In Vivo Tracking of Gene Delivery Modules to Central Nervous System using a Novel Susceptibility MRI Contrast Agent

Y. Kim[1], C. Liu[1], S. Huang[1], B. Rosen[1], P. Liu[1]

[1]MGH/MIT/HMS Athinoula A. Martinos Center for Biomedical Imaging, Charlestown, MA, United States

ABSTRACT Magnetically-labeled oligodeoxynucleotides (ODN) with superparamagnetic mono-crystalline iron oxide nanoparticles (MION[(1)]) were infused into the lateral ventricles of healthy mice. Our goal was to investigate feasibility of *in vivo* detection of exogenous contrast agent distribution in central nervous system (CNS) and to evaluate delivery efficacy of target-specific gene transporting modules to brain using MRI. For CNS delivery, we postulated that contrast agents require a biological carrier which corresponds to a specific neuronal target. In this regard, target-specific delivery of MR contrast probes containing *in vivo* genes was investigated. Experimental results indicated that the biologically-conjugated MION can be introduced into brain cortex and possibly to the cytoplasmic space of the neurons in murine hippocampus. Upon comparing target-specific MION-ODN conjugates containing the ODN sequence that is complementary to c-fos mRNA (A26) to the probes which contain a random ODN sequence (R18), the extended retention time and significant migration to cortex were not observed for the R18 probe. Fluorescence-labeled ODN and histological staining of iron using Prussian blue were used as gold standards to corroborate the MRI results. The current study demonstrated the feasibility of efficiently transporting exogenous agents into the extra-cellular and possibly intra-cellular spaces in CNS and the real time visualization using superparamagnetic MR contrast conjugates.

MATERIALS AND METHODS 5'biotinylated and 100% phosphorothioated ODN (s-ODN) of rncfos115 (a 26mer: A26) with complimentary sequence to mRNA of c-fos[(2)] was custom made (Invitrogen Life Technologies and Amitof Biotech) and used to serve as a ligand for the contrast conjugates. The 3'-OH terminus of s-ODN was labeled using terminal transferase in the presence of digoxigenin (dig)-dUTP, which was used for confirming ex vivo presence of ODN complex following MRI experiments. Thereafter, the resulting 5'biotin-s-ODN-3'dig was purified using dextran column and stored in -20^{o}C[(3)]. 5'biotin-s-rncfos115-3'dig was mixed with 5'biotin-s-ODN at a molar ratio of 1:20 before conjugated with MION-NA. Prior to intracerebral ventricular (ICV) infusion, MION-NA (360 nmoles of MION pre-conjugation weight) were covalently bound to 5'biotin-s-rncfos115 (6 nmol) for one hour at room temperature, followed by addition of d-biotin (10 µmol) and storage for one hour (MION-rncfos115). As for constructing non-targeting control probes, s-ODN of rncfos115 was replaced with an ODN of random sequence (a 18mer: R18). MRI was performed using a 9.4T magnet with T2* weighted gradient echo pulse sequence (TR/TE=500/[2.3, 3, 4, 6]ms, FOV=1.5x1.5cm^2, NA=4, and number of slices/thickness=20/0.5mm.) T2* maps were obtained using a linear least square fit for three different animal groups of which each (n=5-10) was infused with MION only, targeting (rncfos115), and non-targeting (R18) contrast conjugates. MRI acquisitions were performed immediately (~30min), 1 day, and 3 days following the infusion. ROIs were placed over the cotralateral cortex to the infusion site (Figure 1) for the analyses of regional R2* (1/T2*). Additional MRI of ex vivo brains was performed using a 14T magnet with a gradient echo sequence (TR/TE=50/18ms, NA=12m, and isotropic voxel of 40x40x40µm^3).

RESULTS AND DISCUSSION Among three different contrast conjugates infused, only targeting MION-sODN was retained by the cortex, while other agents (non-targeting and MION) were cleared. Specific uptake of magnetic probes labeled with target-specific s-ODN containing c-fos complimentary mRNA was evident in the acquired R2* maps. The temporal dependence of uptake in the contralateral cortex showed that R2* values of specific (A26) and non-specific contrast conjugates (R18) were decreased immediately following the infusion (Data not shown); however, the maximum difference in R2* was reached on the day=3 between two probes as shown in Figure 1. Additionally, for the collected brains on the 3rd day, the accumulation of A26 in the Cornus Ammonis (CA) regions of hippocampus was readily observed in the T2*-weighted images using a 14T magnet and fluorescence images (Figure 2). In this respect, it can be suggested that the retention time in the cortex is an important variable to create localized imaging contrast. These results imply not only the introduction and wide distribution of imaging probes in brain (Figure 1) but also specific retention of such probes, possibly at the transcript level (Figure 2). With this advancement, *in vivo* tracking of gene delivery module and delivery efficiency of target specific contrast conjugates could be assessed in real time obviating postmortem analysis.

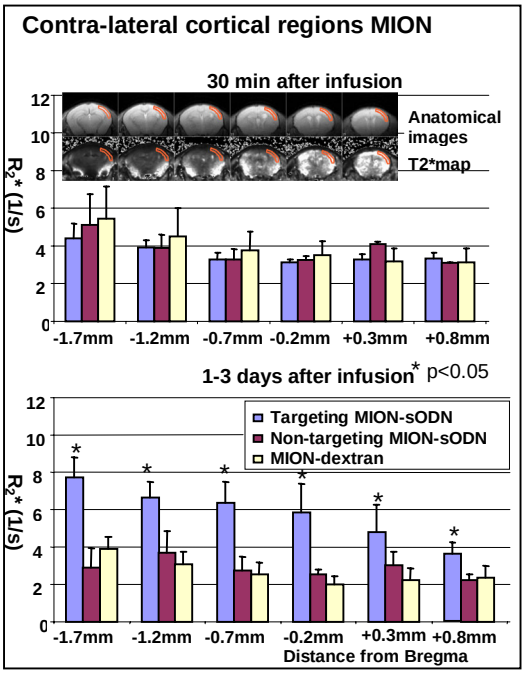

Figure 1. distribution of contrast conjugates: comparison between targeting and non-targeting contrast conjugates.

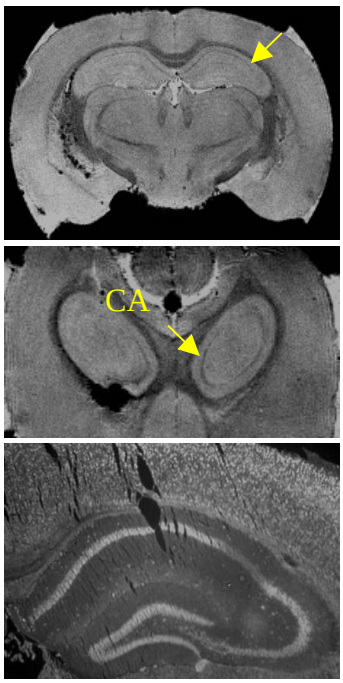

Figure 2. Top panel and middle panel: T2 contrast of CA regions using target-specific probe (A26), axial and coronal slices, respectively. Bottom panel: Fluorescent imaging of cortex and hippocampus

REFERENCES

1. Weissleder, R., Elizondo, G., Wittenberg, J., Rabito, C. A., Bengele, H. H. & Josephson, L. (1990) *Radiology* **175,** 489-93.
2. Liu, P. K., Salminen, A., He, Y. Y., Jiang, M. H., Xue, J. J., Liu, J. S. & Hsu, C. Y. (1994) *Ann Neurol* **36,** 566-76.
3. Cui, J. K., Hsu, C. Y. & Liu, P. K. (1999) *J Neurosci* **19,** 1335-44.