^{19}F MRI Detection of Acute Cardiac Allograft Rejection with In Situ Perfluorocarbon Labeling of Immune Cells

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INTRODUCTION

The current gold standard for diagnosing and staging rejection after organ transplantation is biopsy, which is not only invasive but also prone to sampling errors. We are developing non-invasive methods for staging allograft rejection after heart transplantation by MRI. One approach is to label immune cells with an appropriate contrast agent and detect the accumulation at the rejecting graft. We present a new method of detecting the inflammation associated with organ rejection via *in situ* immune cell labeling with a perfluorocarbon nanoemulsion. A cellular ^{19}F MRI tracer agent has the advantage over gadolinium- or iron-oxide-based contrast agents since there is no endogenous signal from unlabeled tissues. In this approach, the accumulation of inflammatory cells at the sites of rejection can be located, unambiguously, by ^{19}F MRI (1,2). The labeled cells can then be placed in their corresponding anatomical context with a conventional ^{1}H image. In this study we have used a rat heterotopic working heart and lung transplantation model of acute rejection. Accumulation of ^{19}F label is readily detected in the rejecting graft 24 hours following direct intravenous injection of the perfluorocarbon nanoemulsion.

METHODS

An abdominal heterotopic working heart and lung transplantation model was used with Dark Agouti to Brown Norway rat pairs (3). The transplanted hearts develop Mild (Grade 1A or B) rejection by post-operation day (POD) 2.5-3.5; Grade 2 rejection on POD 4.5-5.5; and moderate to the severe (Grade 3A) rejection after POD 6-7. At 24 hours prior to MRI, a 1 ml bolus of 20% v/v perfluoro-15-crown-5 ether nanoemulsion (VS-580H, Celsense, Inc., Pittsburgh, PA) was injected intravenously. Immune cells, presumed to be macrophages, can take up the nanoemulsion particles in circulation and home to the site(s) of inflammation. EKG and respiration gated imaging was performed on a Bruker AVANCE 7 Tesla system. Following conventional ^{1}H MRI pilot scans to localize the transplanted heart, the RF coil was re-tuned and spin-density-weighted ^{19}F images were collected to detect the labeled cells. The ^{19}F image was rendered in pseudo-color and overlaid onto the registered ^{1}H image. *Ex vivo* MR spectroscopy and imaging of fixed tissues along with a ^{19}F reference standard was performed at 11.7 Tesla to further quantify the amount and distribution of labeled immune cells (2).

RESULTS AND DISCUSSION

We have previously shown that immune cells, particularly macrophages, that accumulate in the rejecting organ can be detected non-invasively with *in vivo* MRI after direct intravenous injection of iron-oxide particles such as USPIO, SPIO and MPIO (3,4,5). The iron-oxide labeled cells result in areas of hypointensity in T_2*-weighted images, and thus quantification of infiltrated cells can be challenging. In contrast, the ^{19}F labeled cells provide positive signal only where the labeled cells are found, and the signal intensity is directly correlated with the amount of label and perhaps the severity and stage of rejection. Thus, the use of ^{19}F tracer agents may offer a new approach for gaining deeper insights into the immunobiology of organ rejection and may serve as a biomarker for preclinical therapeutic testing.

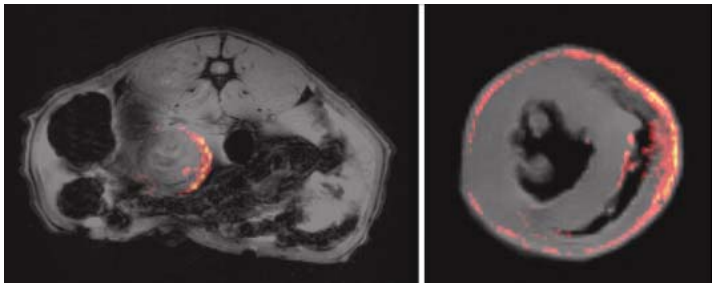

Figure 1. MRI of the heart graft undergoing rejection. Left, *In vivo* MR images from a heart graft in the abdomen on day 6 post transplantation (2.5 mm slice thickness, 0.3 x 0.3 mm resolution, TR/TE = 1000/6 ms, and NEX =1 for ^{1}H and NEX = 4 for ^{19}F). The anatomical ^{1}H image is grayscale and the ^{19}F image is rendered in hot-iron scale showing the accumulation of perfluorocarbon labled macrophages in the transplanted heart. Right, The heart was fixed and both ^{1}H and ^{19}F images were collected at 11.7 Tesla (1.5 mm slice thickness, 0.26 x 0.26 mm resolution, TR/TE = 1000/7 ms, and NEX =2).

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