Detection of macrophage accumulation after heart transplantation in a rat using a novel nano-sized iron oxide particle with high relaxivity

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INTRODUCTION

The gold standard for detecting organ rejection after transplantation is biopsy, which is not only invasive but also prone to sampling errors. We have been developing and optimizing MRI techniques to assess organ rejection after transplantation by monitoring the accumulation of immune cells at the rejection site using various iron-oxide-based contrast agents [1-3]. There are two schemes to label cells with contrast agents. One is *in vivo* labeling by direct intravenous injection, which is clinically convenient and particularly useful for phagocytotic cells, such as macrophages, which have been shown to be involved in transplant rejection. The other is *ex vivo* labeling by isolating specific cell type and labeling them in culture. We investigated here the sensitivity of a novel, biodegradable, non-cytotoxic, nano-sized iron-oxide-based contrast agent with high relaxivity in cellular MRI via *ex vivo* labeling. Specifically, we detected the accumulation of the labeled macrophages at the rejecting heart in a rat heterotopic heart and lung transplantation model of acute rejection.

METHODS

Ex vivo cell labeling: The novel nano-sized ultrasmall super-paramagnetic iron oxide (USPIO) particle (ITRI-IOP) used in this study consists of a 10 nm iron oxide core and a coating with a monolayer of polyethyleneglycol (PEG). The PEG coating prevents the iron oxide particles from aggregation, and also makes the particles highly hydrophilic. The whole particle size of ITRI-IOP is around 60 nm. The longitudinal and transverse relaxivities of these particles are 52.4 and 304.7 $s^{-1}mM^{-1}$, respectively. Macrophages were isolated from spleen of the recipient strain and labeled in culture for 12 hrs with an iron concentration of 25 µg/ml. The viability of labeled macrophage was examined by Trypan blue. Perl's Prussian Blue staining was used to confirm the presence of iron inside the cells.

Rat heart transplantation model and MRI: An abdominal heterotopic working heart and lung transplantation rat model was used. ECG and respiration gated, single slice gradient echo imaging at the mid-ventricular wall was performed on day 5 post transplantation at 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=1.5 mm and NA=8. After a prescan of the transplanted heart, approximately 2×10^7 ITRI-IOP labeled macrophages were injected through a catheter into the femoral vein. Then, *in vivo* MRI was resumed with the similar imaging parameters until 24 hrs post cells injection. At the end of experiment, the rat was scarified and the heart was fixed for further *ex vivo* MRI at 4.7 T and 11.7 T (Bruker AVANCE) with the same imaging parameters as *in vivo*. Hematoxylin and eosin (H&E) staining for pathology and Perl's Prussian Blue staining for iron were performed.

RESULTS

Prior to the injection of ITRI-IOP-labeled macrophages, the myocardial signal is uniform except hypointensity artifact on the epicardium caused by coronary veins (Fig.1A). After the injection, punctuate spots of hypointensity induced by the iron-loaded macrophages are observed on the mid-wall of the myocardium (Fig.1B). *Ex vivo* images at 4.7 and 11.7 T show abundance of punctuated spots of hypointensity most likely due to the accumulation of the ITRI-IOP-labeled macrophages (Fig.1C, D). Histological analysis and iron staining on the corresponding tissue section confirms the presence of macrophages and iron (Fig.2).

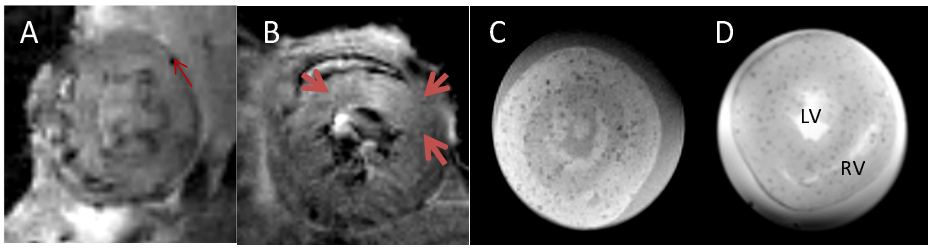

Fig. 1: (A) *In vivo* MRI at 4.7 T before injection (arrow shows artifact in the epicardium caused by coronary vein); (B) *In vivo* MRI at 4.7 T 24 hrs post injection (arrows show hypointensities caused by iron oxide labeled cells); (C) *Ex vivo* MRI at 4.7 T 24 hrs post injection (more and well distributed hypointensities are shown); (D) *Ex vivo* MRI at 11.7 T.

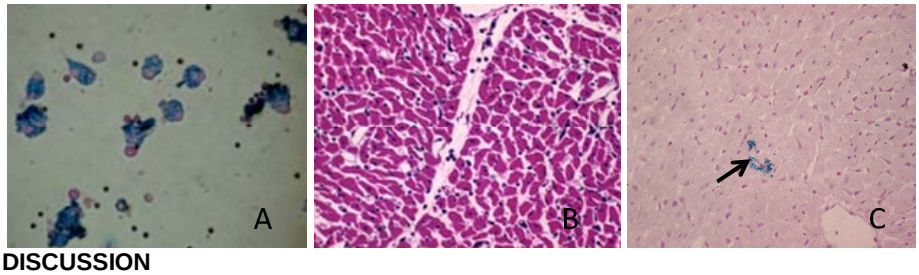

Fig. 2: (A) Perl's Prussian Blue staining of iron in macrophages labeled *ex vivo* by ITRI-IOP; (B) H&E staining of myocardial tissue, most of the black spots shown are nuclei of the immune cells (mostly macrophage); (C) Perl's russian Blue staining shows iron (arrow) in the myocardial tissue.

DISCUSSION

A non-toxic, biodegradable and sensitive contrast agent is highly desirable for *in vivo* cellular MRI. In this study, we have detected *in vivo* that macrophages labeled *ex vivo* with a nano-sized, high-relaxivity iron oxide particles (ITRI-IOP) showed similar punctuate patterns of hypointensity as those using micron-sized particles [3] in the rejecting heart. Future studies with different immune cells specifically labeled with this novel particle may help interpret the migration and role a specific immune cell plays and help understand the immune response mechanism in organ rejection and other inflammatory responses.

REFERENCES: (1) Kanno et al., J Thorac Cardiovasc Surg 120:923-34 (2000) (2) Dodd et al., Biophys. J. 76: 103-9(1999) (3) Wu et al., P*NAS.* 7;103(6):1852-7(2006)