Macrophage Accumulation Associated With Rat Cardiac Allograft Rejection Detected by Magnetic Resonance Imaging With Ultrasmall Superparamagnetic Iron Oxide Particles

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Background—Acute cardiac allograft rejection continues to be the cause of graft loss and contributes to the morbidity and mortality after cardiac transplantation. In this study, we report a new method for detecting organ rejection in transplantation with an MR-based technique using dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) particles. These particles (≈27 nm in diameter) are known to shorten relaxation times in MRI experiments.

Methods and Results—A new rat model of heterotopic heart and lung transplantation has been developed for MRI experiments. Allotransplantations (DA→BN) were performed (n=8), with syngeneic transplantations (BN→BN) serving as controls (n=8). MR images were obtained with a gradient echo method. At postoperative day 7, allotransplants developed moderate rejection as determined histopathologically. A significant reduction in MR signal intensity was observed after USPIO injection into rats with allotransplanted hearts. Syngeneic transplants showed no differences in MR signal intensity before and after USPIO injections. After injection of USPIO particles at postoperative day 6, a group of allotransplanted rats was treated with cyclosporin A (3 mg/kg). Animals treated with cyclosporin A for 7 days showed no reduction in MR signal intensity after USPIO reinjection at day 14, whereas animals treated for 4 days showed a significant decrease in MR signal intensity in the transplanted hearts indicative of acute graft rejection. Pathological analysis of these animals revealed that dextran-coated USPIO particles were taken up by the infiltrating macrophages that accumulated within the rejecting cardiac graft.

Conclusions—This MRI method offers promise as a noninvasive method for detecting transplant allograft rejection. (Circulation. 2001;104:934-938.)

Key Words: magnetic resonance imaging ■ rejection ■ diagnosis ■ transplantation

Heart transplantation is now an established treatment for end-stage cardiac disease not only in adult patients but also in children. The survival rate has improved because of improvements in organ preservation and immunosuppressive treatments; however, allograft rejection remains a major complication after transplantation. Endomyocardial biopsy is used routinely for cardiac transplant rejection surveillance,1,2 and it is the “gold standard” for determining cardiac rejection. Endomyocardial biopsy is an invasive process, however, associated with finite risks of morbidity and mortality. In addition, if a pattern of rejection shows a focal distribution, false-negative results can be obtained because of sampling errors. In addition, children might have distress related to serial invasive procedures.3 Therefore, many attempts have been made to develop noninvasive approaches for detecting transplant rejection, eg, an analysis of heart rate variability,4 pulsed Doppler tissue imaging,5 monitoring of peak filling rate with acoustic quantification echocardiography,6 high-resolution intramyocardial electrograms,7 serum protein analysis,8,9 and detection with 111In-labeled lymphocytes.10 None of these approaches, however, have yet gained widespread clinical use. A sensitive and noninvasive method for detecting rejection is still being sought.

We assessed an MRI technique with in vivo macrophage labeling with dextran-coated ultrasmall superparamagnetic iron oxide (USPIO) particles (≈27 nm in diameter) as a new modality to diagnose cardiac rejection. USPIO particles enhance relaxation times in MRI. This property has been exploited in the use of USPIO particles in MR lymphography,11 which detects the accumulation of USPIO particles in the cytoplasm of macrophages within lymph nodes, and also in labeling T cells in vitro12,13 for tracking purposes in vivo.14 Recently, our laboratory reported that USPIO particles are useful to detect the accumulation of macrophages in a rat model for renal allograft rejection15 and lung transplantation.16 Larger superparamagnetic particles of iron oxide,
which are 100 to 1000 nm in diameter, are rapidly taken up by the mononuclear phagocytic system and cleared from blood within minutes after intravenous infusion, whereas USPIO particles are not immediately trapped by the mononuclear phagocytic system of the liver and spleen. USPIO particles have a longer half-life of ~2 hours in rat blood and are found to be present in lymph nodes.

In this article, we report a new detection method for cardiac rejection that uses an MR technique with dextran-coated USPIO particles. It is a simple, noninvasive, highly sensitive, and safe method without the use of radioisotopes.

Methods

Animals

All rats used in the experiments were male, 2 to 3 months of age, and weighed 220 to 250 g each. Animals were housed individually and provided food and water ad libitum. Animal protocols were approved by the Institutional Animal Care and Use Committee of Carnegie Mellon University. All animals received humane care in compliance with the Guide for the Care and Use of Laboratory Animals, published by the National Institutes of Health (publication No 96-03, revised 1996). Inbred Brown Norway (BN: RT1n) and DA (RT1a) rats were obtained from Harlan Sprague Dawley (Indianapolis, Ind). Rats were obtained over a period of 24 hours after infusion of dextran-coated USPIO particles. The imaging sequence consisted of a gradient echo sequence, triggered to ECG and ventilator (60 strokes/min, 10 mL/kg), with TR/TE 500/10 ms, flip angle equal to Ernst angle, slice thickness 1 mm, field of view 6.0 cm, data matrix size 256×130 (zero-filled to 256×256), and scan time 5 minutes. ECG leads were placed on both of the hind limbs of the rat with the transplant to pick up the heartbeat from the transplanted heart more effectively. The change of MRI signal intensity was measured in whole ventricular wall in each transplanted heart. The MR signal intensity of the heart was normalized to that of the leg muscle, because USPIO particles were not readily taken up by muscular tissue, according to Gellissen et al. 18

Dextran-coated USPIO particles were synthesized in our laboratory according to the method of Palmacci and Josephson17 with slight modifications. The MR relaxivities R₁ (spin-lattice relaxation rate constant, 1/T₁, per mole of Fe in USPIO) and R₂ (spin-spin relaxation rate constant, 1/T₂, per mole of Fe in USPIO) measured at 4.7 T were 3.8×10⁴ and 9.1×10³ (mol/L)/s, respectively. For in vivo studies, dextran-coated USPIO particles were diazylzed against PBS solution and diluted to a concentration of 18 μmol Fe/mL, and 0.8 mL of the suspension (ie, ~3 mg Fe/kg body wt) was injected intravenously for each study.

At 6 days after transplantation, dextran-coated USPIO particles were injected intravenously as mentioned above, and the animals were subjected to MRI. Twenty-four hours later, these animals were again placed inside the magnet and scanned. The regions of interest were defined manually with Bruker software. MR signal intensity in the entire ventricular wall in the plane was measured. After injection of USPIO particles at postoperative day (POD) 6, 10 animals with allotransplants were given CsA for 4 (POD 7 to 10) (n=5) or 7 (POD 7 to 13) (n=5) days and reinjected with USPIO particles on POD 14.

Heart and Lung Transplantation

Under anesthesia with injection of 35 mg/kg body wt of sodium pentobarbital IP, 500 U/kg body wt of heparin was injected. In the syngeneic group (n=8), an en bloc donor heart and lung were taken from a BN rat and transplanted to another BN rat. In the allogeneic group (n=26), a graft from a DA rat was transplanted to a BN rat. This group was divided into 2 groups: one group (n=8) was treated with 3 mg·kg⁻¹·d⁻¹ cyclosporin A (CsA), and the other group (n=18) was not given CsA. Gift survival was monitored every day by palpating contraction of the transplanted heart.

Operative procedures have been described elsewhere. 16 In brief, after the chest wall of the donor rat was opened, the left lung was ligated and excised. The ayzygos vein with the left superior and right superior vena cavae was ligated and divided. The descending thoracic aorta was transected, and 10 mL of cold University of Wisconsin solution (UW solution, Dupont Pharma) was infused into the inferior vena cava until the fluid draining from the aorta was clear, followed by ligation and division of the inferior vena cava. The ascending aorta was dissected and transected at the portion between the left common carotid artery and the left subclavian artery, followed by ligation and division of the right brachiocephalic artery and the left common carotid artery. After removal of the heart and lung from the donor, the right lung was washed 3 times through the bronchus with UW solution containing penicillin G. The grafts were then placed into cold UW solution for ~5 minutes until transplantation. Next, the left inguinal portion of the recipient rat was opened and dissected to make enough space for the transplanted organs. The left lower part of the abdominal wall was opened in a transverse fashion from the left femoral vessels to the midline. The abdominal organs were retracted to the right, and both the aorta and the inferior vena cava just beyond the bifurcation were dissected. The vessels were clamped, and an appropriate opening of the aorta was made to receive the aorta of the graft in an end-to-side fashion. Rhymthic heartbeats commenced spontaneously as the heart and the lung regained circulation after removal of the clamp. After hemostasis of the surgical field, the abdominal wall was sutured, with care taken not to kink or obstruct the aorta of the graft.

MRI Experiments

MRI measurements were carried out on a 4.7-T/40-cm Bruker AVANCE DRX MR instrument equipped with 15-cm, 10-gauss/cm shielded gradients. In vivo MR images of transplanted heart-lung were obtained over a period of 24 hours after infusion of dextran-coated USPIO particles. The imaging sequence consisted of a gradient echo sequence, triggered to ECG and ventilator (60 strokes/min, 10 mL/kg), with TR/TE 500/10 ms, flip angle equal to Ernst angle, slice thickness 1 mm, field of view 6.0 cm, data matrix size 256×130 (zero-filled to 256×256), and scan time 5 minutes. ECG leads were placed on both of the hind limbs of the rat with the transplant to pick up the heartbeat from the transplanted heart more effectively. The change of MRI signal intensity was measured in whole ventricular wall in each transplanted heart. The MR signal intensity of the heart was normalized to that of the leg muscle, because USPIO particles were not readily taken up by muscular tissue, according to Gellissen et al. 18

Pathological Analysis and Immunohistochemistry

After an MR experiment was completed, the transplanted hearts were extirpated, fixed in 3.7% formaldehyde, and embedded in paraffin for 5-μm sections. Hematoxylin-eosin staining and Perl’s Prussian blue staining were performed in the Transplantation Pathology Laboratory of the University of Pittsburgh Medical Center. Histological analysis for pathological grading of heart rejection, which is based on the criteria established by the International Society for Heart and Lung Transplantation, was also performed by this laboratory in a blinded manner. Monoclonal anti-rat macrophage antibody (ED1, Serotec Ltd) was used as a primary antibody for macrophages. Immunohistochemistry was carried out with the ABC staining system (Santa Cruz Biotechnology, Inc) according to the manufacturer’s protocol.

Statistical Analysis

The results are presented as mean±SD. The results were analyzed by ANOVA with StatView software (SAS Institute Inc). A value of P<0.05 was considered to be statistically significant.

Results

In Vivo MRI Experiments on the Rat Heart Transplantation Model

For MRI experiments, we used a newly developed rat model of nonworking heart transplantation. 16 In this model, an en bloc heart and lung graft is transplanted to the groin area of the recipient to detect MR signals effectively, shielded from abdominal and respiratory interference. MRI represents a noninvasive diagnostic modality. Abdominal gas, bowel peristalsis, and respiratory motion, however, can interfere with the MR signal in the conventional experimental model of heterotopic heart transplantation. Therefore, as we mentioned in an earlier article, 16 we have developed a new experimental model in which the graft is transplanted to the groin area of the recipient animal. Representative images of each of the
experimental groups are shown in Figures 1 and 2. A decrease in MR signal intensity is detected in the images of the allogeneic transplanted heart (n = 8) 24 hours after infusion of dextran-coated USPIO particles compared with the preinfusion signal (Figure 1). In contrast, there is no significant MR signal change in the syngeneic transplanted heart (n = 8) before and after USPIO infusion (Figure 2). In the CsA-treated group (n = 8), a significant decrease in MR signal is also seen in the transplanted heart (Figure 2). Figure 3 summarizes the changes of MR signals in each group during the experiment. Although there is no significant difference of the MR signal intensity among the groups before the USPIO infusion, MR signal intensity is significantly reduced in both the CsA-treated and the nonimmunosuppressed allografts. The signal intensity of the CsA-treated allografts is intermediate between isografts and nonimmunosuppressed allografts. During the experiment, the signal intensity in the muscle of the hind limb did not change at any time after USPIO infusion, whereas that of the spleen decreased right after USPIO infusion as well as 24 hours later (results not shown).

After infusion of the dextran-coated USPIO particles at POD 6, a group of animals with allotransplants was given CsA for either 4 days (POD 7 to 10) (n = 5) or 7 days (POD 7 to 13) (n = 5), and dextran-coated USPIO particles were reinjected on POD 14. The animals treated with CsA for 7 days showed no MR signal reduction 24 hours after the USPIO injection, whereas the animals treated for 4 days showed a significant decrease in MR signal intensity in the heart, indicative of graft rejection (Figure 4).

Pathological and Immunohistochemical Analyses of the Cardiac Allograft
After MRI experiments at POD 7, the animals were euthanized, and both transplanted and native hearts and lungs were retained. The organs were fixed with 3.7% formaldehyde and processed for 3-μm sections. Pathological analysis of the allografts showed a moderate rejection (grade 3A equivalent), whereas the allografts with CsA treatment showed mild rejection (grade 1B or 2 equivalent). The syngeneic group showed no pathological evidence of rejection.

Perl’s Prussian blue staining revealed that the distribution of iron particles has an excellent correlation with the macrophages, ie, ED1+ cells (Figure 5). A superimposed image of both ED1 staining and Perl’s Prussian blue staining demonstrates that stained iron particles are observed in the cytoplasm of the ED1+ cells. As shown in Figure 6, ED1+ cells were significantly increased in allografts compared with isografts. These cells stained positive with Perl’s Prussian blue in all groups. This suggests that ED1+ cells are actively

Figure 1. MR images showing effect of infusion of dextran-coated USPIO particles (3 mg Fe/kg body wt) in an allotransplant. MR images of long-axis and short-axis views of rat heart graft are shown in A and B and C and D, respectively. A and C, MR images of graft at POD 6 before USPIO infusion; B and D, images taken 24 hours after infusion. White and black arrows indicate heart and lung, respectively. Representative images.

Figure 2. MR images showing effect of infusion of dextran-coated USPIO particles (3 mg Fe/kg body wt) in an isograft (A and B) and cyclosporin A–treated allograft (C and D). MR images of short-axis view of graft are shown. A and C, MR images at POD 6 before USPIO infusion; B and D, images taken 24 hours after infusion. Rat heart is indicated by arrow in each panel. Representative images.

Figure 3. Summary of measurements of MR signal intensity in transplanted rat heart. MR signal intensity of whole heart wall was measured in short-axis image of each transplanted heart. Data are mean ± SD, shown as percentage vs preinjection value. Allografts (*) showed significantly lower MR intensity vs isografts (×) at 1, 2, and 24 hours after USPIO infusion. CsA-treated allografts (○) also showed significantly lower MR intensity vs isografts but higher signal intensity relative to nonimmunosuppressed allografts.
involved in the rejection process. These results indicate that the iron particles phagocytosed by the macrophages modulate MR signal intensity at the rejection site of the allograft.

Discussion

In this study, we describe a novel, noninvasive method to detect cardiac allotransplant rejection by use of MRI. We have shown that intravenous injection of dextran-coated USPIO particles can be used for in vivo labeling of macrophages, which accumulate at the rejection sites after allogeneic organ transplantation. Macrophages labeled with USPIO particles induce a significant decrease in MR signal intensity in the allograft, and the degree of signal attenuation has an excellent correlation with the pathological rejection grade. Acute and chronic organ rejections remain a critical concern in caring for transplant recipients. An accurate method to diagnose rejection is necessary for appropriate management of transplanted patients. Currently, endomyocardial biopsy is the most reliable technique in making the diagnosis of rejection clinically. The invasive nature of endomyocardial biopsy, however, is associated with risks of morbidity and mortality from procedure-related complications, such as cerebral ischemia, cardiac perforation, and trauma to the tricuspid valve apparatus. Results from biopsy are also subject to possible false-negative results due to sampling errors, particularly if rejection occurs in focal or patchy distribution. Therefore, a reliable noninvasive technique for global detection of acute and chronic rejection is highly desirable.

We previously reported that in vivo labeling of macrophages with dextran-coated USPIO particles could be detected by MRI in rat transplantation models of kidney and lung. This gives rise to the possibility of a noninvasive method to detect rejection in organ transplantation. Although both T cells and macrophages are involved in the process of acute rejection, as demonstrated previously, USPIO particles are taken up mainly by macrophages and not as much by T cells. This is probably because of the much lower labeling efficiency of USPIO particles for T cells compared with macrophages.

Thus, in theory, the strategy that we used here reflects mainly macrophage-related changes in graft rejection. Because CsA affects not only T-cell but also macrophage accumulation indirectly, however, probably via a T-cell–mediated mechanism, macrophage-related information might reflect immunological events that have occurred in the allograft. Thus, we have shown an approach to detect acute cardiac rejection with MRI by use of macrophages labeled with USPIO particles in vivo. This labeling procedure clearly shows a difference in the MR signal intensity between

Figure 4. MR signal changes after CsA treatment. A group with allotransplants was treated with CsA for either 7 days (n=5) or 4 days (n=5) after initial MRI experiments showed decrease of MR signal intensity according to degree of graft rejection. On POD 14, transplanted rats were reinjected with USPIO particles, and then MRI experiments were performed. Animals treated 7 days showed minimal changes in MR signal intensity 24 hours after reinjection (A and B), and animals treated 4 days showed MR signal intensity that was significantly decreased after USPIO injection (C and D). MR images of short-axis view of graft are shown. MR images before USPIO infusion are at A and C, and images taken 24 hours after infusion are at B and D.

Figure 5. Immunohistological counts of ED1+ cells. ED1+ cells were counted in ≥10 fields per section per rat (n=6 in each group). Fields were randomly chosen, and counting was performed in a blinded manner at a magnification of ×400. Data are presented as mean±SD.

Figure 6. Immunohistochemical analyses of allograft. Perl’s Prussian blue staining shows distribution of iron-containing cells (A). B shows distribution of ED1+ cells. Comparing B with A, distribution of iron-containing cells correlates well with presence of ED1+ cells. This is further demonstrated in C by superimposed images from A and B. Arrows indicate ED1+ cells containing iron. Magnification ×400; reduction 333%.
allograft and isograft. It should be noted that 24 hours after injection of dextran-coated USPIO particles, there appears to be an excellent correlation between the pathological rejection grade and the degree of reduction in the MR signal intensity in the heart as well as in the lung, as previously reported. 16

Although this noninvasive MRI technique is promising, it should be noted that there are important limitations at present, as we mentioned in previous articles. 15, 16 Macrophages are involved not only in rejection but also in other antigen-nonspecific pathogenesis, such as reperfusion injury or infection. 24, 25 Thus, we need to pay attention to the fact that the information from MRI may not fully reflect the antigen-specific immunological response of T cells. Despite these limitations, in vivo labeling of macrophages does offer a potential clinical application for detection of cardiac rejection. Although a number of noninvasive approaches for detecting organ transplant rejection have been proposed, none of these approaches have yet been accepted for clinical use. This MRI-based method has the exciting potential to provide an accurate and reliable diagnosis of graft rejection noninvasively.

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