Combined positive contrast and relaxation in the rotating frame for molecular imaging of in-vivo SPIO labeled cells

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Abstract:

Contrast agents incorporating superparamagnetic iron-oxide (SPIO) nanoparticles have become promising probes for *in-vivo* molecular imaging of cells using MRI. We propose the combination between positive contrast (PC) techniques [1] and relaxation in the rotating frame (Tρ) schemes [2] to fine-tune the levels of image contrast to that of the SPIO concentrations. In addition, this combination of methods may increase the specificity for SPIO of the positive contrast technique and could be used for quantification purposes. We tested these experiments on phantoms and model animals.

Introduction:

Conventional MRI detects SPIO as a loss of signal (negative contrast) in gradient-echo sequences due to magnetic susceptibility anisotropy. However, quantification of negative contrast (NC) and imaging of organs such as lungs is problematic. Positive contrast could overcome these problems, though the specificity to SPIO detection might be a limitation [3]. We sought to find ways to improve the PC performance with regard to specificity, range of contrast levels and speed. In particular, our purpose was to use these techniques to visualize SPIO labeled macrophages in order to monitor the response of the host organisms to infection with *Pseudomonas aeruginosa* (PA). This type of infection is the most common cause of sepsis in burned patients and among those rendered neutropenic as well as the most common bacterial cause of lung infection in cystic fibrosis patients [4-6].

Methods:

We studied five burn injured-mice and infected with PA and compared them to controls. Prior to the mice measurements, the experiments were tested on a phantom. We implemented the new methods and acquired images on a 4.7 T horizontal bore magnet (20 cm bore, Magnex Scientific) using a Bruker Avance console and a 7 cm (ID) linear birdcage volume coil (Bruker). R.f. field homogeneity was better than 10% within a sphere of 3 cm radius in the center of the large volume coil.

Positive contrast was achieved with selective water (400 Hz bandwidth) and fat suppression (800 Hz bandwidth) sinc10H pulses followed by spoiling gradients (1 ms, 100 mT/mm). The images were recorded using spin-echo (Nr = 1) or turbo spin-echo (Nr = 2) with the same time parameters (TR = 2s, TE = 7ms). A block for relaxation in the rotating frame was incorporated in the RARE [7] sequence using an MLEV-4 scheme [8] with WURST-8 [9] adiabatic inversion pulses. The r.f field amplitude and mixing time (t_{mix}) were varied in the range 0-2 kHz and 0-50 ms (Nρ = 0-4), respectively. The combined PC and Tρ (PC+ Tρ) pulse sequence [10] is given in Figure 1. PC alone is obtained by omitting the MLEV-4 block from the diagram.

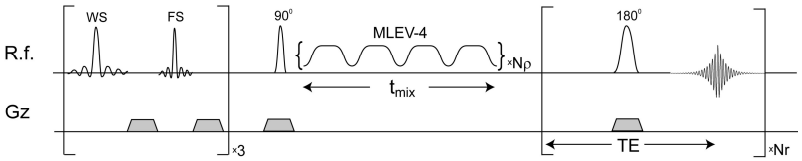

Figure 1. Pulse sequence for positive contrast and relaxation in the rotating frame. The water and fat suppression module is repeated 3 times. Relaxation in the rotating frame is achieved under MLEV-4 (Nρ = 0-4) adiabatic irradiation (WURST-8). RARE (Nr = 2) or spin-echo (Nr = 1) are used as a read-out schemes (read-out and phase encoding gradients are not shown).

Typically 10 axial images (1 mm thickness, 1 mm gap) were acquired in the burned and infected region with FOV of 3x3 cm, matrix size of 128x128, and 4 scans were averaged for signal to noise. Typical measurement time was 2-3 h per mouse. Control images were also acquired with gradient-echo (FLASH), spin-echo and turbo spin-echo (RARE). Mice were anesthetized (1% isoflurane) and imaged 24h post burn-infection and 18h post SPIO (Ferumextron-10, 500μg Fe, tail IV) bolus injection.

Results:

We investigated PC alone and in combination with both longitudinal (T1ρ) and transverse (T2ρ) relaxation in the rotating frame. Our observations indicate qualitatively that: (i) it is possible to obtain PC for SPIO (Ferumextron-10) concentrations in the range 0.5-5 mM, (ii) positive contrast levels are not linear over the entire concentration range (more sensitive detection for 0.5-1 mM), (iii) turbo spin-echo (Nr = 2) can be used to speed-up image acquisition compared to spin echo (Nr = 1), (iv) use of T2ρ benefits more than T1ρ the combination with PC, (v) by adjusting the r.f. field amplitude and mixing time it is possible to fine-tune the contrast for specific SPIO concentrations, (vi) combination of PC and T2ρ (PC+T2ρ) provides less artifacts than the PC alone for *in-vivo* mice imaging, and (vii) the methods are sensitive to the accumulation of SPIO labeled macrophage at the infection site, as proven by test and control experiments. Comparisons of control images with PC and PC+T2ρ are shown in Figure 2 for the phantom, and in Figure 3 for the mice (one test mouse and two control mice).

Discussions:

We believe that we improved our specificity for SPIO detection and the ability to follow the *in-vivo* macrophage accumulation and progression of infection. We have additional parameters (r.f amplitude and mixing time) that can be varied to obtain image contrast and quantify SPIO concentration. Further development includes the estimation of the relaxivities in the rotating frame for SPIO and mice tissues and the use of this information in a quantification procedure. In the long run, we hope that the developed methods will provide important data to guide therapeutic drug design of a new class of anti-infective compounds that are being developed in our lab. Furthermore, with adequate improvement, the proposed approach may be relevant also to clinical studies.

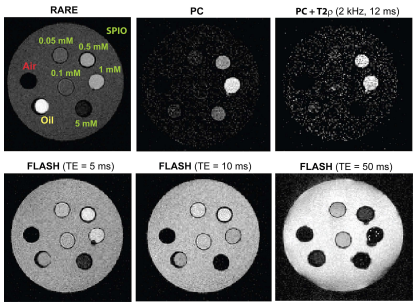

Figure 2. Axial images of the phantom. Results obtained with RARE (Nr = 2), PC (Nr = 2), PC+T2ρ (Nr = 2, 2kHz, 12ms) and FLASH are shown. The phantom (Falcon tube) contains 5 SPIO tubes (0.05-5 mM), one oil tube and an air tube immersed in water (saline solution).

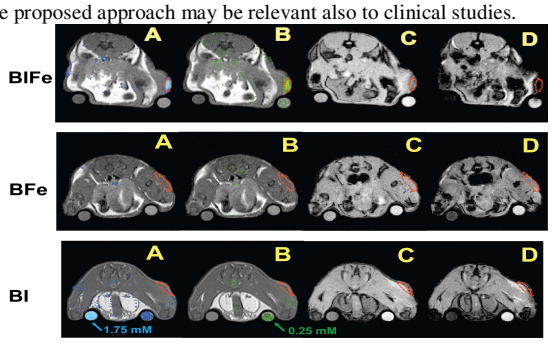

Figure 3. Axial images of a test (BIFe = Burn + Infected + SPIO) and two controls mice (BFe = Burn + SPIO; B+I = Burn + Infected). Overlays of the RARE (Nr = 2) anatomical images with: (A) PC (Nr = 2), (B) PC+T2ρ (Nr = 2, 2kHz, 6ms), (C) FLASH (TE = 5 ms), (D) FLASH (TE = 10 ms) are shown. ROI are drawn in red on the burned side. Expected correlations between PC and NC are found only for the BIFe test mouse (2 control tubes of 1.75 and 0.25 mM SPIO were placed under the mice).

References:

[1]. Cunningham, C.H., et al, Magn. Res. Med., 2005, 53:999-1005.

[2]. Michaeli, S., et al., Magn. Res. Med., 2005, 53:823-9.

[3]. Farrar, C., et al, NMR in Biomed., *in press.*

[4]. Rahme, L.G., et al., Science, 1995, 268:1899-902.

[5]. Fabian, T.C., Am. J. Surg., 2000, 179(2A suppl):18S-23S.

[6]. Gang, R.K., et al., Burns, 1999, 25(7):611-6.

[7]. Henning J., et al, Magn. Res. Med., 1986, 3:823-33.

[8]. Levitt, M.H., et al., J. Mag. Reson. 1982, 47(2):328-30.

[9]. Kupce, E., et al., J. Magn. Reson. Ser. A 1995, 115(2):273-76.

[10]. Andronesi, O.C., et al., *manuscript in preparation.*