Non-invasive assessment of chronic cardiac allograft rejection in a rat model with *in vivo* MR imaging of immune cells labeled with MPIO particles

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INTRODUCTION:

Chronic cardiac allograft rejection (CCAR) constitutes a major obstacle to the long-term success of heart transplantation. Studies indicate the involvement of immune cells in development of the CCAR. Thus, it could be possible to assess CCAR by labeling these cells with MRI contrast agent and then monitoring the accumulation in the rejecting graft. In this study, immune cells (mainly macrophages) were labeled *in situ* with micrometer-sized paramagnetic iron oxide (MPIO) particles, and their accumulation in the rejecting allograft was monitored non-invasively with MRI. The aim of this study was to assess the feasibility of using MRI to detect CCAR.

METHODS:

1. Animal model: An abdominal heterotopic working heart and lung transplantation was implanted using PVG.1U ($RT1.A^uB^uD^uC^u$) into PVG.R8 ($RT1.A^aB^uD^uC^u$) rat pairs. The transplanted hearts exhibit chronic rejection by post-operation day (POD) 20 and the most extensive by POD 100.
2. Contrast agent labeling: Immune cells, mostly macrophages, were labeled by direct i.v. injection of MPIO particles (0.9 µm in diameter) at a dose of 3 mg Fe/kg one day prior to MRI.
3. MRI methods: EKG and respiration gated T2*-weighted cine imaging on a Bruker AVANCE DRX 4.7-T/40-cm system was used for *in vivo* imaging with following parameters: repetition time (TR) = one respiration cycle (≈1 s); echo time (TE) = 8 ms; field of view (FOV) = 3–4 cm; slice thickness = 1 or 1.5 mm; in-plane resolution = 156 µm. *Ex vivo* MR Microscopy (MRM) was performed following MRI experiment at 11.7T using a Bruker AVANCE DRX 11.7-T/89-mm system with a Micro2.5 gradient insert with the following parameters: TR = 500 ms; TE = 8 ms; isotropic resolution = 40 µm.

RESULTS AND DISCUSSION:

In-vivo MRI of heart grafts were started one day or one week after intravenous injection of MPIO, and then subsequently imaged once a week for a period of up to 16 weeks to assess the feasibility of using MRI to monitor the rejection by target the labeled macrophages. Representative T_2*-weighted *in-vivo* MR image of a POD 112 allograft are shown in Figure 1A, some very distinct, dark spots of hypointensity are exhibit on the different regions of the graft. This indicates that MPIO-labeled macrophages have accumulated in the graft as reported in our previous study of acute rejection in rats (1, 2). This MRI data are confirmed with MRM (Figure 1B) and ED1 macrophages staining (Figure 1C). The macrophage-concentrated areas are correlated with CCAR detected by H&E (Figure 1D).

CONCLUSIONS:

Our data showed that the distribution of macrophage can be non-invasively assessed using *in vivo* MRI and the accumulation of macrophages over time in the rejecting allograft hearts can be used as a determinant of late cardiac allograft outcome. This approach may further improve the understanding of the immune cells involved in chronic rejection and as well as the management of heart transplantation.

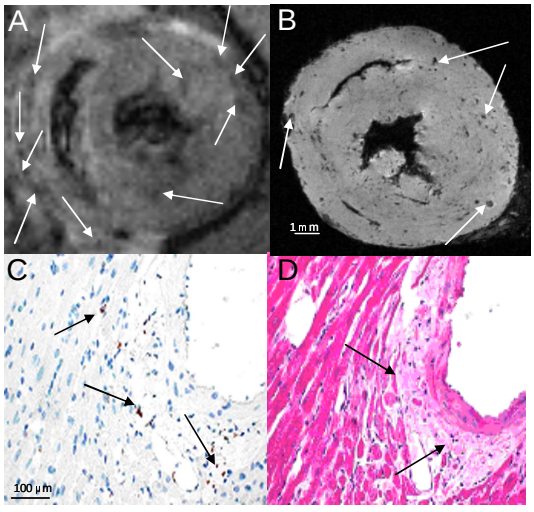

Figure 1. T2*-weighted *in vivo* MRI (A) for an allograft on POD112. Very sparse punctate and distinct contrast spots can be seen, as indicated with white arrowheads, which represent MPIO labeled macrophages accumulated in the rejecting graft. This was confirmed with MRM (B) and ED1 macrophages staining (C). The macrophage-concentrated areas are correlated with chronic cardiac allograft rejection detected by H&E (D).

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