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

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Introduction—There is strong interest in MR imaging methods for in vivo MR imaging of gene transcription (Hajitou 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-ODN 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 activation (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 lipoid 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-fosB mRNA 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 Combi® 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 phosphatidyl choline, Cholesterol, DOTAP - 1,2-dioleoyl-3-trimethylammonium propane (methyl sulfate salt) and PEG-PE 2000 poly(ethyleneglycol)-distearyl phosphoethanolamine (6:3:1, 1.5g/mouse) were mixed in chlorof orm (2ml) with spin coated with Avidin (1g/mouse) at 30°C and vacuum. Following evaporation micelles were formed following hydration and vortex. The lipid coated spin 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 non-burned mice injected with USPIO. Six hours post-burn 500 mg of Ferumoxtran-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 to 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 x 3 cm FOV, 128 x 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 to SNR images. The image intensity at each voxel was measured within ROIs in burned and control groups (n=6 per group, P<0.05).

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 administrable 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.

References
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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).