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

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Introduction– We developed a novel molecular magnetic resonance (MR) imaging method to monitor accumulation of macrophages at the infection site infection. Positive-contrast molecular MR imaging provides an alternative to negative-contrast molecular MRI imaging, exploiting the chemical shift induced by ultra-small superparamagnetic iron-oxide (USPIO) nanoparticles to nearby water molecules. We introduced a novel combination of off-resonance (ORI) positive-contrast MRI and $T_{2\rho}$ relaxation in the rotating frame (ORI-$T_{2\rho}$) for positive-contrast MR imaging of USPIO [1,2]. We used this new method for testing a new anti-infective compound M50 following *Pseudomonas aeruginosa* infection.

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 leg thermal injury of 5% total burn surface area was produced on the right thigh muscle. Six hours post-burn and infection 500 mg of Ferumextron-10 suspension was injected by intravenous injection in the tail vein. Mice were randomized into one experimental and one control group (N=6 per group). The experimental group consisted of burned and infected mice, injected with USPIO and injected with the anti-infective compound M50. The control group consisted of burned and infected mice injected with USPIO.

The mice were imaged 12 hour post-burn. 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). Positive and negative contrast MRI was performed. Positive contrast MRI was performed using an off-resonant imaging (ORI) method implemented in a RARE sequence (also known as Fast Spin Echo, FSE) with RARE acceleration factor two. The ORI-T2ρ sequence was implemented in a RARE sequence with acceleration factor 2 via insertion of an MLEV-4 block with HS4 adiabatic pulses for relaxation in the rotating frame. The RF amplitude and the mixing time of the adiabatic pulses in the ORI-T2ρ have been optimized for *in vivo* mouse imaging . Selective water and fat suppression was 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. Negative contrast MRI was achieved with a series of FLASH images with increasing echo time for T_2* weighting, with typical values $\alpha = 35^{\circ}$, TR = 500 ms, TE = 4, 6, 8, 12, and 14 ms. The same slice prescription was used for all sequences. Anatomical reference images were acquired with RARE or proton-density weighted FLASH (fast-low angle shot) imaging. Typically, 10 axial slices were acquired in the burned region (1 mm thickness, 1.5 mm gap, 3 × 3 cm FOV, 128 × 128 matrix size, 8 averages). Typical MR imaging time was 2.5 hr per mouse.

Results– We tested ORI-$T_{2\rho}$ in phantoms and imaged *in vivo* the accumulation of USPIO-labeled macrophages at the infection site in a mouse model of burn trauma and infection with *P. aeruginosa*. The USPIO nanoparticles were injected directly in the animals in solution, and macrophage labeling occurred *in vivo* in the animal model. We observed a significant difference between ORI-$T_{2\rho}$ and ORI, which leads us to 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 future research. We used this ORI-$T_{2\rho}$ method to image the effects of our anti-infective compound M50 (Fig. 1A). Figure 1 shows negative-contrast images in pseudocolor, thresholded to signal greater than three in (dimensionless) SNR units, and superimposed on anatomical reference images, which were acquired using FLASH. The images were transformed to SNR images and thresholded in the same manner (in units of image standard deviation) for comparisons. Figure 1B shows that the signal detection in the controls was significantly different from the signal detection in the M50-treated group.

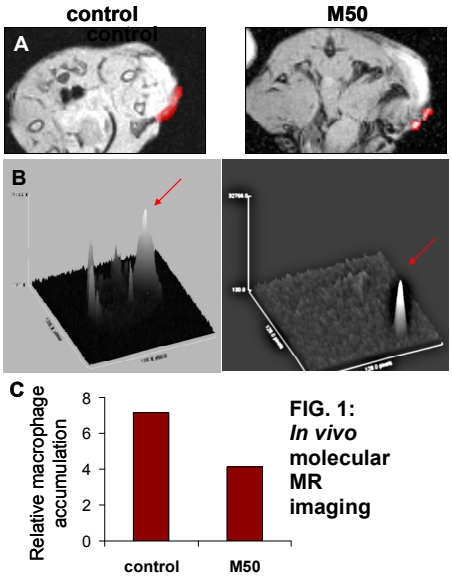

FIG. 1: *In vivo* molecular MR imaging

A. *In vivo positive contrast MR images, using our novel ORI-T2ρ approach, of mice infected with PA14 in the absence (control) and presence of M50. The images were transformed to SNR images and thresholded (in units of image SD). Images are shown in pseudocolor, thresholded to signal greater than three in (dimensionless) SNR units, and superimposed on a FLASH image. 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. Care was taken to choose similar slices at the same anatomical location in all mice.* ***B.*** *3-dimensional graphs of pixel intensities show an intense peak in the burn area for the control mouse, which is reduced with M50.* ***C.*** *Signal was measured in units of SNR (threshold 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. The control group is statistically different from mice receiving M50.*

Discussion- Our results show that compound M50 attenuates the signals coming from macrophages that accumulate at the infection site and thus support the compound's anti-infective action. These results may have direct implications in the longitudinal monitoring of infection, and open perspectives for testing our novel anti-virulence compounds.

References

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