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EXPEDITED REVIEW

Magnetic Resonance Imaging of Targeted Catheter-Based Implantation of Myogenic Precursor Cells Into Infarcted Left Ventricular Myocardium

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OBJECTIVES This study was designed to test the hypothesis that myocardial implantation of myogenic precursor cells (MPC) loaded with iron oxide can be reliably detected in vivo by cardiac magnetic resonance imaging (MRI).

BACKGROUND In vivo imaging of targeted catheter-based implantation of MPC into infarcted left ventricular (LV) myocardium is unavailable.

METHODS The study was conducted in seven farm pigs (four with anterior myocardial infarction), in which autologous MPC were injected through a percutaneous catheter allowing for LV electromechanical mapping and guided micro-injections into normal and infarcted myocardium. Cardiac MRI was used to detect implanted MPC previously loaded with iron oxide nanoparticles.

RESULTS Magnetic resonance imaging data were compared with LV electromechanical mapping and cross-registered pathology. All 9 injections into normal and 12 injections into locally damaged myocardium were detected on T2-weighted spin echo and inversion-recovery true-fisp MRI (low signal areas) with good anatomical concordance with sites of implantation on electromechanical maps. All sites of injection were confirmed on pathology that showed in all infarct animals iron-loaded MPC at the center and periphery of the infarct as expected from MRI.

CONCLUSIONS Targeted catheter-based implantation of iron-loaded MPC into locally infarcted LV myocardium is accurate and can be reliably demonstrated in vivo by cardiac MRI. The ability to identify noninvasively intramyocardial cell implantation may be determinant for future experimental studies designed to analyze subsequent effects of such therapy on detailed segmental LV function. (J Am Coll Cardiol 2003;41:1841–6) © 2003 by the American College of Cardiology Foundation

Over the last decade, several studies have used cell transplantation to limit postinfarction fibrous scar formation and subsequent congestive heart failure by grafting of myogenic precursor cells (MPC) into left ventricular (LV) myocardium (1–4). In experimental studies, MPC implanted into ischemic damaged myocardium have generated functional tissue (1,4). The feasibility and effectiveness of MPC transplant into infarcted myocardium were recently demonstrated in humans (5,6). In these studies, autologous MPC were implanted into a postinfarct scar using direct epicardial injections during coronary artery bypass grafting. This approach requires sternotomy that is associated with significant morbidity and mortality. Also, it combines the use of two distinct therapeutic procedures, which may be confusing when analyzing the role of MPC transplant in the improvement of LV function. The development of catheter-based techniques for intramyocardial cell implantation would represent a significant step towards the clinical emergence of this promizing therapy. Besides, a noninvasive in vivo imaging technique able to attest proper implantation of MPC would be determinant for accurate analysis of subsequent therapeutic effects. We assessed, through the use of cardiac magnetic resonance imaging (MRI), the feasibility and accuracy of targeted catheter-based myocardial injections of iron-loaded MPC into normal and infarcted myocardium in pigs.

METHODS

Experimental model/cell cultures. Seven farm pigs (25 to 35 kg) were premedicated with intramuscular ketamine (15 mg/kg), anesthetized with Propofol (0.35 mg/kg intravenous [IV] bolus, and 0.05 mg/kg/min), and ventilated. Skeletal muscular biopsy (right sternocleidomastoid) was performed in all animals. Immediately after biopsy, anterior myocardial infarction was induced under deep anesthesia in four animals by a 90-min balloon occlusion of the mid-left anterior descending artery followed by reflow (6F sheath introduced in the left carotid artery). Animals received 5,000 UI heparin and 250 mg aspirin IV and were then allowed to recover.

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Abbreviations and Acronyms

Gd-DTPA	= gadolinium-enhanced diethlenetriaminepentaacetic acid
IV	= intravenous
LV	= left ventricular
MPC	= myogenic precursor cells
MRI	= magnetic resonance imaging

Skeletal muscle–derived MPC cultures were carried out as previously described (7,8). Briefly, muscles were mechanically minced, washed, and incubated in digestion medium (HAM F12-HEPES containing 1.5 mg/ml pronase E and 0.03% EDTA) during 40 min at 37°C. Cells were isolated from tissue debris after wash-out, slow centrifugation, and filtering. They were then seeded in HAM-F12 containing 15% fetal calf serum. Cell expansion was enhanced by addition of human basal fibroblast growth factor (10 ng/ml) and insulin-like growth factor 1 (50 ng/ml). Cultures in Cell Factory (Nunc, Roskilde, Denmark) allowed the production of $\sim 10^9$ cells in four weeks. The MPC were then incubated between 4 and 36 h with various concentrations of nanoparticles of iron oxide (Endorem, Guerbet, France) (0 to 4 mg iron/10^6 cells). Potential toxicity of iron oxide towards MPC was checked either immediately, or between 4 and 20 days after incubation, using the WST-1 proliferation kit (Roche Diagnostics, Manheim, Germany). Iron Oxide uptake by the MPC was checked by Perls stain, and optimal conditions were defined as 24 to 36 h incubation with 4 mg iron/10^6 cells. After incubation with Endorem, MPC were extensively washed with phosphate buffered saline, trypsinized, and recovered in serum-free medium containing 0.5% porcine serum albumin before catheter injection (8).

Catheter injections. Four weeks after muscular biopsy, a nonfluoroscopic endoventricular electromechanical mapping was performed in all seven animals (Noga-Star, Biosense Webster, Diamond Bar, California), allowing for the detection of necrotic myocardium in infarct pigs defined as reduced electrical (>5 mV) and mechanical activity (local shortening <5%) (9,10). This catheter is implemented by a 27-gauge needle at its extremity that enables guided microinjections into LV myocardium (11). The lack of potential

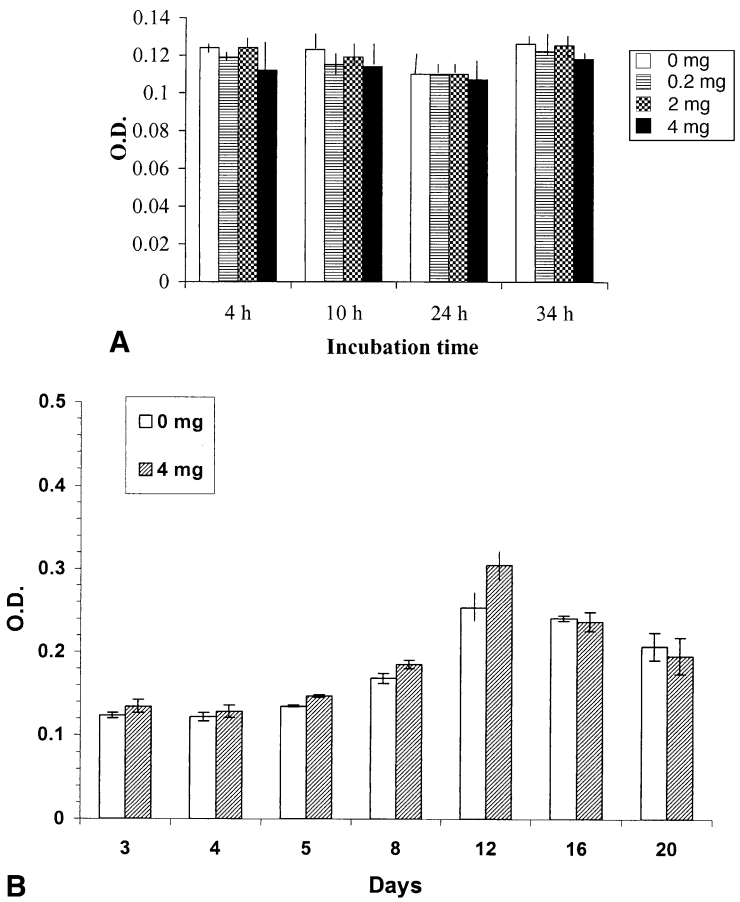

Figure 1. **(A)** Short-term toxicity of nanoparticles of iron oxide (Endorem) towards myogenic precursor cells (MPC) was evaluated immediately after incubation with various Endorem concentrations (0 to 4 mg iron/10^6 cells) and during different incubation times (4 to 36 h). Cell viability was determined with the WST-1 Roche Diagnostics proliferation kit (Manheim, Germany). The optical density represents mitochondrial enzymatic activity that is proportional to the number of viable cells. There was no evidence of short-term toxicity of Endorem towards MPC. **(B)** Long-term toxicity of Endorem towards MPC that were incubated with 4 mg iron/10^6 cells during 24 h. Cell viability was evaluated at 3- to 20-day cultures using the WST-1 kit. Results are expressed as mean optical density ± SD of two experiments performed in triplicate. Compared with control **(white bars)**, there was no evidence of long-term toxicity of Endorem **(gray bars)** towards MPC in culture. OD = optical density.

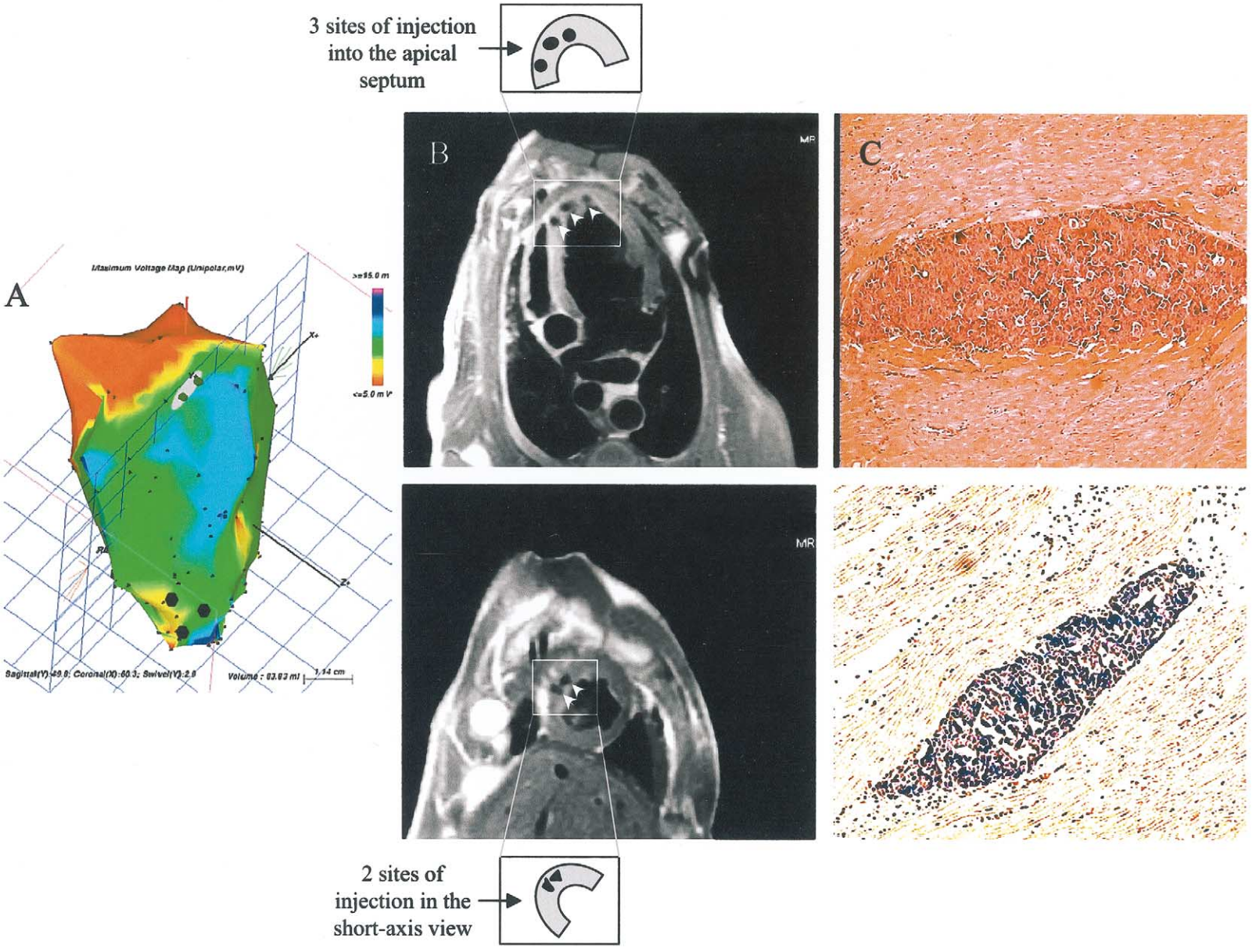

Figure 2. **(A)** Left ventricular (LV) voltage map in a healthy animal in which three percutaneous catheter-based injections of iron-loaded myogenic precursor cells (MPC) were performed at the LV apex **(black dots)**. **(B)** Corresponding magnetic resonance imaging 90 min after MPC implantation (long axis and apical short axis black-blood T2-weighted turbo spin echo imaging) showing the three sites of injection **(arrows)** on the four-chamber view into normal myocardium with good anatomical agreement (two sites of injection were intersected on the two-chamber view, **arrows**). **(C)** Corresponding hematoxylin **(upper panel)** and Perls stain **(lower panel)** of implanted iron-loaded MPC into healthy apical LV myocardium (×100).

Table 1. Sites of Implantation on Electromechanical Maps and MRI

Pig Number	Targeted Injections By Electromechanical Maps	MRI
Normal animals		
1	2 septum, 1 apex	2 septum, 1 apex
2	1 lateral, 2 apex	1 lateral, 2 apex
3	3 mid-septum	3 mid-septum
Infarcted animals (septo-apical infarcts)		
4		3 apical septum
5	The 4 infarct animals had 1 injection	3 apical septum
6	in the center and 2 at the periphery of the infarct	1 apical septum, 1 mid-septum, 1 apex
7		3 apical septum

Electromechanical maps and MRI were visually assessed by two independent observers blinded to each other's data.
MRI = magnetic resonance imaging.

cell damage caused by the passage through the catheter and needle has been previously evaluated by comparing cell mortality and myogenic capacities of MPC at entry and distal extremity of the device (8). Guided transendocardial injections (0.4 ml each, at the concentration of 100.10^6 cells/ml, 3 to 5 mm into the myocardium) were performed at three different locations in healthy pigs and three locations within the ischemic tissue (one in the center, and two in the periphery) in the four infarct animals. Pigs were then transported to MRI facilities.

MRI. Ninety minutes after cell grafting, cardiac MRI (1.5 T Siemens Symphony, Erlangen, Germany) was performed in anesthetized animals in the right antecubital position with electrocardiographic gating and short breath-hold acquisitions. A flexible cardiac phased-array coil was wrapped around the chest for signal acquisition. The LV long axis was localized. For detection of implanted cells, single-phase black-blood T1- and T2-weighted turbo spin echo MRI was performed in the apical four-chamber and two-chamber views and in a series of six LV short-axis slices from base to apex. Imaging parameters were typically: field of view 280 mm; repetition time = 2RR intervals; echo time 25 ms for T1 and 65 ms for T2; image matrix 256 × 160; slice thickness 6 mm. For infarct size imaging, a single-phase two-dimensional inversion-recovery true-fisp (steady-state free precession, ssfp) pulse sequence (field of view 280 mm; inversion time 200 to 280 ms, slice thickness 6 mm) was acquired in the same views 15 min after gadolinium-enhanced diethlenetriaminepentaacetic acid (Gd-DTPA) injection (0.1 mM IV).

Pathology. Pigs were euthanized under deep anesthesia with IV potassium chloride 3 h after cell injections for pathology. The heart was excised, and the LV was cut from base to apex into six ~6 mm-thick short-axis slices for cross-registration with MRI. The junction between the right ventricular free wall and the inferior septum was used as a landmark, and myocardial segments were numbered from 1 to 17 according to the recommendations of the American Heart Association for cross-registration between imaging and pathology (12). Myocardial segments in which injections were performed, as well as remote tissue used for control, were thin cut and stained with hematoxylin-eosin and Perls stain.

The animals were used in accordance with the Guide for the Care and Use of Laboratory Animals (DHHS publ. No. NIH 85-23, revised 1996, Office of Science and Health Reports, Bethesda, Maryland).

RESULTS

In vitro cell viability. There was no immediate toxicity of iron oxide towards MPC (Fig. 1A), nor in cultures up to 20 days (Fig. 1B). We have recently shown that cell passage through the catheter was not associated with significant cell mortality (8).

Accuracy of percutaneous guided micro-injections. In healthy animals, LV maps showed normal electrical and mechanical activity. All nine injections within normal myocardium were detected on T2-weighted spin echo MRI as low signal areas (iron oxide) (Figs. 2A and 2B) (Table 1). On pathology, all sites of injection were found and corresponded to cross-registered low signal areas by MRI. Perls stain revealed a huge number of implanted MPC that were loaded with iron and organized as densely packed mononucleated myoblasts (Fig. 2C). There was no visible lesion in adjacent myocardium and no MPC in remote myocardium either by MRI or on Perls stain.

In the four infarct pigs, LV maps showed dramatically reduced electrical and mechanical activity in the anterior wall. Iron-loaded cell injections into the infarct region were all detected by T2-weighted spin echo MRI. Fifteen minutes after Gd-DTPA, inversion-recovery true-fisp MRI showed in all animals a delayed hyperenhancement containing three distinct low signal regions (Figs. 3A and 3B). All infarcts were located in the apical interventricular septum and represented 6.6 ± 2.1% of total LV myocardial area by planimetry on MRI. Overall, the 12 sites of injection were found by MRI within the hyperenhanced infarcted region (Table 1). There was a nice agreement with cross-registered pathology that showed in all animals the presence of iron-loaded MPC at the center and periphery of the infarcted tissue as expected from MRI (Fig. 3C).

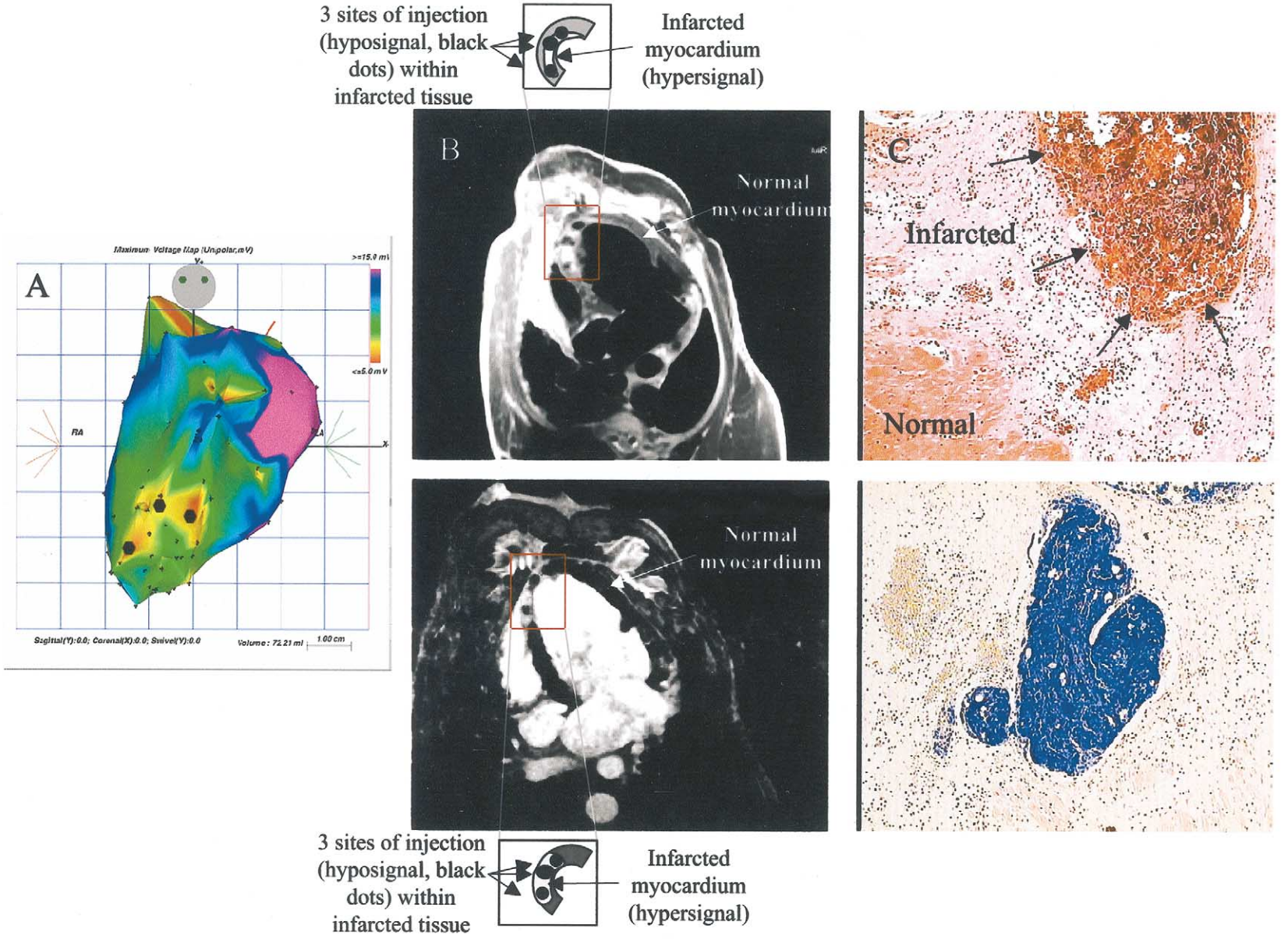

Figure 3. **(A)** Left ventricular voltage map in an infarct animal in which three percutaneous catheter-based injections of iron-loaded myogenic precursor cells (MPC) were performed into locally damaged myocardium (one in the center and two at the periphery, **black dots**). **(B)** Corresponding magnetic resonance imaging 90 min after MPC implantation (long axis black-blood T2 turbo spin echo imaging, **upper panel**; inversion-recovery true-fisp in the same view, **lower panel**) showing three sites of injection **(arrows)** into hyperenhanced damaged tissue with nice anatomical agreement. **(C)** Corresponding hematoxylin **(upper panel)** and Perls stain **(lower panel)** of implanted iron-loaded MPC **(arrows)** into ischemic myocardium (×100).

DISCUSSION

The main results of the study showed: 1) sites of implantation of MPC loaded with iron oxide can be reliably detected in vivo by cardiac MRI; and 2) precise targeted catheter-based implantation of MPC into locally infarcted myocardium is feasible and accurate.

So far, only pathology has enabled identification of the integration of recently implanted cells into LV myocardium (13,14). The demonstration that cardiac MRI is accurate for noninvasive in vivo identification of iron-loaded cell implantation may be determinant for future experimental studies, particularly for accurate interpretation of follow-up functional studies. Potential effects of implanted cells on subsequent recovery of LV function can be analyzed accurately, provided one can make sure that cells were properly implanted at targeted locations within ischemic myocardium. Indeed, MRI offers the unique opportunity to confirm in vivo targeted myocardial implantations of MPC without the need for sacrifice of the animals. All sites of implantation were accurately identified by MRI and further confirmed by pathology with good anatomical agreement. At utilized concentrations, iron consistently generates susceptibility artifact that represents the basis for signal detection by MRI. Implanted cells appear as low signal area on T2-weighted images, and sites of injection are also clearly seen within infarcted myocardium on delayed contrast-enhanced MRI.

This work also demonstrates that percutaneous catheter-based delivery of MPC into locally infarcted myocardium is accurate and complements recent studies that have described intramyocardial catheter-based injection of plasmid (15), adenovirus (16), or more recently bone marrow cells in pigs (17) or patients with ischemic heart disease (18).

Study limitations. The contrast in the image relies on a susceptibility artifact, which implies that there is no linear relationship between the intensity of the signal and the number of implanted cells. Therefore, the size and transmural distribution of the injection cannot be accurately assessed. Although we have previously demonstrated that cell viability is preserved after up to two weeks when implanted cells are recovered from the implanted tissue 3 h after injections (8), future studies are warranted to determine long-term viability, structural and architectural organization, and functional capacity of implanted cells. To date, there are no data about the potential toxicity of direct injections of iron-loaded cells into human myocardium, therefore, this method is not suitable for use in patients.

CONCLUSIONS

After myocardial infarction, precise targeted catheter-based implantation of MPC into locally infarcted myocardium is feasible and accurate and can be reliably and noninvasively identified in vivo by cardiac MRI.

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