CytoCEST: Cells as CEST agents

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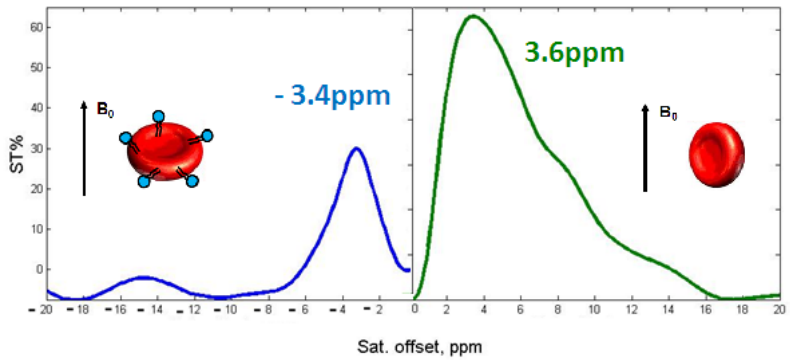
Purpose: Among the proposed MRI contrast agents (CAs), CEST (Chemical Exchange Saturation Transfer) ones have received much attention, in virtue of their ability, unique in the field of ^{1}H-MRI agents, to generate a frequency encoded contrast. Through this mechanism, the contrast can be switched on and off at will, changing the radiofrequency pulse, thus making possible the simultaneous detection of multiple agents even co-localized in the same region. Unfortunately the main drawback of CEST agents is the low sensitivity, lower than conventional MRI CAs. The sensitivity of CEST agents depends on the number of equivalent mobile protons irradiated. Therefore, systems bearing huge numbers of exchanging protons should have large sensitivities (e.g.. LipoCEST) (1,2). In this work we have developed a labeling procedure allowing to exploit the huge number of intracellular water protons to generate a CEST contrast. The separation between the NMR signal of "bulk" and intracellular water protons is provided by entrapping inside the cell a paramagnetic shift reagent (SR).

Methods: Human erythrocytes or J774A.1 cells (murine macrophages) have been labeled with Shift reagents (SRs) like Ln-HPDO3A complexes (where Ln=Dy, Tm, Yb, Eu, Gd) by using different internalization routes. Erythrocytes have been loaded by using hypotonic swelling (3) and macrophages by using pinocytosis, electroporation or hypotonic swelling. The visualization of cells has been possible by acquiring the Z-spectra at 7T on a Bruker Avance 300MHz spectrometer. A typical RARE spin–echo sequence (RARE factor varying from 2 to 32 depending on the T2 of the sample) with an echo time of 3 ms and a TR value of 5 s has been used (isotropic 64 x 64 acquisition matrix with FOV of 10 mm and slice thickness of 1 mm). The whole sequence has been preceded by a saturation scheme consisting of a continuous rectangular wave pulse 2 s long with a B2 intensity of 3μT. The Z-spectra have been interpolated by smoothing splines to identify the zero-offset on a pixel-by-pixel basis of the bulk water and, then, to assess the correct ST% value over the entire range of frequency offsets investigated.

Results: The Z-spectra of erythrocytes labelled with DyHPDO3A by hypotonic swelling shows a saturation Transfer of *ca*. 50% at 3.6 ppm from water signal. By making the labelled erythrocytes interact with paramagnetic micelles with a sign of magnetic anisotropy opposite to that of cell membrane, the Z-spectrum changed and displayed a ST of *ca*. 30% at -3.4 ppm from water signal (fig.1).



Discussion: The water resonance, in the presence of the paramagnetic SR, undergoes a shift that is due to two main contributions: i) the dipolar and ii) the bulk magnetic susceptibility (BMS) effects. The former contribution requires a chemical interaction between the paramagnetic complexes and the water molecules, while the latter effect depends on the shape of the "container" in which the SRs are confined. Cells are not spherical compartments (in particular erythrocytes) so they should be able to orient themselves into a magnetic field. The driving force for this orientation is represented by the magnetic anisotropy of the phospholipidic membrane. The herein reported shift is namely due to BMS contribution in agreement with what expected for non-spherical shaped cells. The ability to switch the sign of BMS after the interaction with the paramagnetic micelles is due to the change in the orientation of erythrocytes in the magnetic field.

Conclusions: The herein reported work shows that upon loading of paramagnetic shift reagents it is possible to use intracellular water protons as mobile proton pool to generate a very efficient CEST contrast.At the best of our knowledge this is the first proof of concept demonstrating the possibility to use cells themselves as CEST agents. The use of cellular system as CEST agent has two main advantages: i) the extreme biocompatibility of this system and ii) the very high sensitivity.

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