Development of multimodal imaging probes for neuroanatomical connectivity studies in vivo by means of MRI

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Introduction: In order to improve our understanding of brain function, a detailed knowledge of effective anatomical connections between different brain regions is of great importance. Unfortunately the requirement for highly invasive technologies precludes most studies in living subjects. In contrast to the conventional techniques, volume imaging with MRI-visible neuronal tracers provides a complete description of large-scale three-dimensional (3-D) networks.[1] The recent attempt to conjugate a commonly used paramagnetic complex Gd-DOTA with classical neuroanatomical tracers such as cholera toxin subunit B (CTB) or biocytin demonstrate the high potential of such systems to act as a contrast agent.[2,3] Here we have developed an efficient neuronal tracer that can allow a complete investigation of the neuronal networks using both MR and Optical imaging techniques. Functionalized Dextran-tetramethylrhodamine (**Dextran-TR**, Mwt 10 kDa) which is used extensively in neuroanatomical research served as a core molecule (Chart 1). It was conjugated to a Gd^{3+} complex of a modified AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid) ligand acting as MR reporter.

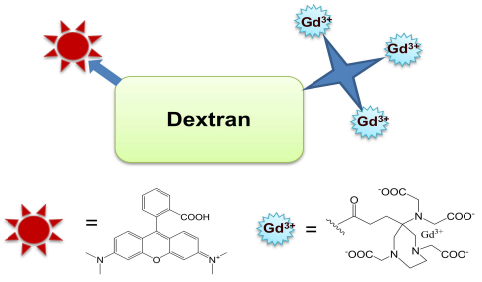

Chart 1. Schematic illustration of the new tracer molecule

Methods: We developed the AAZTA type ligand with an additional acid function which enables a further conjugation of the final Gd^{3+} complexes with the commercial **Dextran-TR** molecule using peptide coupling reagents. Longitudinal and transverse relaxation times (T_1 and T_2) measurements were performed on the final molecule at magnetic field strengths of 7T and 3T, at 25°C and pH 7.4. Cell studies were performed on differentiated mouse neuroblastoma cells (N18). Male Sprague-Dawley and Long-Evans rats (200-250 g) were used for *in vivo* studies. The animals were anesthetized with 2.0% isoflurane and placed in a stereotaxic frame. The tracer (400nL of 20% solution in PBS buffer) was stereotaxically injected using a 0.5 µL Hamilton syringe at a rate of 0.5 nL/min under general anaesthesia, using aseptic techniques. Each rat was imaged up to five times: 1h, 4d, 6d, 9d and 14d after tracer injection. We used a modified driven equilibrium Fourier transform (MDEFT) method to obtain T_1-weighted anatomical images. The scan parameters were: TR = 22ms, TE = 3ms, FA =20°, ID = 1000ms and four segments. The geometric parameters of the 3D scans were: matrix 240x170x126 and FOV 48x34x25.2mm resulting in a voxel size of 0.2x0.2x0.2mm. For each measurement 8 MDEFT images were acquired and averaged.

Results and discussion: Synthesized **GdAAZTA-Dextran-TR** molecule bears both MR and Optical reporter. According to the data provided by the supplier for the dextran / fluorophore and our determinations for dextran / Gd^{3+} ratios we estimated the Gd^{3+} / fluorophore ratio to be 3 /2. Both MR and Optical methods were used for the characterization of the tracer molecule. Fluorescence microscopy of the cells incubated with different concentrations of the tracer molecule clearly demonstrated its effective internalization and localization mainly within the cell body of the neuronal cells. In our studies we injected the **GdAAZTA-Dextran-TR** into the primary motor cortex (M1) of rats. The MRI data obtained 9d post-injection is presented in Fig. 2. It can be clearly seen that the internalized tracer reduced T_1 relaxation times of the brain tissue, which results in an enhanced signal intensity at the injection site (Fig. 2, top panel). Voxel-wise statistical analysis revealed a signal enhancement in several well-known subcortical targets of the primary motor cortex (M1), including the dorsolateral thalamus area (Po) and Caudate putamen (CPu) (yellow arrows, Fig. 2). Moreover, in the images one can observe a cluster of significant voxels that, most likely corresponds to the pyramidal tract, because the trace clearly originates from the injection site in M1 and tracks toward the CPu region (blue arrows, Fig. 2). The highest signal intensity in the regions of interest was found in the time range between 6 and 14 days which is in agreement with the literature data for dextran based tracer molecules.

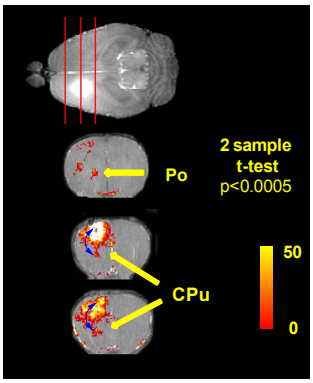

Fig.2 Injection **of GdAAZTA-Dextran-TR** into the rat M1 cortex. Tracing ability and accumulation in the area of interest is shown 9d post-injection

Conclusions: A Gd^{3+} based paramagnetic dextran conjugate has been developed, which enables the tracking of neuroanatomical connectivity in the brain by both MR and Optical imaging. Cell studies and subsequent *in vivo* experiments in rodents demonstrate high potential of the new molecule and its applicability for *in vivo* connectivity studies. Such molecules can enable a diversity of new experimental studies on various neuroscientific issues associated with longitudinal investigations on brain plasticity.

References: [1] J. H. Lee, A. P. Koretsky, *Curr Pharm Biotechl.,* 2004; 5(6), 529-537; [2] C.W-H. Wu, O. Vasalatiy, N. Liu, H. Wu, S. Cheal, D.-Y. Chen, A.P. Koretsky, G.L. Griffiths, R.B.H. Tootell, L.G. Ungerleider, *Neuron*, 2011, 70, 229-243; [3] A. Mishra, A. Schüz, J. Engelmann, M. Beyerlein, N. K. Logothetis, S. Canals, *ACS Chem.Neurosci.,* 2011, published online, DOI: 10.1021/cn200022m