Cellular MR Imaging of Immune Cells infiltration as a Marker for Assessment Allograft Outcome in a Chronic Cardiac Allograft Rejection Rat Model

Q. YE[1], Y. L. Wu[1], L. M. Foley[1], T. K. Hitchens[1], H. S. Zhang[1], D. Eytan[1], C. L. Chen[2], and C. Ho[1]

[1]NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Industrial technology research institute, Taiwan

INTRODUCTION:

Non-invasive, repetitive and reliable methods for *in-vivo* assessment of cardiac allograft status are highly desirable since biopsy is invasive and prone to sampling errors because of the limited size and location of tissue available. We are developing non-invasive means for detecting allograft rejection by using MRI techniques (1). With cellular MRI, we can monitor the accumulation of immune cells labeled with iron particles (2, 3). In this study, immune cells are *in situ* labeled with micrometer-sized paramagnetic iron oxide (MPIO) particles. We then monitor the progression of immune cell accumulation in the rejecting heart allograft to assess the progression of chronic cardiac allograft rejection (CCAR) by MRI.

METHODS:

1. Animal Model: This study used an abdominal heterotopic working heart model by transplanting an en bloc donor heart and lung into the abdomen of recipient with PVG.1U (RT1.A^{u}B^{u}D^{u}C^{u}) to PVG.R8 (RT1.A^{a}B^{u}D^{u}C^{u}) rat pairs as allograft and PVG.R8 to PVG.R8 pairs as isograft. Since there is only single gene mis-match between donor and recipient (4), the allografts develop chronic rejection without any immunologic manipulation of recipient.
2. Cell labeling: Immune cells (mainly macrophages) were *in situ* labeled via i.v. injection of MPIO particles encapsulated with dragon green fluorescence at a dose of 3 mg Fe/kg, 24 hours prior to initial *in-vivo* MRI.
3. MRI and Pathology: The heart grafts were longitudinally imaged by *in-vivo* MRI for up to 120 days on a Bruker AVANCE 4.7-T system at designed time points, and EKG-respiratory gated T2*-weighted gradient echo sequence was used to monitor the accumulation of MPIO-labeled cell with the in-plane resolution of 156 µm. At the endpoint of the study, grafts were harvested for high-resolution 3D Magnetic Resonance Microscopy (MRM) on a Bruker 11.7-T/89-mm system. Fluorescence microscopy for detecting MPIO, pathology for verifying MRI data were performed on graft tissue following *ex-vivo* MRI.

RESULTS AND DISCUSSIO:

Our data revealed that macrophage infiltration associated with CCAR can be detected by cellular MRI during rejection from post-operation day (POD) 20 to beyond POD 100. A representative *in-vivo* MR image of an allograft on POD 21 is shown in Fig. 1A. A few dark spots of hypointensity are observed on different myocardial regions of the graft. In contrast, on POD 112 allograft, many dark spots detected by *in-vivo* MRI (Fig. 1B). These dark spots can bee further seen with *ex-vivo* MRI (Fig. 1C and D) at 11.7 T and these MRI detected dark spots were caused by macrophages labeled with MPIO particles confirmed by fluorescence microscopy (Fig.1E and F). There are more density and number of fluorescence spots are observed in POD 112 allograft (Fig.1 F) than in POD 21 allograft (Fig. 1 E). Immunohistochemical staining on the corresponded tissue section with anti-rat ED1 antibody shows less macrophages infiltration in the early phase of chronic rejection allgraft (Fig. 1G) than in the POD 112 graft under went sever chronic rejection (Fig. 1H). This progression of macrophage infiltration correlated with our MRI observation which also coincided with CCAR progresses. The H&E staining indicated mild interstitial inflammation and slightly intimal thickening shown in POD 21 graft (Fig. 1I) and more concentrated macrophage accumulated in the endocardium and adventitia and more sever intimal thickening in POD 112 graft (Fig. 1J).

CONCLUSIONS:

We can detect a progression of macrophage infiltration as the CCAR progresses using cellular MRI. Our MRI results correlate well with pathology. This study demonstrates the feasibility of non-invasive detection of chronic rejection in our rat cardiac allograft using *in-vivo* MRI.

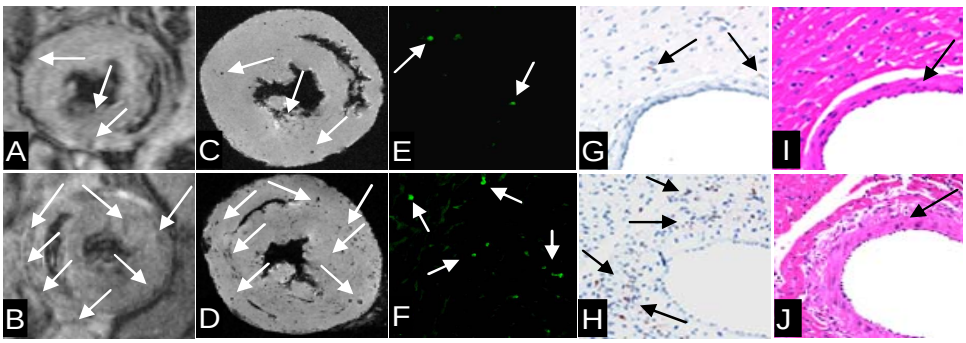

Figure 1. (A) T2*-weighted *in-vivo* MR image of a POD 21 allograft, a few dark spots of hypointensity are exhibit on the different regions of the graft, On POD 112 allograft, there are many more dark spots were detected (B). These dark spots can be further see with *ex-vivo* MRI at 11.7T(C and D). There are many more dark spots show in POD 112 graft (D). These MRI detected dark spots were caused by macrophages labeled with MPIO particles confirmed by fluorescence microscopy of Dragon green. Macrophages staining with anti-rat ED1 antibody accumulated in the graft tissue (G and H) which coincided with CCAR progression indicated by H&E staining (I an J).

ACKNOWLEDGMENTS

We thank Ms. Lisa McGaw for assistance with animal care. This work is supported by grants from the National Institutes of Health (P41EB001977 and R01HL-081349)

REFERENCES:

1. Ho, C. & Hitchens, T. K. (2004) Curr Pharmaceut Biotechnol, 5: 551-566.
2. Wu, Y.-J. L., Ye, Q., Foley, L. M., et al. (2006) Proc Natl Acad Sci, 103: 1852-57.
3. Ye, Q., Wu, Y.-J. L., Foley, L. M., et al. (2008) Circulation, 118:149-156.
4. Shirwan, H., Mhoyan, A., Yolcu, E. et al. (2003) Transpl Immunol, 11: 179-85.