Macrophage tracking with heteronuclear proton MRI

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Target audience: MR physicists, biologists, chemists, and physicians interested in novel methods for cell tracking

Purpose:

In vitro labeling and administration of cells is a common strategy in *in vivo cell* tracking by MRI. Usually, for cell tagging Gd- or Fe-based contrast agents are employed and contrast changes that result in the vicinity of these markers are detected. Either positive or negative contrast changes can be detected by locally altered relaxation times. However, other sources of contrast or artefacts often hamper the unambiguous identification of the labeled cells. To overcome this lack of specificity, a number of techniques have been proposed, including labeling with ^{19}F-containing compounds and detecting the heteronuclear fluorine signal, which has virtually no background *in vivo*. However, detection of ^{19}F signal requires dedicated instrumentation. Here, we show that heteronuclear proton MRI can be employed for cell tracking. We directly detect the strongly shifted resonance of Thulium-1,4,7,10-tetramethyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (Tm-DOTMA) [1,2], which was used to label bone marrow-derived macrophages (BMDM).

Methods:

The 12 magnetically equivalent methyl protons of Tm-DOTMA have a chemical shift of ~100 ppm and a very short T_1 of 5.3ms. Using ultra-short echo time (UTE) MRI with narrow frequency excitation and narrow acquisition bandwidth this resonance becomes detectable independently from the water signal (Fig.1). Phantom experiments were performed on aqueous solutions of Tm-DOTMA, Dy-DOTMA, and Tb-DOTMA at concentrations of 0.25 mM or 10 mM. *Cell labeling*: BMDM were labeled with 15 or 20 µmol Tm-DOTMA / 10^6 cells by incubation for 24 h. Trypan blue exclusion test showed no significant toxicity. Due to the self-fluorescence of Tm-DOTMA (ex: 253 nm / em: 460 nm), successful labeling could be visualized with fluorescence microscopy. Non-localized *in vitro* MR spectroscopy of labeled cells showed an average of 9 -10 x 10^{10} Tm-DOTMA molecules per cell. *In vivo*: Inflammation was induced by a subcutaneous injection of 100 µl acrylamide gel containing 30 µg LPS / ml. Either 1 d prior to (n=2) or 2 – 4 h after (n=6) injection of the gel, the mice received 4 – 6 x 10^6 labeled cells i.v.. To detect the migrating BMDMs MRI was performed on a Bruker Biospec 94/20 with a 35 mm ^{1}H volume coil over a period of 8 days. To avoid relaxation losses a 3D UTE sequence was employed, applying a 365 µs gaussian excitation pulse to achieve selective excitation of the desired resonance line. The short T_1 of Tm-DOTMA allowed for efficient sampling with high flip angles (Ernst angle was used) and very short TR of 3.2 ms. Further parameter: TE=0.19 ms, 32^3 matrix, spatial resolution 1.25 x 1.25 x 2.5 mm^3, acquisition bandwidth: 25 kHz.

Results:

Phantom experiments showed that the Tm-DOTMA signal was detected with threefold higher signal to background ratio if UTE was used compared to gradient echo methods (Fig. 2). Using different Lanthanoid-DOTMA complexes multi-color MRI could be realized due to the largely different resonance frequencies of the respective methyl groups. In vivo, Tm-DOTMA signal was detected in the bladder (day 1) and in liver, spleen and gel pellets over 8 days. Within 2 h scan time signal-to-background values ranged from 5 in the liver to 3 in the gel pellets (Fig.3), showing that detection of the Tm-DOTMA signal in BMDMs that had migrated to the inflammation was possible during the 8 days of examination. From a reference tube with a 0.25 mM Tm-DOTMA solution placed next to the mice (top right in Fig. 3), the in vivo detection limit was estimated to be slightly below 10^4 BMDMs. Origin of the signal from migrated BMDMs was confirmed by a combination of histology and laser ablation inductively-coupled plasma mass spectrometry (LA-ICPMS), showing both BMDMs and Tm around the injected gel.

Conclusions:

These results show the feasibility of obtaining virtually background-free signal of BMDMs labeled with Tm-DOTMA using 3D UTE. Migration of BMDMs to the site of inflammation could be observed and the label detected for more than one week. This approach of heteronuclear proton MRI may provide a versatile tool to follow labeled cells *in vivo* and thus facilitate molecular MRI without the need for extra MR equipment.

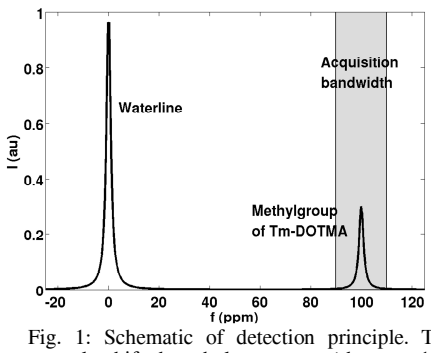

Fig. 1: Schematic of detection principle. The strongly shifted methyl resonance (shown x 10^4) is excited and detected separately from the water line.

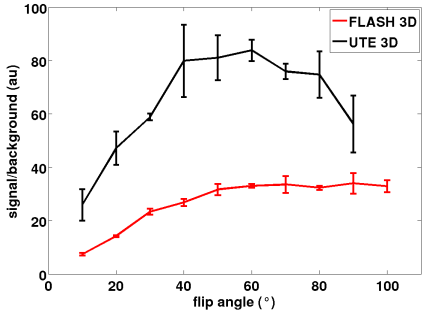

Fig. 2: Flip angle dependence of signal/ background (in a.u.) measured in a 10 mM Tm-DOTMA solution at 9.4 T.

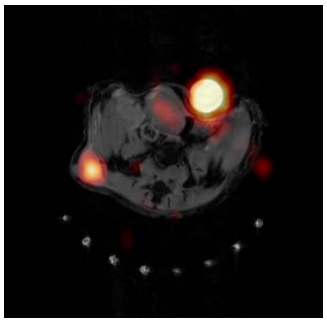

Fig. 3: In vivo Tm-DOTMA image (yellow-red color) of labeled BMDMs in a mouse with LPS-gel pellet, overlaid onto a conventional T2w MRI. Top right: reference tube, 0.25 mM solution of Tm-DOTMA.

References: 1. Hekmatyar, et al. (2005) Magn Reson Med 53:294-303. 2. Delli Castelli, et al. (2010) J Control Release 144:271-9.