Imaging paraCEST Agents Using Frequency Labeled Exchange Transfer (FLEX) MRI

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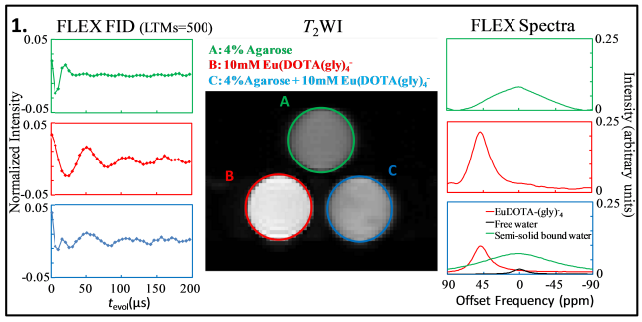
Introduction: Currently, there is an increasing effort underway to design paramagnetic chemical exchange saturation transfer (paraCEST) agents for biological target identification by MRI such as temperature, glucose, pH, nitric oxide, and enzyme activity (1-3). The main benefit of paraCEST agents over their diamagnetic counterparts is that they can be engineered to have large chemical shift differences ($\Delta\omega$ >10 ppm) between the resonances of the exchanging pools and the water resonance. Consequently, saturation pulses can be applied well away from the water resonance, thus avoiding off-resonance saturation of bulk water. In addition, faster exchanging compounds can be used while still adhering to the slow exchange limit ($k_{ex} < \Delta\omega$) necessary for CEST. This allows for more saturated spins to be transferred within a given time frame, resulting in an increase of transfer efficiency as expressed by the proton transfer ratio (PTR). However, detection of paraCEST compounds with such rapid exchange rates ($k_{ex} \sim 10^4$ s^{-1}) requires a high B_1 level to achieve at least partial RF saturation before exchange occurs. Unfortunately, this comes with an increase in conventional magnetization transfer (MT) effects in biological tissues, which, despite the large $\Delta\omega$, can still complicate the analysis in paraCEST studies. Frequency labeled exchange transfer (FLEX) (4) is a new approach that detects exchanging protons using frequency transfer instead of saturation transfer. Using spectroscopy, it has been shown that FLEX allows detection of rapidly exchanging water molecules k_{ex} >10,000s^{-1} without interference of MT effects by separating these out using FLEX time domain analysis (5,6). This presents the potential for imaging the distribution of functional paraCEST agents in biological tissues. The aim of this study was to implement the FLEX technique on preclinical MRI systems and to evaluate the possibility for imaging of paraCEST agents.

Material & Methods: The FLEX approach has been described in detail previously (4-6). Briefly, the pulse sequence contains a preparation time (t_{prep}) consisting of a series of n label-transfer modules (LTMs). Within each LTM, exchangeable solute protons are frequency labeled and transferred to water. In the labeling part, a pair of selective $90_x/90_{-x}$ RF pulses is applied, in between which chemical shift evolution of the exchangeable protons occurs over a period, t_{evol}. Signal amplification occurs because fresh z-magnetization is present for the solute protons at the start of each LTM, allowing multiple opportunities to transfer labeled protons to the water when applying multiple modules during the preparation time. The PTR can be expressed as Eq. 1, where χ_s is the fractional concentration of the solute (s) protons and λ_s the labeling efficiency. In contrast to CEST, which detects the decrease of water signal, FLEX measures modulation in the water signal as a function of t_{evol} in a manner depending on the frequency difference between the offset frequency (o1) of the 90_x excitation pulse and the solute resonance ($\Delta\omega_{so1}$). The effect on the water signal can be described (Eq. 2) by a free induction decay (FID). Three phantoms in tris buffer (pH=7) were prepared for MRI acquisition. One contained a 10 mM paraCEST solution of EuDOTA-(gly)$^-_4$, a second the same paraCEST solution mixed with 4% agarose in order to generate MT effect similar to that observed in biological tissue, and a third a 4% agarose solution as a control. All MRI data were acquired at 37°C on a Varian 9.4T MRI system. FLEX images were measured by multiple LTMs, followed by a fast spin-echo acquisition with a TR of 5 s, a TE of 8.65 ms, a FOV of 1.5 cm, a slice thickness of 5 mm, and a matrix size of 64 *64 mm. The FLEX FID signal was acquired by varying t_{evol} from 1μs to 196 μs using time intervals of 5 μs (dwell time). The o1 of labeling pulse = 95 ppm was calculated to avoid water excitation (0 ppm) (7) and keep high efficiency for labeling paraCEST agents (50 ppm). The other parameters for FLEX data acquisition were: t_{exch} = 400 μs and number of LTMs = 0, 100, 300, 500, 700, 1000, and 1500. Signal differences with respect to the average water signal after decay were normalized with respect to the water signal without LTMs.

$$PTR_s = x_s \cdot \lambda_s \cdot (1 - e^{-k_{ex,s} \cdot t_{exch}}) \cdot \sum_{i=1}^{n} e^{\{-1+(i-1)/n\} t_{prep}/T_{1w}} \quad [1]$$

$$I_w = \sum_s PTR_s \cdot e^{-(k_{ex,s}+1/T_{2s}^*) \cdot t_{evol}} \cdot \cos(\Delta\omega_{so1} \cdot t_{evol}) \quad [2]$$



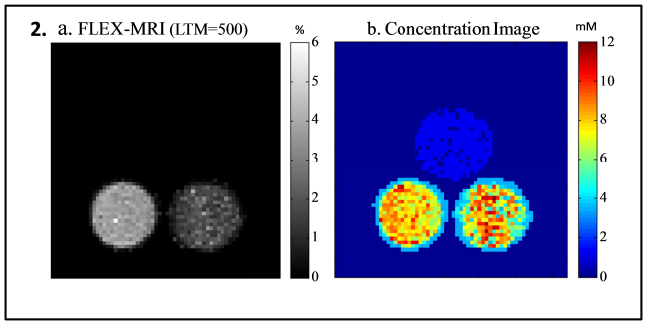


Fig. 1. FLEX FIDs (LTM=500) and corresponding Fourier-transform spectra in three different phantoms (Agarose only: green FID; paraCEST solution: red FID; paraCEST solution mixed with agarose: blue FID). Fig. 2. FLEX-MRI (LTM=500) and calculated concentration image of paraCEST agent.

Results & Discussions:

The FLEX FIDs and spectra in the three samples are shown in Fig.1. The rapid initial decay in the FLEX FID of the agarose phantom corresponds to the broad band FLEX spectrum (green line) of the short T_2* component of the semi-solid MT effect. The water signal modulation in the paraCEST agent solution modulated at a frequency $\Delta\omega_{so1}$ of 18,000Hz and generated a peak at 50 ppm (red line). Notice that the 50 ppm resonance in the FLEX spectrum (red line) is much larger than the free water (0 ppm) due to the limited excitation of bulk water. In the combined sample (paraCEST agent mixed with agarose), the FLEX FID (blue line) indicates a rapid signal drop due to MT in the first 20-30 μs, followed by a more gradual decay. Using time domain analysis with the prior knowledge (Eq.2) about frequencies of the bound solute water (18,000 Hz) and free water (38,000 Hz) components, the fitted FLEX spectrum could be separated into three signal components (green: MT effect; red: paraCEST agent; black: free water). FLEX-MRI obtained by pixel-by-pixel time domain fitting (Fig. 2a) shows the paraCEST signal in phantoms with the paraCEST agent but not in the agarose-only sample. The mismatch of signal magnitude between the pure solution (2.9%) and solution mixed with agarose (1.5%) is likely due to a shorter T_{1w} and a potential increase in partial saturation of the water pool due to MT exchange broadening. Fortunately, by fitting the FLEX signal as a function of number of LTMs according to the FLEX theory (Eqs.1,2), we determined the concentration of the paraCEST agent to be identical, 6.6 mM for pure solution and 6.4 mM for agarose phantoms (Fig. 2b). The concentration underestimation is probably due to exchange transfer of additional dephased signal that was not returned to the z-axis after the evolution time, which accumulates when applying multiple LTMs.

Conclusion: The first images from FLEX-MRI experiments on a paraCEST agent solution at physiologic temperature and pH are presented. The FLEX approach allowed separation of MT and exchange transfer effects in the time domain and provided quantitative concentration images. These findings demonstrate the potential for in vivo imaging of paraCEST agents.

Reference: 1. J. Zhou, et al., PNMRS, 48: 109-136, 2006. 2. A.D. Sherry et al., Annu Rev Biomed Eng, 10: 391-411, 2008. 3. S. Aime, et al., Acc Chem Res, 42: 822-831, 2009. 4. J.I. Friedman., et al., JACS, 132:1813-1815, 2010. 5. C.Y. Lin, et al., Proceedings of 19th ISMRM, 709, 2011. 6. van Zijl et al., MRM, 65:927-948, 2011. 7. J. Cavanagh, et al., in Protein NMRS: principle and practice, 912, 2007.