

Assessment of Macrophage Depletion on Acute Cardiac Allograft Rejection by MRI

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INTRODUCTION

Acute cardiac allograft rejection is an inflammatory process that results in the damage and loss of function of the graft tissue. This process involves the accumulation of recipient leukocytes, namely lymphocytes and macrophages, at the site of rejection. Although abundant macrophage infiltration is observed in cardiac allograft rejection, the contribution of these cells to the rejection process and the tissue damage that results remains unclear. This study is designed to investigate the role these cells play in our rat model of acute cardiac rejection by selective depletion of circulating macrophages with liposomal-clodronate treatment. In this model, without any treatment, rejection begins on post-operative day (POD) 3.5, and becomes severe on POD 7. We have previously shown that the nanometer-sized USPIO and micron-sized MPIO superparamagnetic iron oxide particles are taken up by macrophages in the circulation and then home to the site of rejection, detected non-invasively by cellular MRI [1]. Here, the effects of macrophage depletion were examined using MRI both to track iron oxide-labeled cellular infiltration of the graft following a direct intravenous injection, and to assess changes in graft function throughout the course of acute rejection.

METHODS

The transplantation model used in this study employed Dark Agouti (DA) to Brown Norway (BN) rat pairs. Transplant recipients received a bi-ventricular working heart and lung graft transplanted into the abdomen. Animals received liposomal-clodronate intravenously one day before transplantation surgery, on post-operative day (POD) 3 at the onset of rejection, and on POD 6. Iron oxide-based contrast agents, either USPIO or MPIO, were given intravenously on POD 4 at 4.5 mg/kg body weight, while serial *in vivo* imaging was performed at 7 Tesla on days 4 through 7. We used T₂*-weighted imaging to detect immune-cell infiltration at sites of rejection by monitoring the accumulation of the iron oxide-labeled cells, while simultaneously using cardiac cine-tagging and strain analysis to detect regional myocardial function loss resulting from rejection [2]. Organs were harvested on POD 7 and imaged at high resolution at 11.7 Tesla before undergoing histopathological examination.

RESULTS

We have previously shown that macrophages can be labeled *in vivo* by direct i.v. injection of iron oxide-based contrast agents, and their accumulation at the site of rejection can be detected non-invasively by MRI. By POD 5, corresponding to moderate rejection, areas of hypointensity from labeled macrophage accumulation can be seen in the graft, corresponding with the severity of rejection (Figure 1A,C). In animals receiving treatment, however, little to no hypointensity is seen at this point, indicating a reduction in macrophage accumulation in the transplanted organ (Figure 1B,D). This difference is seen with both MPIO and USPIO labeling of macrophages, although the patterns of hypointensity vary between the two. Similarly, by POD 7, graft function is typically very poor as determined by the reduced ejection fraction and wall movements seen by cine-tagging experiments on the graft. Clodronate-treated animals, however, showed little functional deterioration from POD 3 to POD 7.

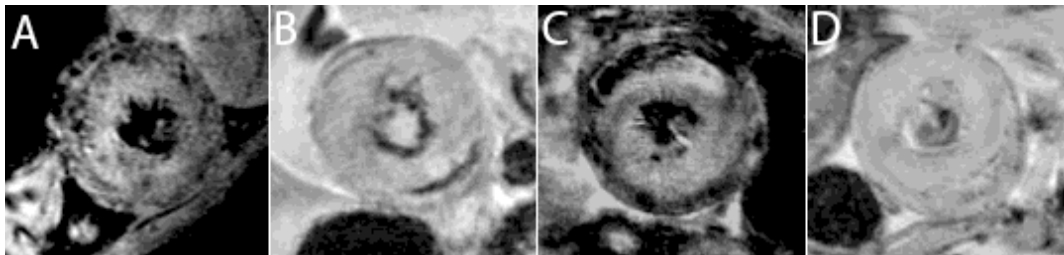


FIGURE 1. *In vivo* MRI of untreated (A, C) and clodronate-treated (B, D) allograft hearts undergoing rejection on POD 5, 24 hours following direct i.v. injection of 4.5 mg/kg BW MPIO (A, B) or USPIO (C, D). Accumulation of MPIO (A) and USPIO (C) labeled macrophages can be seen with T₂*-weighted contrast, more concentrated in the epicardium of the graft heart (1.5 mm slice thickness, 156 μ m in-plane resolution, TR/TE 500/8 ms) [1]. Little or no hypointensity can be seen in MPIO (B) and USPIO (D) injected, treated animals (1.5 mm slice thickness, 156 μ m in-plane resolution, TR/TE 130/6).

CONCLUSION

This study indicates that macrophages contribute to tissue damage during acute allograft rejection, and that their selective depletion using liposomal-clodronate may attenuate the damaging effects of rejection in rat cardiac allografts.

REFERENCES

1. Wu *et al.* 2006 *Proc Natl Acad Sci USA.* 7;103(6):1852-7.
2. Wu *et al.* 2009 *J Am Coll Cardiol Img.* 2:731-741.

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