Comparison of Iron-oxide- and Perfluorocarbon-based Cellular Contrast Agents for Detecting Immune Cell Infiltration in Models of Organ Transplant Rejection

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INTRODUCTION

Cellular imaging is an emerging and important field in magnetic resonance. The ability to non-invasively detect the trafficking and accumulation of different cell types *in vivo* has broad implications for both a better understanding of biological processes and the clinical diagnosis and treatment of numerous diseases. Using rodent models, we are developing cellular imaging methods for detecting and staging organ rejection after transplantation. Achieving our goal requires the selection of an appropriate contrast agent and imaging method to detect the cells of interest. Several types of cellular contrast agents exist that provide fundamentally different image contrast: Iron-oxide-based agents provide T_2* contrast, Gadolinium-based agents give T_1 contrast, and fluorine-based tracer agents provide spin-density weighted signal in ^{19}F images. Thus, to optimize our cellular imaging, we evaluated the advantages and disadvantages of different cellular contrast agents in our rat model systems. We have used two transplantation models in this study, an orthotopic kidney transplant model in rat, and a heterotopic working heart and lung transplantation model in the abdomen of a rat. In these models, without immunosuppressive treatment, rejection begins on post-operation day (POD) 3 and 4, respectively, and becomes severe on POD 5 and 6, respectively. This provides a window of opportunity to follow the progression of immunological rejection by cellular MRI. Rats are given a direct intravenous injection of contrast agent that is taken up by macrophages in circulation, which subsequently migrate to the sites of rejection. Both nano- and micron- sized iron-oxide particles were used and compared with results obtained with a perfluorocarbon nanoemulsion agent.

METHODS

The transplantation models in this study used Dark Agouti (DA) and Brown Norway (BN) rat pairs. Kidney transplant recipients receive an orthotopic transplant of the left kidney [1]. Heart transplant recipients receive a bi-ventricle working heart graft transplanted into the abdomen [2]. In both cases, DA to BN transplantation serves as the experimental allograft that experiences acute rejection, and BN to BN transplant serves as the isograft control that does not experience rejection. Rats, 24 hours prior to MRI, are given a direct intravenous injection of either an iron-oxide based or fluorine-based agent. For iron oxide agents, a dose of 4-6 mg Fe per Kg BW is given of either dextran coated ultra-small iron oxide (USPIO) particles or micron-sized (polystyrene-divinyl benzene) iron oxide particles (MPIO) (Bangs Laboratories, Fishers IN). The fluorine agent given is delivered in a 1 ml bolus of 30% v/v perfluoro-15-crown-5 ether nanoemulsion (VS-580H, Celsense, Inc., Pittsburgh, PA). MRI is carried out at 4.7 T or 7 T. A T_2*-weighted gradient echo sequence is used to detect iron-oxide labeled cells and a spin-echo sequence is used to detect the ^{19}F-labeled cells. Organs are also imaged ex vivo at 11.7 T.

RESULTS AND DISCUSSION

We have previously shown that the immune cells, particularly macrophages, accumulate in the rejecting organ and can be detected non-invasively with *in vivo* MRI after direct intravenous injection of contrast agents [1,2]. Figure 1 shows examples of imaging macrophage accumulation in both the kidney (A,B) and heart transplant models (D,E). Iron-oxide labeled cells result in areas of hypointensity in conventional T_2*-weighted images, Figure 1A. Because of the high sensitivity of ^{1}H imaging and the "blooming effect" of iron oxide, the labeled cells can be detected at high resolution, and it is even possible to detect single cells *in vivo*. However, T_2* can result from other sources of susceptibility artifacts, such as hemorrhaging and at tissue interfaces, therefore, quantification can be challenging. Alternatively, ^{19}F-labeled cells provide a positive signal only where the labeled cells are found, figures 1B, 1E, and a conventional ^{1}H image is used for anatomical context. The ^{19}F signal intensity is directly correlated with the amount of label [3], however, because this tracer agent is directly detected, image sensitivity to tissues containing sparse cell numbers needs to be further explored. It is important to understand the advantages and disadvantages of different types of contrast agents for cellular imaging of inflammatory events and we believe that Iron-oxide- and ^{19}F-based agents can provide complementary information.

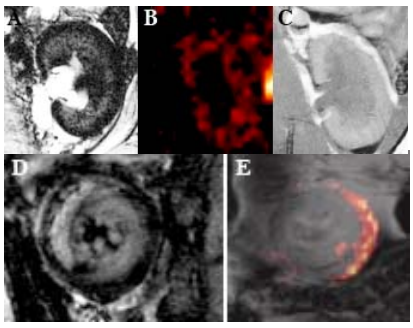

Figure 1. MRI of a kidney (A-D) and heart (D,E) grafts undergoing rejection 24 hrs following direct i.v. injection of USPIO (A,D) or ^{19}F nanoemulsion (B,E). The T2*-weighted image in (A) shows hypointensity mostly in the cortex of the kidney due both to the accumulation of USPIO-labeled macrophages and hemorrhaging of the kidney [1]. The ^{19}F image in (B) is rendered in hot-iron pseudo color and shows similar accumulation of ^{19}F labeled macrophages in the cortex of the rejecting kidney, panel (C) shows corresponding anatomical image (2 mm slice thickness, 0.3 x 0.3 mm resolution, TR/TE = 1000/6 ms, and NEX =2 for ^{1}H and 1 x 1 mm resolution, NEX = 64 for ^{19}F). Panel (D) shows accumulation of USPIO labeled macrophages with a T2*-weighted contrast, more concentrated near epicardium of the transplanted heart. Panel E shows composite ^{19}F and ^{1}H image identifying the accumulation of ^{19}F labeled macrophages mostly near the heart epicardium. The ^{19}F image is rendered in hot-iron pseudo color.

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