Transcription MRI Detects Altered Cerebral Gene Expression in Live Stroke Animals

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Introduction Stroke and cardia arrest are the No 1 cause of disability and productivity loss in the nation; it is an enormous health problem. The expression of immediate early genes is thought to be associated with cell repair/viability of the brain after stroke and heart attack. Detecting gene expression at the transcription level using MR imaging will provide significant clinical benefits. We reported the development of MR contrast probe using a biotinylated phosphorothioate-modified oligodeoxynucleotide (sODN) with NeutrAvidin (NA)-tagged superparamagnetic iron oxide nanoparticles (SPION). We infused male C57black6 mice intra-cerebroventricularly (ICV) with one of the two conjugates of SPION: (1) SPION-cfos, a targeting conjugate which contains an sODN of 26 bases with sequence complementary to c-fos mRNA, and (2) SPION-βactin, a control conjugate of sODN with a sequence complementary to a housekeeping gene, β-actin. Studies in molecular biology have shown that, shortly after the stroke insult, the number of cerebral c-fos transcript is greatly elevated whereas β-actin transcript is not. We reported here the capability of these customized SPION conjugates to detect altered gene transcription in the brain as an acute response to stroke using *in vivo* MRI.

Methods SPION and sODN were conjugated via the NA-biotin linkage. Male C57black6 mice were anesthetized using katamine (80 mg/kg, i.p.) and xylazine (16 mg/kg, i.p.). Animals were infused with the contrast probe (SPION = 84pmol/kg in 2ul sodium citrate, pH = 8) via ICV route (LR:-1; AP:-0.2; DV:-3 mm, bregma). Cerebral ischemia was induced 4 hours after the ICV infusion with the transient occlusion of the bilateral carotid arteries (BCAO) for 30 minutes, after which the occlusion was released for blood reperfusion. Two days later, we acquired MR images in live animals (with pure O_2 plus 2% halothane [800 ml/min flow rate]) using a 9.4Tesla magnet. We acquired R_2* maps using serial GEFI sequences (TR/TE=500/3, 4, 6, 8, 10ms, FOV=1.5cm, 128×128, α=30). Region of interest was placed in the somatosensory cortex contralateral to the infusion hemisphere both anterior and posterior to the infusion site. Averaged R2* values were compared between the stroke and sham operated animals within the group that received either SPION-cfos or SPION-βactin. The brain samples were then collected for validation using *ex vivo* MR microscopy (14T, R2* maps: 3D Multi-GE, TR/TE=50/4, 7, 13, 18ms, FOV=1.28cm, 256×256×128, α=30; T2* weighted image: 3D FLASH, TR/TE=50/18ms, FOV=1.28cm, 256×256×128, α=20). In separate groups of animals, no MR contrast probe were infused but the same BCAO and sham operation were given to induce c-fos mRNA transcription which was validated using conventional, but advanced molecular biological assays (in situ reverse transcription PCR [RT-PCR] and in situ hybridization).

Results We observed significant R_2* elevation due to increased retention of SPION-cfos in the somatosensory cortex ($p<0.05$) after cerebral ischemia by 30-min bilateral common carotid occlusion (BCCAO), compared to sham operated animals. We did not observe significant R2* elevation between the sham operated and stroke animals which were infused with SPION-βactin (Figure 1). *Ex vivo* R_2* maps obtained from either sham or stroke animals infused with SPION-cfos (Figure 2), positively correlated to the results from the c-fos mRNA map obtain from in situ hybridization (Figure 3). While c-fos transcription level is well below detection by in-situ hybridization technique at normal condition (Fig. 3A), c-fos transcription level is much enhanced after stroke induction (Fig 3B). This elevation is observed on the R_2* map as regions of elevated R_2*. Furthermore, we observed that in the hippocampus, localized T_2 signal reduction (Figure 4, lower left), correspond to elevated c-fos transcriptions as detected using in situ RT-PCR (Figure 4, lower right). Particular regions of interest include the neuronal formation of the pyramidal cell layers and dentate gyrus (DG).

Conclusions This ODN-based contrast probes may be used to explore the potential of MRI for real-time detection of transcripts, offering a new approach to direct, minimally invasive transcription assessment of phenotyping in the brain under neurological disease states (NSR01045845, P41RR14075 & the MIND Inst).

Figure 1. Elevation of SPION Retention (R2* values) in Live Animals

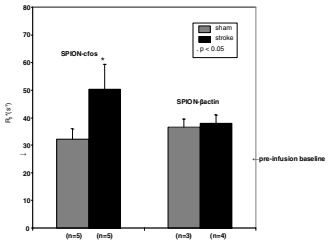

Figure 2. R_2* maps of ex vivo samples

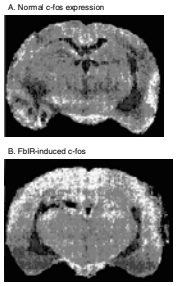

Figure 3. in situ hybridization of gene expression

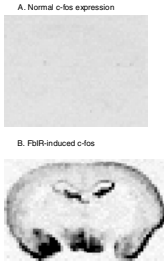

Figure 4 Hippocampal SPION-cfos retention with or without BCCAO