

In Vivo Magnetic Resonance Imaging of Mesenchymal Stem Cells in Myocardial Infarction

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Background—We investigated the potential of magnetic resonance imaging (MRI) to track magnetically labeled mesenchymal stem cells (MR-MSCs) in a swine myocardial infarction (MI) model.

Methods and Results—Adult farm pigs (n=5) were subjected to closed-chest experimental MI. MR-MSCs (2.8 to 16×10^7 cells) were injected intramyocardially under x-ray fluoroscopy. MRIs were obtained on a 1.5T MR scanner to demonstrate the location of the MR-MSCs and were correlated with histology. Contrast-enhanced MRI demonstrated successful injection in the infarct and serial MSC tracking was demonstrated in two animals.

Conclusion—MRI tracking of MSCs is feasible and represents a preferred method for studying the engraftment of MSCs in MI. (*Circulation*. 2003;107:2290-2293.)

Key Words: magnetic resonance imaging ■ myocardial infarction ■ cells ■ contrast media

Because of the limited regenerative capacity of the heart muscle, mesenchymal stem cells (MSCs), found in bone marrow, may have enormous therapeutic potential for limiting infarct size and restoring cardiac function after irreversible ischemic injury. Most techniques for the study of stem cell transplantation in animal models require histological analysis to determine the fate and migration of cells.¹⁻⁵ Thus, the number and location of MSCs delivered using intramyocardial delivery techniques can only be estimated postmortem. The recent ability to label MSCs⁶ with magnetic resonance imaging (MRI)-visible contrast agents should enable serial tracking and quantification of MSC transplantation noninvasively with high spatial resolution. Thus, the purpose of this study was to determine whether magnetically labeled MSCs injected intramyocardially could be detected and tracked noninvasively by MRI in a swine model of myocardial infarction.

Methods

Animal Model

Animal studies were approved by the Institutional Animal Care and Use Committee. The evening before creation of a myocardial infarction (MI), 5 farm pigs (25 to 35 kg; Archer Farms, Belcamp, Md) received aspirin (325 mg) and diltiazem (180 mg sustained release) orally. Animals were sedated with acepromazine, ketamine,

and atropine; induced with thiopental; and then intubated and mechanically ventilated with oxygen and isoflurane. To create MI, cardiac catheterization was performed via a 9F carotid sheath, and a 2.0 or 2.5×20-mm coronary angioplasty balloon (Cordis, Inc) was inflated in the left anterior descending (LAD) artery beyond the first diagonal branch to occlude LAD flow for 60 minutes, followed by reperfusion. After allowing for stabilization, allogeneic MSCs were given by intramyocardial injection using a deflectable guiding catheter and a helical needle (Biocardia, Inc). In 4 animals, MR-MSCs were injected; 1 control animal received nonlabeled MSCs.

Magnetically Labeled MSCs

Swine mesenchymal stem cells were isolated and cultured as previously described.⁷ The MSCs were culture-expanded 2 or 3 passages *in vitro*, yielding up to 400 million cells, which were frozen and thawed for use. In two studies, before freezing, the MSCs were fluorescently labeled with DiI (1,1'-dioctadecyl-3,3,3'-tetramethylindocarbocyanine perchlorate) and DAPI (4',6-Diamidino-2-phenylindole), which preferentially stain cell membranes and nuclei, respectively.

The swine MSCs were magnetically labeled by incubation with ferumoxides injectable solution (25 μ g Fe/mL, Feridex, Berlex Laboratories) in culture medium for 24 to 48 hours with 375 ng/mL poly-L-lysine (PLL; average MW=275 kDa) added 1 hour before cell incubation. Magnetic labeling was histologically assessed using Prussian Blue.

After magnetic labeling, Feridex-labeled MSCs (MR-MSCs) were washed in phosphate-buffered saline (PBS, pH=7.4), trypsinized,

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washed, and resuspended in 0.01 mol/L PBS at a concentration of 7 to 10×10^6 cells/mL.

MR Imaging

All imaging was performed under general anesthesia on a 1.5T MR scanner (CV/i, GE Medical Systems) using ECG gating, with the animal in right decubitus and a 4-channel phased-array coil wrapped around the chest wall. All images were acquired during suspension of the ventilator.

To assess MR-MSC detection, animals were imaged within the first 24 hours after intramyocardial MR-MSC injection. In addition, one animal was reimaged at 1 week and another at 3 weeks to detect MR-MSC migration. Pulse parameters for cardiac gated, fast gradient-recalled echo (FGRE), and double-inversion recovery, fast spin echo (FSE) were optimized to detect MR-MSCs.

The size and location of the myocardial infarction was assessed by using delayed-enhancement MRI (DE-MRI) with image parameters of 7.8-ms repetition time (TR); 3.4-ms echo time (TE); 25° flip angle (FA); 256×192 image matrix; 5-mm slice thickness; 32-kHz bandwidth (BW); 28-cm field-of-view (FOV); 2 slice averages (NSA); and 250-ms inversion delay (TI). Gd-DTPA (Magnevist, Berlex Laboratories; 0.2 mmol/kg bolus) was injected intravenously; DE-MRI was acquired 15 minutes after contrast. The number of injection sites visualized by MRI was determined by a consensus of two observers blinded to the location or number of injections. The MR-MSC lesion volume was determined using planimetry of FGRE MRI (Cinetool, GE). The contrast of the MR-MSC hypointense lesion from the FGRE images, acquired within 24 hours of MR-MSC injection and at 1 week after injection, was determined using the difference in signal intensity between the hypointense and normal myocardium divided by signal intensity of the normal region.

Postmortem Analysis

After humane euthanasia, the heart was excised and sliced along the short-axis plane. Histology corresponding to MRI slices that demonstrated MR-MSCs lesions was performed.

Results

In Vitro Magnetic Labeling of MSCs

MSCs appeared to be unaffected by magnetic labeling, and viability immediately before injection, as determined by trypan blue exclusion, was >95%. Prussian Blue staining of PLL-Feridex-labeled MSCs revealed the presence of numerous iron-containing vesicles or endosomes in the cytoplasm.

Optimized MR Imaging Parameters

Hypointense lesions attributable to MR-MSCs were better visualized on FGRE images than on FSE images (Figure, A and B). FGRE image parameters were as follows: TR/TE/FA=6.0 ms/1.6 ms/20°; 512×512 image matrix; 5-mm slice thickness; 32-kHz BW; 28-cm FOV; and 4 NSA. Images were acquired in the same imaging planes using an ECG-gated FSE pulse sequence with blood suppression. Optimized imaging parameters were as follows: TR/TE=1463 ms/85 ms; echo train length=32; 256×256 image matrix; 62.5-kHz BW; and 2 NSA. Due to the apical location of the MR-MSC injections, the hypoenhancing lesions were best appreciated on long-axis images (Figure, A and B).

Intramyocardial Injections

A total of 50 intramyocardial injections (Table) were performed under x-ray fluoroscopy. One injection in each of four animals was intentionally placed far from the infarct territory. Twenty-four of the 34 MR-MSC injections were visualized by MRI, but none of the nonlabeled injections were seen. One

of four injections placed remote from the infarction could not be detected with MRI. DE-MRI confirmed the presence of MR-MSC injection sites within the infarct (ie, hypoenhancing lesion within hyperenhancing infarcted tissue; Figure, C).

Images of MR-MSC injection sites within 24 hours after injection appeared as ovoid hypoenhancing lesions with sharp borders (Figure, D). At 1 week and 3 weeks after injection, the delineation of the borders was less clear (Figure, E). At 1 week after MR-MSC injection, all lesions that were detected within 24 hours after injection could still be detected. The size of the lesions expanded by 15% over 1 week. The contrast of the lesion decreased by $24 \pm 4\%$ in concert with the lesions' expansion at 1 week. However, at 3 weeks after injection, some wall thinning had occurred in the region to the extent that only 25.8% of the original MR-MSC lesions could be detected. Multiple hypointense lesions that were present within 24 hours of MR-MSC injection could no longer be appreciated at 3 weeks. Instead, a hypointense signal was appreciated along the rim of the endocardial border adjacent to the area of wall thinning and myocardial infarction.

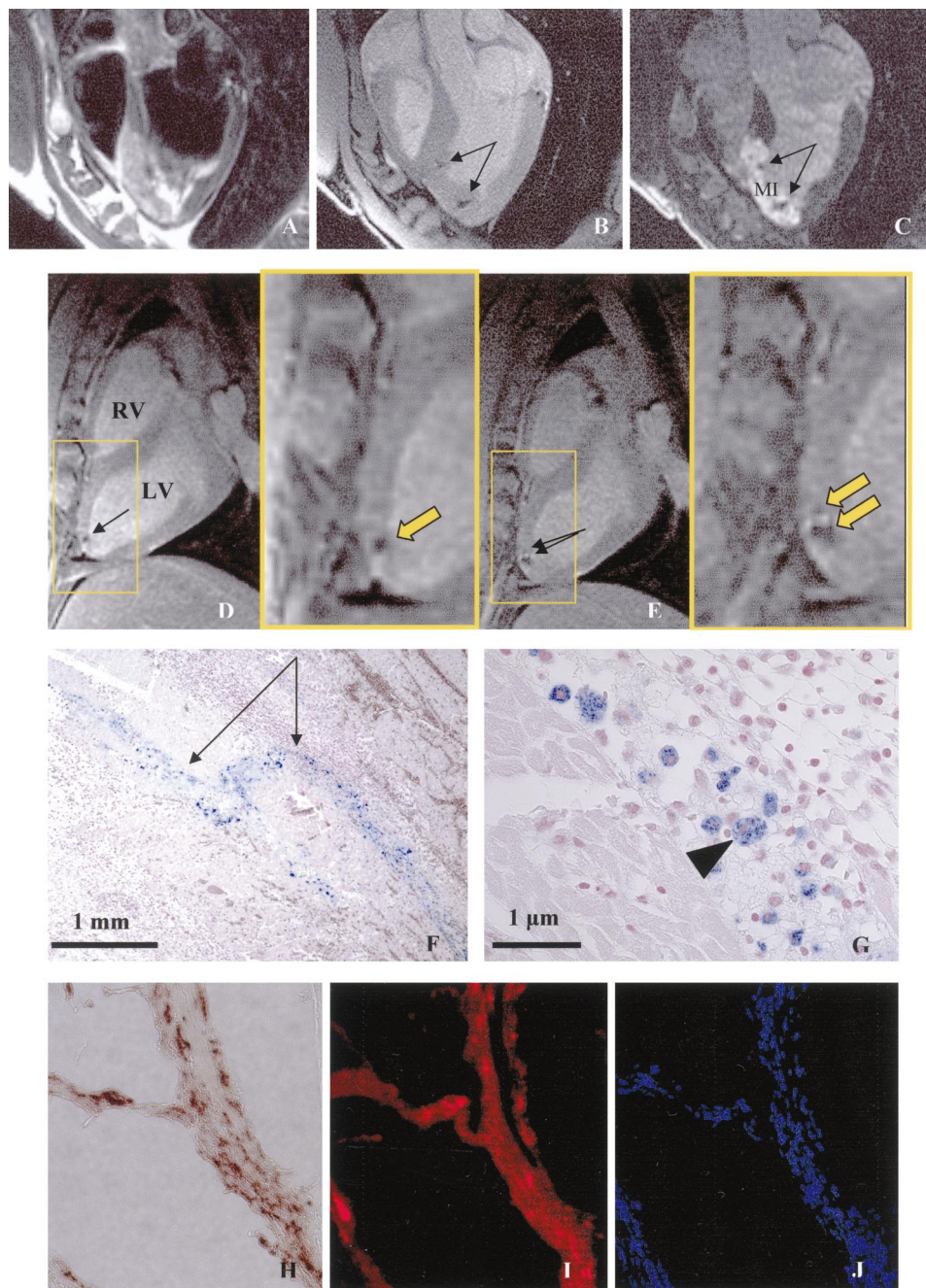
Postmortem Analysis

Prussian blue staining consistently showed dense intracellular iron labeling in large round cells, with iron exclusion from the nucleus. Cells were detected in sections corresponding to lesions seen on MRI. In addition, fluorescently labeled MSCs were demonstrated on histological sections adjacent to Prussian Blue staining for Feridex-labeled MSCs (Figure, F through J), indicating that Feridex remained within the originally labeled cells. Intramyocardial injection sites could not be appreciated grossly postmortem. However, distinct "needle-tract" lesions representing MR-MSCs were present on the Prussian blue-stained sections (Figure, F).

Discussion

A high degree of magnetic labeling of MSCs was achieved using poly-L-lysine as a transfection agent. PLL coating of the Feridex effectively chaperones the iron oxide nanoparticles across the cellular membrane.⁸ Cell viability remained >95% after Feridex/PLL labeling, with no increased apoptosis at 3 days after labeling, no alterations to proliferation, or increased toxicity for at least 9 days as determined by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) assay.⁸

A major difficulty with x-ray fluoroscopic delivery of MSCs for cardiac regeneration is the inability to determine whether an injection was successful. Labeling MSCs with an MR contrast agent allows determination of the size and location of each intramyocardial injection to determine the extent of MSC retention. This labeling technique uses an approved contrast agent with a non-species-specific transfection technique, the imaging protocol uses a standard clinical MRI scanner, and the swine model uses devices under development for human trials in a closed-chest MI model that is minimally invasive and thus more comparable to human MI pathology than open-chest rodent models. Thus, this study demonstrates the feasibility of MR-MSC labeling for future clinical trials of cellular cardiomyoplasty.



Representative hypointense lesions in FSE (A), FGRE (B), and DE-MRI (C) of MR-MSC injection sites (arrows) within 24 hours of injection. MR-MSCs were injected in the infarct (MI, hyperintense region in C). Long-axis MRIs showing hypointense lesions (arrows) caused by MR-MSCs acquired within 24 hours (D) and 1 week (E) of injection with inset at right demonstrating expansion of lesion over 1 week. Needle tract (arrow) of MR-MSCs is demonstrated in histological section at 1 week after injection with Prussian Blue staining (F) as cells with blue iron inclusions (arrowhead) that are excluded from nucleus (G). Iron inclusion from DAB-enhanced Prussian Blue staining (H) matches co-labeling with Dil (I) and DAPI fluorescent dyes (J) on adjacent histological sections at $\times 20$ magnification at 24 hours after MSC injection in another animal, indicating Feridex is still contained within original MSCs. LV indicates left ventricle; RV, right ventricle.

In previous swine MI studies, 10 to 20 injection sites of ≈ 20 million cells per site have demonstrated the ability of swine MSCs to engraft in the infarct, differentiate toward a cardiomyocyte phenotype, and improve global cardiac function compared with control animals.⁹ In the present study, we were able to detect MSC injection sites at approximately one third to one half the therapeutic dose of previous studies,

lending promise to the ability to detect small numbers of MSCs by MRI.

Although this decreased dose of MSCs did not prevent wall thinning at 3 weeks in one animal, serial tracking of MSC lesions in two animals showed increasing lesion size with a concurrent decrease in lesion contrast that could result from the following: (1) MSC division with decreasing Feridex

MRI Visualization of Intramyocardial MSC Injections

Study	MSCs/mL of PBS	No. of 1-mL Injections	Total MSCs Injected	MRI Visualized
1	7×10^6	4	2.8×10^7	3
2	7×10^6	6	4.2×10^7	4
3	7×10^6	11	7.7×10^7	7
4	1×10^7	13	1.3×10^8	10
5*	1×10^7	16	1.6×10^8	0

*Unlabeled MSCs.

concentration/MSC; (2) migration of MSCs with decreasing Feridex concentration/voxel; or (3) a combination of MSC migration and division. Decreased contrast could result from MSC death and removal but would not explain the expanded lesion volume without reuptake of iron by macrophages, which contradicts the histological picture in this study. Future studies will further delineate the fate of injected MR-MSCs and will incorporate noninvasive tagging methods to monitor myocardial function after MSC engraftment in the MI. The recent report of autologous intracoronary bone marrow transplantation in MI patients¹⁰ demonstrates the need for noninvasive techniques to monitor MSC delivery and response to treatment in future clinical trials.

Limitations

In this study, using a limited number of injections per animal, we were unable to visualize by MRI $\approx 30\%$ of the injections performed under x-ray fluoroscopy in each animal.

Because of the large T2 effects exerted by Feridex, only a small quantity of labeled MSCs (≈ 10 to 20 MSCs/voxel) is required to cause a hypointense lesion. The “missed” MR visualization of injection sites could be explained by four possibilities: areas where (1) intramyocardial delivery failed; (2) an extremely low MR-MSC concentration was injected; (3) the saline medium alone (without MR-MSCs) was injected; or (4) multiple injections occurred at the same anatomical location.

Conclusion

MR tracking of MR-MSCs is feasible and represents a method for noninvasively tracking the quantity and location of intramyocardial delivery after MI. The ability to perform serial assessment of infarct size and regional cardiac function by MRI while tracking MR-MSCs offers a method to study the optimum protocols for delivery of MSCs for cardiac regeneration.

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References

1. Jackson KA, Majka SM, Wang H, et al. Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. *J Clin Invest.* 2001;107:1395–1402.
2. Wang JS, Shum-Tim D, Galipeau J, et al. Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages. *J Thorac Cardiovasc Surg.* 2000;120:999–1005.
3. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature.* 2001;410:701–705.
4. Toma C, Pittenger MF, Cahill KS, et al. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation.* 2002;105:93–98.
5. Kocher AA, Schuster MD, Szabolcs MJ, et al. Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function. *Nat Med.* 2001;7:430–436.
6. Bulte JW, Douglas T, Witwer B, et al. Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. *Nat Biotechnol.* 2001;19:1141–1147.
7. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science.* 1999;284:143–147.
8. Frank JA, Miller BA, Arbab AS, et al. Clinically applicable labeling of mammalian (stem) cells by combining (FDA)-approved superparamagnetic iron oxides and commonly available transfection agents. *Radiology.* In press.
9. Shake JG, Gruber PJ, Baumgartner WA, et al. Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects. *Ann Thorac Surg.* 2002;73:1919–1925; discussion 1926.
10. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002;106:1913–1918.