CEST and FLEX MRI for detection of CNS graft rejection

Sujith V Sajja[1,2], Guanshu Liu[1,3], Nirbhay Yadav[1,3], Jiadi Xu[3], Antje Arnold[1,2], Anna Jablonska[1,2], Michael McMahon[1,3], Peter van Zijl[1,3], Jeff Bulte[1,2], Piotr Walczak[1,4], and Miroslaw Janowski[1,5]

[1]Dept. of Radiology and Radiological Science, Johns Hopkins University School of Medicine, Baltimore, MD, United States, [2]Cellular Imaging Section and Vascular Biology Program, Institute for Cell Engineering, Johns Hopkins University, Baltimore, MD, United States, [3]F.M. Kirby Research Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States, [4]Department of Radiology, University of Warmia and Mazury, Olsztyn, Poland, [5]NeuroRepair Department, Polish Academy of Sciences, Warsaw, Poland

Target audience: Physicians and scientists interested in stem cell-based therapy and CNS graft rejection.

Purpose: "Conventional" T1 and T2 weighted MRI has been an attractive tool for non-invasive visualization of anatomy and function of CNS, but many processes at the molecular level are currently undetectable through conventional MRI techniques. The continuous advancement in MR methodology is allowing us to overcome this barrier. For example, a recent study by Zhou et al., 2013, has shown that chemical exchange saturation transfer (CEST) of tissue proteins can differentiate between regrowth of glioma and radiation necrosis[1]. In addition, endogenous exchange of amide protons (pH sensitive) in these proteins was imaged in a human brain[1]. Encouraged by this robust technology, we used MR-based CEST and frequency-labeled exchange transfer (FLEX)[2] for studying the process of stem cell graft rejection in the brain, which is not identifiable using "conventional" MRI. Cellular therapies for CNS disorders are of tremendous interest, but current methods for the evaluation of graft survival are clinically inadequate. As transplanted stem cells may require several months to differentiate and become functional, it is critical to know the fate of cells during that time-course to predict therapeutic outcome or tailor the means for graft survival within the paradigm of personalized medicine. The aim of this study was to develop a novel, clinically applicable approach for non-invasive detection of cellular graft infiltration by immune cells, which could be a surrogate marker for graft rejection using CEST and FLEX MRI.

Methods: Glial-restricted progenitor cells (GRPs) that can remyelinate the axons and were shown to have tremendous therapeutic potential in dysmyelination were used in this study for engraftment. Grafts were placed using a stereotaxic procedure, which is a mainstay of clinical applications. The survival of GRPs derived from luciferase (luc+) mice was evaluated by bioluminescence imaging (BLI) on every second day for up to two weeks using IVIS SpectrumCT optical imager (Parkin Elmer) (modality available in small animals only). Balb/c (immunocompentent) mice were used as rejecting animals, and age-matched rag-/- (immunodeficient) mice (n=5/group) were used as control (non-rejecting) animals. T_{1w}, T_{2w}, T^*_{2w} and CEST/FLEX MRI were obtained at days 1, 7, and 14 post-transplantation. MRI was performed using a Bruker BioSpec 11.7 T MR scanner, equipped with a phase-array coil and one 72 mm volume coil (Bruker). CEST MRI was performed with saturation offset of -8 ppm to 8 ppm (0.4 ppm increment) using a 2 µT,3-second-long continuous wave (CW) saturation RF pulse. Following CEST MRI, FLEX MRI was performed and the data obtained was FLEX associated changes. On-resonance FLEX experiments (FLEX baseline) were performed by varying t_{vol} from 0.0 to 1.45 ms in steps of 0.05 ms, LTMs=152, t_{exch}=10 ms. Imaging parameters for all experiments had a readout consisting of a rare factor=20, TR= 6 s, TE = 4 s, slice thickness =1 mm, FOV= 15mm×15mm consisting of 128×128 acquisition matrix. Animals were sacrificed for post-mortem assessment at two weeks and immunohistochemistry was performed to identify immune cell infiltration of the graft using anti-CD4, CD8, CD68, and CD45 and staining for luciferase to determine graft cell viability.

Results: BLI signal showed a ~92% decrease for Luc+ cells two weeks after transplantation. Time course analysis of Luc+ signal using BLI (B) is shown in panel K of the figure (WT- Balb/c mice ; Rag-/- - Rag2 immunodeficient mice). Immunohistochemistry revealed high immune cell infiltration with CD45+ and CD68+ cells at the transplantation site in immunocompetent Balb/c mice, but not in immunodeficient rag-/- mice. Representative images of CD45+ (B at 5x, D at 20x) staining, A at 5x ,C at 20x shows counterstaining with DAPI (scale bar for A,B: 200µm) at 14 days post transplantation in Balb/c animals. Representative images of CD68+ (G at 5x, I at 20x) staining, F at 5x, H at 20x show counterstaining with DAPI at 14 days post transplantation Balb/c animals. E at 5x and J at 10x shows Luc+ GRP cells at day 1 post transplantation. T1, T2, and T2* showed no imaging contrast. However, a ROI based analysis showed that the CEST signal as quantified by MTR_{asym} at the non-transplantation site is higher than that on the contralateral side of the brain from day 1 to day 14. There is also a clear difference in CEST MRI contrast between Balb/c and Rag-/- animal model. Graphs (M,N) in the figure shows the representative CEST signal (mean MTR_{asym}) of WT-Balb/c and Rag-/- immunodeficient mice (blue, TS-Transplanted Site; green CTS – Contralateral Site) at 1 day post-transplantation. Color graphs (G,H) shows the representative CEST images at Day7 of WT and Rag-/- mice. Longitudinal CEST assessment at 0.5 ppm shows a decrease in MTR_{asym} on TS (Blue bars) but an increase in CTS (brown bars) in both WT (I) and Rag-/- (J)mice. Longitudinal assessment showed a significant decrease in FLEX contrast in rag-/- mice at 7 days and 14 days following transplantation but not in WT animals.

Discussion: CEST MRI can potentially be employed to detect immune response following cell transplantation, as evidenced by the changes of CEST contrast at the transplantation site (day 1 to day 14). This may be attributable to immune rejection of the graft, since the bioluminescence showed a dramatic decline in transplanted cells (day 1 to day 14). The evidence of high immune cell infiltration (CD45+ and CD68+) at the transplantation site further supports graft rejection, which would also contribute to a change of CEST contrast from day 1 to day 14. Changes in FLEX could reflect the differences in inflammatory process between WT and rag-/- mice.

Conclusion: CEST MRI in combination with FLEX can potentially be used to detect the CNS graft rejection that can be clinical amenable.

Reference: 1. Zhou J, Tryggestad E, Wen Z, et al., Nat Med. 2011; 17(1):130-4.

2. Nirbhay N. Yadav, Craig K, et al., Magn Reson Med. 2013; 69(4): 966–973.