Detecting the Migration and Accumulation of Macrophages in An Acute Rejection Model of Heart-lung Transplantation in Rats by in vivo MRI Using A New Nano-sized Iron Oxide Particle

H. Zhang[1], Q. Ye[1], C-L. Chen[2], K. Hitchens[1], W-Y. Hsieh[3], L. Liu[1], Y. Wu[1], L. Foley[1], H-H. Shen[2], J. Wang[2], and C. Ho[1]

[1]NMR Center for Biomedical Research, Carnegie Mellon University, Pittsburgh, PA, United States, [2]Biomedical Engineering Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan, [3]Material and Chemical Research Laboratories, Industrial Technology Research Institute, Hsinchu, Taiwan

INTRODUCTION

Iron-oxide-based cellular MRI has been used to assess organ rejection after transplantation by monitoring the accumulation of immune cells at the rejection site [1]. There are two approaches to label targeting cells with iron-oxide particles. One is *in vivo* labeling by direct intravenous injection, which is convenient and particularly useful for phagocytotic cells, such as macrophages, which have been shown to be involved in acute transplant rejection. The other is *in vitro* labeling by isolating a specific cell type and labeling them in culture. Though *in vitro* labeling has higher sensitivity of MRI, most of the ongoing studies use focal injection after *in vitro* labeling interested cell types, in which the migration and accumulation of the targeting cells at the focus sites might not be convincingly demonstrated via in vivo MRI [2]. In this study, we investigated the migration and accumulation of the labeled macrophages in the transplanted allograft heart with a new, nano-sized iron-oxide-based contrast agent ITRI-IOP via *in vitro* labeling and venous infusion in a rat acute rejection model.

METHODS

In vitro macrophage labeling by ITRI-IOP: Our nano-sized (around 60 nm) ultrasmall super-paramagnetic iron oxide particle ITRI-IOP is coated with a monolayer of polyethyleneglycol (PEG). The longitudinal and transverse relaxivities of these particles are 48.9 and 321.2 $s^{-1}mM^{-1}$, respectively. Macrophages were isolated from spleen of the recipient rat strain and labeled by ITRI-IOP in culture for 12 hrs with an iron concentration of 25 μg/ml. Perl's Prussian Blue staining was used to confirm the presence of iron inside the cells.

Rat heart transplantation model and MRI: An abdominal working heart-lung transplantation rat model with acute rejection was used (n=9). ECG and respiration gated, single slice gradient echo imaging at the mid-ventricular wall was performed on day 5 post transplantation on a Bruker AVANCE 4.7 T system (Bruker, Billerica, MA) with the following parameters: TR = one respiration cycle (1 s), TE=8.1 ms, FOV=4×3.6 cm, in plane resolution= 156×156 μm, ST=1.2 mm, and NA=8. After a pre-scan of the transplanted allograft heart, approximately 2-30×10^6 ITRI-IOP-labeled macrophages were infused through a catheter into the femoral vein. Then, *in vivo* MRI was carried out with the similar imaging parameters immediately after and approximately 24 hrs post cells infusion. At the end of experiment, the rat was sacrificed and both the allograft and native hearts were fixed for further *ex vivo* MRI at 4.7 T and 11.7 T (Bruker AVANCE) using the same imaging parameters as *in vivo* studies and high resolution MR microscopy (51×51×130 μm). Pathological analysis was performed to examine our MRI results.

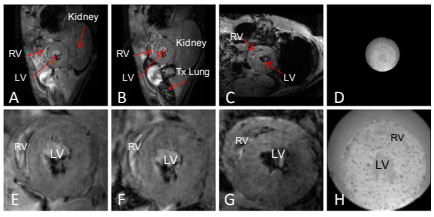

Fig. 1. Images of transplanted heart at 4.7 T. Top row, original transplanted heart images in rat abdomen; bottom row, zoomed in images of the heart. (A and E) *In vivo* images before; (B and F) 10 min after; (C and G) 22 hrs after infusion of 15×10^6 ITRI-IOP-labeled macrophages. (D and H) *Ex vivo* images of the same heart at 4.7 T about 24 hrs post infusion.

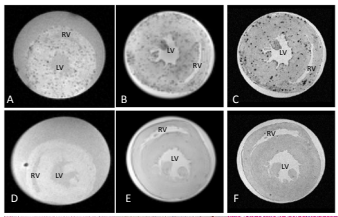

Fig. 2. *Ex vivo* images of transplanted and native hearts. Top row: transplanted heart; bottom row: native heart. (A and D): Using the in vivo imaging parameters at 4.7T (156×156 μm×1.5mm); (B and E): Using the in vivo imaging parameters at 11.7T (156×156 μm×1.5mm); (C and F) High resolution microscopy at 11.7T (51×51×130 μm).

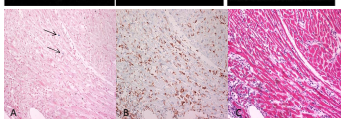

Fig.3. Pathology results. (A): Prussian blue stain show iron (arrows); (B): $ED1^+$ stain show macrophages as brown; (C): H&E staining show mononuclear cells and rejection in the corresponding myocardial tissue section.

RESULTS

Prior to the infusion of ITRI-IOP-labeled macrophages, the myocardial signal is uniform (Fig.1A and E). Immediately after the infusion, the transplanted lung turned into dark due to the iron-loaded macrophages in the blood circulation (Fig.1B). About 22 hrs post infusion, punctuate spots of hypointensity induced by the iron-loaded macrophages are observed on the myocardium of allograft heart and make the image look noisy (Fig.1C and G). This observation is more obvious in the *ex vivo* image of the same fixed transplanted heart at 4.7T (Fig.1D and H). *Ex vivo* images and microscopy of the transplanted and native hearts at 4.7 and 11.7 T further show abundance of punctuated spots of hypointensity only in the transplanted heart (Fig. 2 A-C, more evident at high field and high resolution), but not in the native heart (Fig. 2 D-F). The punctuate hypointensity spots in the transplanted heart are most likely due to the migration and accumulation of the ITRI-IOP-labeled macrophages from the blood circulation into the site of graft rejection. Pathology results on the corresponding tissue section also confirm the presence of co-localized iron, macrophages and rejection (Fig. 3).

CONCLUSION

In this study, we have detected the migration and accumulation of macrophages labeled *in vitro* by *in vivo* MRI with a nano-sized and sensitive iron oxide particle (ITRI-IOP) in the rejecting transplanted heart, which showed similar punctuate patterns of hypointensity as those using micron-sized particles [3]. Future studies with different immune cells specifically labeled with ITRI-IOP particles may help interpret the migration and role a specific immune cell may play in organ rejection and other inflammatory responses.

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