Combined off-resonance imaging and relaxation in the rotating frame for positive contrast imaging of infection in a murine burn model

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Introduction– There is strong interest in MR imaging methods for non-invasive *in vivo* cell imaging and tracking, in order to monitor accumulation of macrophages in inflammation and infection (1,2,3). To this end, accumulation of macrophages that takes place at the site of burn trauma and infection with *Pseudomonas aeruginosa (PA)* infection is clinically important because it is the most common cause of sepsis in burned patients and in patients rendered neutropenic, as well as the most common bacterial cause of lung infection in cystic fibrosis patients (4). A range of MRI methods have been proposed for *in-vivo* molecular imaging of cells based on the use of superparamagnetic iron-oxide (SPIO) nanoparticles and related susceptibility weighted imaging methods (5,6). Positive-contrast MR imaging provides an alternative to negative-contrast MRI, exploiting the chemical shift induced by the SPIO nanoparticles to nearby water molecules. SPIO imaging with positive-contrast MRI has been achieved using ON-resonant water suppression (IRON) and off-resonant imaging (ORI) methods (7,8). We employed an ORI method and our novel combination of ORI and relaxation in the rotating frame (ORI-$T_{2\rho}$), (9). Specifically, we imaged the accumulation of *in vivo* SPIO-labeled macrophages at the infection site in a mouse model of burn trauma and infection with *PA*. The SPIOs were injected in solution directly in the animals, and macrophage labeling occurred *in vivo* in the animal model, thus the concentration of labeled macrophages was determined endogenously. Our study demonstrates the feasibility of using a novel positive-contrast method to monitor infection *in vivo*, in a clinically relevant mouse model of burn and infection with *PA*.

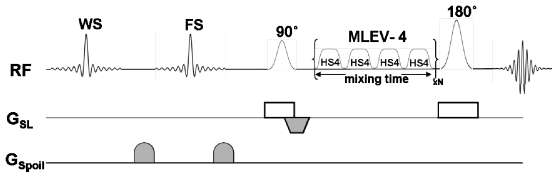

Figure 1. Schematic for ORI-$T_{2\rho}$ pulse sequence. Frequency-selective water- (WS) and fat-suppression (FS) pulses are followed by spoiler gradients to dephase the transverse magnetization. They are followed by a spin-echo imaging sequence. A spin-locking pulse block was inserted between the 90° and 180° RF pulses for relaxation in the rotating frame. The spin-locking block was implemented with the MLEV-4 scheme, using HS4 180° adiabatic full passage pulses.

Materials and Methods– Ultra-small super-paramagnetic iron oxide (USPIO) nanoparticles, known generically as Ferumoxtran-10 commercially and as Combidex® in the U.S. (Advanced Magnetics, Cambridge, MA) were used as the molecular imaging MRI contrast agent. Six weeks old CD-1 mice were anesthetized according and a full thickness thermal injury of 5% total burn surface area was produced on the right thigh muscle. Sixteen mice were randomized into one target and three control groups (N=4 per group). The target group consisted of mice burned, infected, and injected with USPIO, (BIFe), (Fig. 1). The controls groups consisted of: burned mice (B); burned and infected mice (BI); and mice that were burned and injected with USPIO, but not infected, (BFe). The control groups test the hypothesis that neither burn nor infection will alone result in MRI signal. They also test for the hypothesis that iron will not accumulate in the site of burn injury, which is not infected since, in that case, there will not be a sufficient accumulation of macrophages. 100 µl of bacterial suspension containing $2.5x10^6$ colony forming units (cfu) of mvfR (an attenuated mutant of PA that induces higher infiltration of macrophages) was injected intra-muscularly in the burn tissue immediately after the burn. Six hours post-burn 500 mg of Ferumextron-10 suspension was injected to the BFe and BIFe groups by intravenous injection in the tail vein. The mice in all four groups were imaged 24hour post-burn and infection. During MRI, mice were kept anesthetized with a mixture of isoflurane and maintained at 37° C. Imaging was performed in a 4.7 T horizontal magnet (20 cm bore, Bruker Avance console) using a custom-built volume coil (3 cm inner diameter, 10 cm active length). We optimized our previous protocol (9) and acquired images using: **(A)** ORI, **(B)** ORI-$T_{2\rho}$, **(C)** FLASH (α=35°, TR/TE = 500/4 ms), **(D)** FLASH (α=35°, TR/TE = 500/14 ms). The ORI and ORI-$T_{2\rho}$ pulse sequences were built on a RARE (Rapid Acquisition with Refocused Echo) sequence with RARE acceleration factor two. For the ORI-T2ρ sequence, a spin-locking pulse block for relaxation in the rotating frame was inserted between the 90° and 180° RF pulses of the RARE sequence. The spin-locking block was implemented with the MLEV-4 scheme (magnetization inversion was achieved with 180° adiabatic full passage pulses using HS4 adiabatic pulses) and was optimized for mouse imaging (3 ms 180° pulse duration, 12 ms spin-lock duration). Frequency-selective water and fat suppression were achieved using ten-lobed sinc pulses (400 Hz pulse bandwidth for water suppression, 800 Hz bandwidth for fat suppression) followed by spoiling gradients to decohere the transverse magnetization. The water and fat suppression pulses were followed by the spin-echo imaging sequence (ORI), or by the spin-echo sequence with the spin-locking block (ORI-T2ρ). Typical acquisition time was approximately 20 minutes for ORI and 40 minutes for ORI-T2ρ.

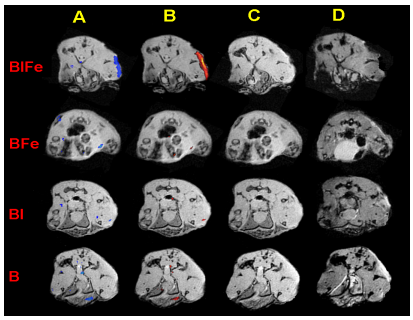

Figure 2. The positive-contrast images are shown, from left to right: **(A)** ORI, **(B)** ORI-$T_{2\rho}$, **(C)** FLASH (TE = 4 ms), **(D)** FLASH (TE = 14 ms). For image processing, regions of interest (ROI) were drawn around the area of the burn and the total (thresholded) signal intensity was integrated within each ROI BIFe: Burn, Infection, USPIO-labeled; BFe: Burn, SPIO-labeled; BI: Burn, Infection; B: Burn.

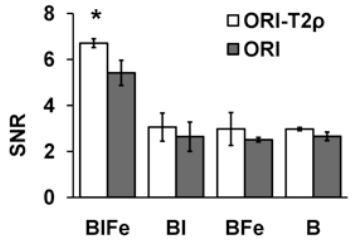

Figure 3. Signal detection in the target and control groups. Signal is measured in units of SNR (thresholded at three standard deviations), measured within ROIs at the level of the burn and infection. The noise threshold was estimated from fitting the image background to a Rician distribution. Error bars shown depict standard error of the mean image intensity in the ROI. Asterisk, denotes significant difference between ORI-$T_{2\rho}$ and ORI (n=4 per group, P=0.04).

Results– Fig. 2 shows positive-contrast images in pseudocolor, thresholded to signal greater than three in (dimensionless) SNR units, and superimposed on T_1-weighted FLASH images; negative-contrast images are also shown. Both ORI and $T_{2\rho}$ images were transformed to SNR images and thresholded in the same manner (in units of image standard deviation) for comparisons between ORI and ORI-$T_{2\rho}$ imaging protocols. Fig. 3 shows that the SNR of all control groups was significantly different from the SNR of BIFe mice (Bonferroni correction for multiple comparisons was applied). In controls, the signal detection was almost zero, because presumably there was no USPIO accumulation, due to absence of infection and hence no macrophage recruitment. In that case, ORI-$T_{2\rho}$ and ORI methods with similar yet different sensitivities did not show significant differences (Fig. 3). In the BIFe mice, we detected a strong signal attributed to USPIO accumulation, which allows the comparison of the two methods. Importantly, in the BIFe group, a significantly stronger signal was detected with ORI-$T_{2\rho}$ as compared to ORI, (P=0.04), (Fig.3).

Discussion– In our study, the pickup of USPIO cells by macrophages was visualized *in vivo*, since there was no *ex vivo* culture and the contrast agent was injected directly into the animals in aqueous solution. Additionally, imaging was performed *in vivo* as well. We observed a very strong statistical difference between the target (BIFe) group and the three control (B, BI, and BFe) groups. This shows that, in principle, positive-contrast imaging can be used to detect infection in a clinically relevant model. Since we observed a significant difference between ORI-$T_{2\rho}$ and ORI (Fig. 3), we suggest that ORI-$T_{2\rho}$ is more sensitive in detecting USPIO signal. To this end, the ORI-$T_{2\rho}$ positive contrast method may prove to be of higher utility in our line of research. In conclusion, our results have direct implications in the longitudinal monitoring of infection, and open perspectives for testing novel anti-infectives, which is our imminent goal.

References

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