Magnetic resonance imaging of *c-fos* gene transcription after burn trauma using a superior contrast agent

V. Righi[1,2], A. Papagiannaros[1,2], J. He[3], G. Dai[2], L. Rahme[3], V. Tugnoli[4], P. K. Liu[2], R. G. Tompkins[5], B. R. Rosen[2], and A. A. Tzika[1,2]

[1]Department of Surgery, NMR Surgical Laboratory, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, [2]Department of Radiology, Athinoula A. Martinos Center of Biomedical Imaging, Boston, MA, United States, [3]Department of Surgery, Molecular Surgery Laboratory, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States, [4]Departement of Biochemistry, University of Bologna, Bologna, Bologna, Italy, [5]Department of Surgery, MGH and Shriners Burn Institute, Harvard Medical School, Boston, MA, United States

Introduction– There is strong interest in MR imaging methods for *in vivo* MR imaging of gene transcription (Hajitu et al). Such imaging would enable MR detection of mRNA alterations in disease. A range of MRI methods have been proposed for *in-vivo* molecular imaging of cells based on the use of ultra-small super-paramagnetic iron oxide (USPIO) nanoparticles and related susceptibility weighted imaging methods (1,2).Although a gene assay technique is established to differentiate the induction profiles of *fosB* and *fosB* mRNA *in vivo* (3), a superior contrast agent that will be administered using systemic as opposed to local administration and assess whether it will target and accumulate at the burn site is not available. To visualize in live tissue the differential *fosB* gene expression profile after burn trauma, we developed MR probes that link T2* contrast agent [superparamagnetic iron oxide nanoparticles (SPION)] with a oligodeoxynucleotide (ODN) sequence complementary to *fosB* or *fosB* mRNA to visualize endogenous mRNA targets via *in vivo* hybridization (4,5,6,7). The presence of this SPION-sODN probe in cells results in localized signal reduction in T2*-weighted MR images, in which the rate of signal reduction (R2*) reflects the regional iron concentration at different stages of amphetamine (AMPH) exposure in live mouse tissue (8). Following the successful transfection of the animals the mRNA expression is imaged and quantified *in vivo*. Specifically, we developed and delivered pegylated lipid coated MR probe with ultra-small super-paramagnetic iron oxide nanoparticles (USPION, a T2 susceptibility agent) coated with polymer modified fusogenic lipids and covalently linked to a phosphorothioate-modified oligodeoxynucleotide (sODN) complementary to *c-fos* mRNA (SPION-cfos) and imaged mice subjected leg burn. Our study demonstrates the feasibility to monitor burn injury using MR imaging of *c-fos*B? transcription *in vivo*, in a clinically relevant mouse model of burn trauma for the first time.

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. Spions were lipid coated using the thin layer method. (Ko et al 2009). Briefly lipids (POPC – Palmitoyl oleoyl phosphatidyl choline, Cholesterol, DOTAP - 1,2-dioleoyl-3-trimethylammonium propane (methyl sulfate salt) and PEG-PE 2000 poly(ethyleneglycol)-distearyl phosphoethanolamine (6:3:1:1, 5µg/mouse) were mixed in chloroform (2ml) with spion coupled with Avidin (1µg/mouse) at 30°C and vacuum. Following evaporation micelles were formed following hydration and vortex. The lipid coated spion was conjugated to c-Fos sODN via avidin biotin linkage and was purified using dialysis. Linkage was verified using SDS gel electrophoresis as described previously (Liu *et al* a,b,c,) 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. Mice were randomized into one experimental and one control group (N=6 per group). The experimental group consisted of burned mice and injected with USPIO. The control group consisted of non-burned mice injected with USPIO. Six hours post-burn 500 mg of Ferumextron-10 suspension was injected by intravenous injection in the tail vein. 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). Negative contrast was achieved with a series of FLASH images with increasing echo time for T_2* weighting, with typical values $\alpha = 35^o$, 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. Negative contrast (FLASH at 4ms and 14ms) images were transformed into SNR images. The image intensity at each voxel was divided by the image noise level. Image noise level was estimated from fitting the histogram of an area in the image background, containing only pure noise voxels, to a Rician distribution.. This procedure is advantageous since it avoids contamination from voxels degraded by Rician noise. Accordingly, the value of three standard deviations was chosen to threshold-out Rician-noise voxels. An ROI was drawn at the level of the burn area for each mouse, and the total above-threshold signal enclosed in the ROI was computed.

Results– 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 RARE. The images were transformed to SNR images and thresholded in the same manner (in units of image standard deviation) for comparisons. Figure 2 shows that the signal detection in the burned group was significantly different from the signal detection in the control group.

Discussion- Here we demonstrate the feasibility to monitor *c-fos* transcription at the site of injury using MR imaging *in vivo*, in a clinically relevant mouse model of burn trauma by developing an improved contrast agent that is administrate systemically. MR imaging of gene transcription has been shown originally in disease models of the central nervous system (6). Our modification increases the specificity of the imaging, avoids the use of lipofectin, a generic non specific agent while the intravenous administration allows visualization systemically of the gene of interest. .We observed a very strong statistical difference between the experimental and control groups. The overall impact of our study is that USPIONs can be used as MR contrast agents for imaging of gene transcription in suitable animal models of disease.

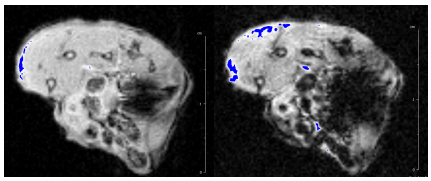

Figure 1. Negative-contrast images, after USPION-cfos injection, shown in pseudocolor, thresholded to signal greater than three in (dimensionless) SNR units, and superimposed on anatomical RARE image. TR/TE =500/4ms (A) and TR/TE=500/14ms (B).

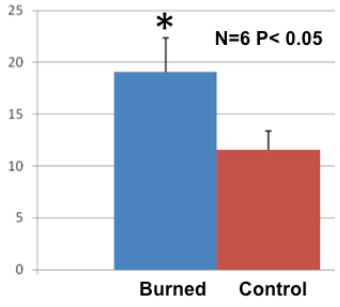

Figure 2. Signal detection in the burned and control groups. Values are means± SE, measured within ROIs in burned and contralateral hind limbs. Error bars shown depict standard error of the mean image intensity in the ROI. Asterisk, denotes significant difference between burned and control groups (n=6 per group, P<0.05).

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