Detection of MRI contrast based on chemical exchange saturation transfer (CEST) in the presence of the T2/T2* contrast agent Feridex

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Introduction

Neural stem cells have the ability to migrate and penetrate glioblastoma multiforme, thus offering new opportunities for drug delivery or homing of cells expressing anti-tumor genes. However, in order to image stem cell migration, their tumor interaction, and tumoral therapeutic response, it is necessary to develop techniques that allow labeling and imaging of both stem cells and tumor cells simultaneously. One option is to use contrast agents that rely on two different relaxation mechanisms. One example of labeling tumor cells is using contrast agents based on Chemical Exchange Saturation Transfer (CEST) [1, 2]. For fast dividing tumor cells, the CEST label is preferably a reporter gene that will produce a constant level of contrast without dilution by dividing cells, as in the case of the lysine rich protein (LRP) reporter gene [3]. Stem cells proliferate much slower and can be labeled with iron oxide nanoparticles [4], which induce a stronger contrast but dilute out with every cell division. In order to assess the feasibility of using these two contrast agents simultaneously, we conducted an *in vitro* study. This study shows that there is a range of concentrations in which the two contrast agents can be distinguished from each other.

Materials and Methods

Glass capillary phantoms filled with poly-L-lysine (PLL, MW 22kDa) and different concentrations of SPIO (Feridex; panels 1A, E) were measured on 11.7 T Bruker MR spectrometer. A spin-echo sequence was used for determination of T2 (TR 3000ms, TE 9.2, 20, 30, 40, 50, 60, 70, 80 and 90 ms) and CEST [5] imaging was performed with the parameters TR/TE 9000/6.35ms, saturation power 0.5µT, saturation time 4000ms, and Δω=± 3.758ppm from the water 1H frequency.

Theory

For applications in which the different contrast agents will be used to label different cells, each contrast material will affect a different population of water assuming slow exchange of water between cells. For measuring the signal S without saturation pulse the following expressions can be used: $S = \sum_i f_i \bullet Si$ and the dependence on the relaxation times described by $S_i = S_0(1-e^{-TR\bullet R_1})e^{-TE\bullet R_2}$.

The following equation applies for the measured CEST effect or proton transfer ratio (PTR) [5]:

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

in which k_{sw} is the contrast agent-water exchange rate, X_{CA} is the fractional concentration of exchangeable protons of the contrast agent, t_{sat} is the saturation time, α is the saturation efficiency, and the term k_{sw} X_{CA} accounts for back exchange of saturated water protons to the contrast agent, which will occur when the exchange rate and/or the concentration of exchangeable protons for the CEST agent are very high. α is determined as: $\alpha = \omega_1^2 / (\omega_1^2 + pq)$ and R1 and R2 contrast agents can affect the PTR through p and q which defined as:

$$p = R_{2s} + k_{sw} - k_{sw}^2 \bullet X_{CA} / (R_{2w} + k_{sw}\bullet X_{CA})$$ and $$q = R_{1s} + k_{sw} - k_{sw}^2 \bullet X_{CA} / (R_{1w} + k_{sw}\bullet X_{CA})$$

Results and Discussion

In order to evaluate the interactions of the two contrast agents an *in vitro* study was conducted. Figure 1 shows two 5 mm NMR tubes, each containing 5 inner capillaries imaged sequentially using two imaging protocols The first protocol was aimed to measure T2 (sensitive to the presence of iron; panels C, G, I) and the second protocol sensitive to CEST and included saturation pulses at different frequencies (panels D, H, J).

Lower concentrations of Fe did not affect the CEST contrast (capillaries 1-4), with the CEST agent clearly detectable. However, at higher Fe concentrations (capillary 5-6) the iron was found to significantly reduce the CEST effect, which is most likely explained by broadening of the water line width. The Fe range in which the Feridex interferes with CEST, 50-500 µg Fe/ml, is orders of magnitude higher than that encountered with Feridex labeled cells *in vivo.* Thus, simultaneous detection of two cell populations labeled with either CEST agents (PLL or the LRP reporter gene) or Feridex should be possible.

Furthermore, it is also evident that PLL does not have much of an effect on the iron-sensitive T2 maps, as the T2 for 250 µM PLL+500 ng Fe/ml Feridex was 31.9±3.5msec, whereas the T2 for 500ng Fe/ml Feridex without the addition of PLL was T2=32.6±5.6 (n=3).

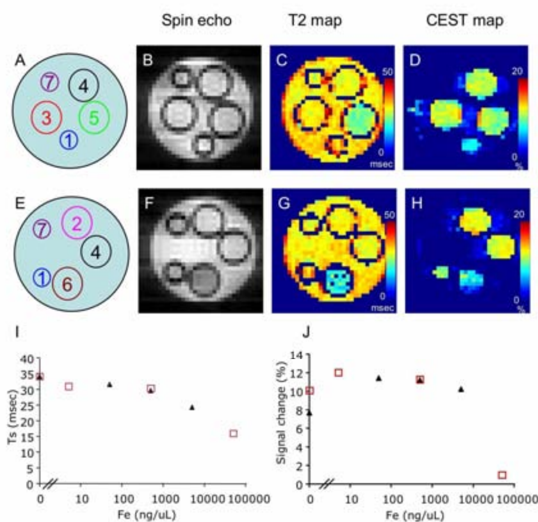

Figure 1. A,E *) Phantom layout: capillaries 1-6 all contain 250 µM Poly-L-Lysine , 22 kDa, with different Feridex™ concentrations of (1) 0 ng/ml, (2) 5 ng/ml, (3) 50 ng/ml, (4) 500 ng/ml, (5) 5 µg/ml, and (6) 50 µg Fe/ml; phantom (7) contains 500 ng Fe/ml of Feridex™ without the addition of Poly-L-Lysine.* ***B,F****) Reference T2 weighted image.* ***C,G****) T2 maps and (****D,H****) difference maps between RF irradiation at Δω=±3.758 ppm from the water frequency.* ***I)*** *T2 dependency of feridex in the presence of Poly-L-Lysine.* ***J)*** *CEST (signal change) dependency on Feridex concentrations in the presence of Poly-L-Lysine.*

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