulk water signal following the chemical exchange [53]. This separation of rotation vs saturation transfer-effects in pulsed CEST experiments was dubbed chemical exchange rotation transfer (CERT). Moreover, it was found that pulsed CEST contrast as a function of the flip angle (θ) is dependent on the

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chemical exchange rate (k_{ex}) of the exchanging mobile proton pool. Gochberg and colleagues have exploited these properties using the ratio of contrast at multiple θ values for assessing chemical exchange rate of endogenous amide and amine protons by keeping constant the transmitted B_1 amplitudes ($B_{avg\ power}$) at different flip angles [54].

Here, we demonstrate the application of a double-angle ratiometric approach on the clinical approved X-ray contrast agent, iodixanol, possessing only one amide proton pool (Fig. 1), for the generation of a new pH-responsive CERT contrast agent. The proposed method, called ratio of pulsed RF angles (RPA), is based on the ratio of CERT contrast at two different θ values by keeping $B_{avg\ power}$ constant. The influence of different $B_{avg\ power}$ levels, duty cycle, temperature, concentration and θ values under a pulsed CEST sequences was also evaluated.

2. Materials and methods

2.1. Numerical simulation

Simulated pulsed CEST-MRI was generated using Matlab (Mathworks, Natick, MA, USA) using the modified Bloch-McConnell equations [45,55,56] for a three pool model (water, hydroxyl and amide protons labeled as w, b and s, respectively) with a field strength of 7 T. Pulsed saturation was modeled using the discretization method, with each Gaussian pulse divided into 64 steps and the spin evolution was modeled assuming a constant B_1 amplitude within each step. The transverse magnetization was set to zero at the end of the inter-pulse period to represent the dephasing caused by crusher gradients, whereas the longitudinal magnetization relaxed toward equilibrium [44].

The variables in the model were set according to the range of values calculated from fitting Z-spectra obtained from phantom #1 (40 mM iodixanol in phosphate buffer solutions titrated in the pH range 5.5–7.9) at 37 °C with CW saturation at several irradiation powers (1, 2 and 3 μT for 5 s) in the range ±10 ppm with steps of 0.1 ppm. Specifically, the following variables were fixed to previously published values [57,58]: longitudinal relaxation time, T_{1w} = 4.0 s, T_{1b} = 1.0 s, T_{1s} = 1.0 s; T_{2w} = 1.5 s, T_{2b} = 0.8 s, chemical shift ω_b = 0.8 ppm, ω_s = 4.3 ppm; or to experimental conditions: amide proton ratio (f_s) = 0.00145 (40 mM ∗ 4/110 M), hydroxyl proton ratio (f_b) = 0.0033 (40 mM ∗ 9/110 M). The following parameters were solved from numerical fitting: exchange rates for amide (k_{ex}) and hydroxyl groups (k_{wb}) and T_{2s} for each pH value.

A range of parameter values were simulated for pulsed CEST-MRI: FA (θ) varied from 45° to 900° with intervals of 15°, T_{1w} (3.0–3.7–4.4 s), T_{2w} (1.5–2.0–2.5 s), T_{1s} (1.0–2.0–3.0 s), T_{2s} (10–20–30 ms), f_s (0.007–0.0011–0.0018), k_{ex} (21–47–108–150 Hz), dc was set at 30% and 50%.

2.2. In vitro

2.2.1. Phantom preparation

Three sets of phantoms were prepared by dissolving iodixanol (Visipaque®, GE Healthcare) in different media. A phantom containing several vials of 40 mM iodixanol in phosphate buffered solution were pH titrated between 5.5 and 7.9 and used for calculating the chemical exchange rates under CW irradiation and for the CERT experiments under Gaussian-train irradiation scheme. A second phantom was prepared by dissolving iodixanol in phosphate buffer solution at pH = 7.2 at different concentrations (2.5–5.0–10.0–20.0–40.0 mM) to investigate the concentration independence of the proposed ratiometric approach. A third phantom was prepared by dissolving 40 mM iodixanol in reconstituted human plasma (Seronorm Human, SERO AS ASKER, Norway) at several pH values (6.3, 6.7, 7.0, 7.4) to mimic *in vivo* conditions with the presence of several proteins and metabolites at physiological concentrations.

2.2.2. Magnetic resonance imaging

Pulsed-CEST experiments were acquired on a 7 T Bruker Avance 300 scanner (Bruker BioSpin, Ettlingen, Germany) equipped with a micro 2.5 MICWB 30 mm quadrature (1H) imaging probe. Z-spectra were acquired sampling the frequency offsets from −10 ppm to 10 ppm, with step size of 0.1 ppm. The pulsed-CEST scheme exploited a series of Gaussian irradiation pulses for the saturation part and a single-shot (with centric encoding) fast spin-echo imaging readout. After each pulse, crusher gradients (with alternating sign) were applied to spoil residual transverse magnetization. Each irradiation pulse had duration τ_P, flip angle θ, interpulse delay τ_D and the pulse train repetition (PTR) is given by $\tau_P + \tau_D$. $B_{avg\ power}$ levels were set to be 0.5, 1.0 and 2.0 μT with different values of duty cycle (dc) of 50% and 30% for a total irradiation time of 5 s. To test the predicted angular dependence, 15 values between 45 and 900° were acquired for each $B_{avg\ power}$ level and dc conditions.

For pulsed-CEST imaging, $B_{avg\ power}$ can be calculated by using the following equation [59]:

$$B_{avg\ power} = \sqrt{\frac{1}{PTR}\int_0^{PTR} B_1^2 dt} = \sqrt{\frac{p_2}{dc}} \cdot \frac{\pi\theta}{180 \cdot \gamma \cdot p_1 \cdot PTR} \quad (1)$$

where $B_{avg\ power}$ is the field strength of a continuous wave irradiation with the same average power as the pulsed-CEST, p_1 is the ratio of the average amplitude to the maximum amplitude of the irradiation pulse, p_2 is the ratio of the average of the square of

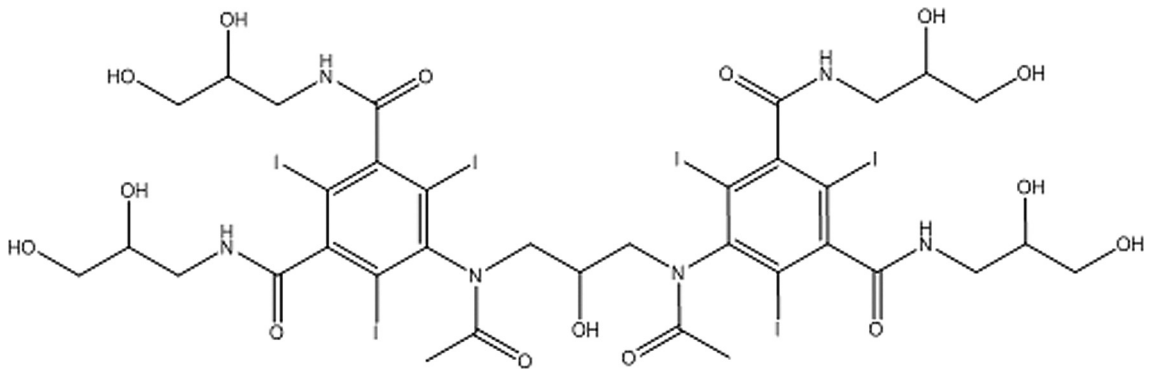

Fig. 1. Chemical structure of the radiographic agent iodixanol.