Permanent Coronary Artery Occlusion: Cardiovascular MR Imaging Is Platform for Percutaneous Transendocardial Delivery and Assessment of Gene Therapy in Canine Model¹

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Purpose:

To provide evidence that vascular endothelial growth factor (*VEGF*) genes delivered transendocardially with magnetic resonance (MR) imaging guidance may neovascularize or improve vascular recruitment in occlusive infarction.

Materials and Methods:

All experimental procedures received approval from the institutional committee on animal research. Dogs with permanent coronary artery occlusion were imaged twice (3 days after occlusion for assessment of acute infarction; a mean of 50 days after occlusion $\pm$ 3 [standard error of the mean] for assessment of chronic infarction). A mixture of plasmid *VEGF* and plasmid *LacZ* ($n = 6$, treated animals) or plasmid *LacZ* and spirodianide ($n = 6$, placebo control animals) was delivered to four sites. MR fluoroscopy was used to target and monitor delivery of genes. The effectiveness of this delivery approach was determined by using MR imaging methods to assess perfusion, left ventricular (LV) function, myocardial viability, and infarct resorption. Histologic evaluation of neovascularization was then performed.

Results:

MR fluoroscopic guidance of injectates was successful in both groups. Treated animals with chronic, but not those with acute, infarction showed the following differences compared with control animals: (a) steeper mean maximum upslope perfusion ($200 \text{ sec}^{-1} \pm 32$ vs $117 \text{ sec}^{-1} \pm 15$, $P = .02$), (b) higher peak signal intensity (1667 arbitrary units ± 100 vs 1132 arbitrary units ± 80 , $P = .002$), (c) increased ejection fraction (from $27.9\% \pm 1.2$ to $35.3\% \pm 1.6$, $P = .001$), (d) smaller infarction size (as a percentage of LV mass) at MR imaging ($8.5\% \pm 0.9$ vs $11.3\% \pm 0.9$, $P = .048$) and triphenyltetrazolium chloride staining ($9.4\% \pm 1.5$ vs $12.7\% \pm 0.4$, $P = .05$), and (e) higher vascular density (as number of vessels per square millimeter) at the border (430 ± 117 vs 286 ± 19 , $P = .0001$) and core (307 ± 112 vs 108 ± 17 , $P = .0001$).

Conclusion:

The validity of plasmid *VEGF* gene delivered with MR fluoroscopic guidance into occlusive infarction was confirmed by neovascularization associated with improved perfusion, LV function, and infarct resorption.

Heat failure due to infarction is a disease of increasing prevalence and poor prognosis. Recent predictions suggest that heart failure will become the leading cause of all disability by 2020 (1). For patients with refractory end-stage heart failure, the best therapeutic intervention is heart transplantation, but this is extremely limited by the supply of acceptable donor hearts, which has reached a plateau of less than 3000 per year worldwide (1). On the other hand, gene therapy continues to show promise as a tool for the investigation and treatment of cardiac diseases. Progress toward clinical applications has been slowed by limited in vivo delivery methods at the gene (2) and interventional (3) levels.

Transendocardial gene delivery aims to (a) achieve a locally effective concentration, (b) minimize systemic side effects, (c) minimize the effect of circulating enzymes on therapy, and (d) eliminate open-chest surgery. Both x-ray fluoroscopy (4,5) and an electromechanically guided approach (NOGA; Biosense-Webster, Haifa, Israel) (4,6,7) have been used to guide transendocardial delivery in ischemic heart disease. Unlike these modalities, magnetic resonance (MR) imaging can help differentiate and delineate acute and chronic infarction (3,8–11), and it offers precision and efficacy advantages

in delivering local therapies over other techniques (3–15).

MR fluoroscopic guidance has been successfully used in delivering stem cells into infarction (9,10,12), but not genes. Results of other studies (4,5,13,16) have shown the salutary effects of vascular endothelial growth factor (*VEGF*) genes in the infarcted myocardium of humans and animals. To our knowledge, however, the effectiveness of *VEGF* genes delivered transendocardially into two sites at the border zone and two additional sites in the core of the infarction with MR fluoroscopic guidance has not been previously validated. The current study provides advancement over our earlier studies of hypoxia-inducible cardiac-specific adenoassociated *VEGF* genes delivered epicardially in reperfused infarction (16,17) in that it involved the use of (a) MR imaging guidance (active and passive catheter tracking) to navigate the endovascular catheter to hit the target and inject the genes into four sites (two at the border zone and two additional sites at the core of the occlusive infarction); (b) a comprehensive MR imaging protocol to assess myocardial perfusion, left ventricular (LV) function, and myocardial viability before and after therapy; and (c) specific immunohistologic staining procedures for the quantification of vascular density in remote myocardium and in the border zone and core of the infarction. The purpose of this study was to provide evidence that *VEGF* genes delivered transendocardially with MR imaging guidance may neovascularize or improve vascular recruitment in occlusive infarction.

Advances in Knowledge

- The therapeutic efficacy of a plasmid vascular endothelial growth factor (*VEGF*) gene, a cytomegalovirus promoter driving *VEGF*₁₆₅ complementary DNA, delivered transendocardially with MR imaging guidance into the border and core of infarction was confirmed by neovascularization, improvement in myocardial perfusion and left ventricular function, and infarct resorption.
- The combination of percutaneous MR fluoroscopic guidance and genes is a minimally invasive technique for neovascularization of occlusive infarction.

Implication for Patient Care

- MR imaging guidance of transendocardial delivery of the *VEGF* gene can enable a substantially reduced level of invasiveness compared with open-chest surgery; it may result in treatment on an outpatient basis, rapid patient recovery, and cost savings to the health care system.

Materials and Methods

Therapeutic Genes

This study tested a plasmid *VEGF* created in house that contained a cytomegalovirus promoter driving *VEGF*₁₆₅ complementary DNA. To create the plasmid *VEGF*, we (H.S. and Y.W.K.) inserted the *VEGF*₁₆₅ complementary DNA in the multicloning sites containing a cytomegalovirus promoter and SV40 poly A flanking sites. A total of four injections of 0.5 mL each were made (M.S. and A.J.) of a mixture of 2 mL of plasmid *VEGF* (2 mg) with *LacZ* (2 mg) (treated animals) or placebo plasmid *LacZ* (2 mg) with dysprosium diethylenetriaminepentaacetic acid *bis*(methylamide) (sprodiamide, GE Healthcare, Milwaukee, Wis) (control animals), respectively.

Gadoterate meglumine (Dotarem; Guerbet, Roissy, France) at 0.1 mmol per kilogram of body weight was used for perfusion imaging. An additional dose of 0.05 mmol/kg was administered to delineate acutely infarcted myocardium. The four-injection protocol used in the current study was similar to that used in a recent dog study by Ferrarini et al (18), who also injected *VEGF* gene into two sites at the border zone and two additional sites in the core of infarction. The *VEGF* gene or plasmid vehicle

Published online before print
10.1148/radiol.2491072068

Radiology 2008; 249:560–571

Abbreviations:

LV = left ventricle
TTC = triphenyltetrazolium chloride
VEGF = vascular endothelial growth factor

Author contributions:

Guarantor of integrity of entire study, M.S.; study concepts/study design or data acquisition or data analysis/interpretation, all authors; manuscript drafting or manuscript revision for important intellectual content, all authors; manuscript final version approval, all authors; literature research, M.S.; experimental studies, M.S., A.M., A.J., M.B., L.D.; statistical analysis, M.S., A.J., M.B., L.D.; and manuscript editing, all authors

Funding:

This research was funded by the National Institutes of Health (grant R01 HL07295).

See Materials and Methods for pertinent disclosures.

was injected into the core of the infarct, which was defined by the volume exhibiting enhancement at viability imaging, while the border zone was considered to be within 5 mm of the region boundary. Short- and long-axis views were used to define each injection site. The contrast material-enhanced MR images and triphenyltetrazolium chloride (TTC)-stained slices were used to guide sampling of tissues for histologic staining and analysis. No staff member of any of the manufacturers mentioned above is an author of this study or had control of the study data.

Experimental Protocol

All experimental procedures received approval from the institutional committee on animal research. This investigation conformed to National Institutes of Health guidelines for the care and use of laboratory animals. Male 8-month-old mongrel dogs (Marshall Bioresources, North Rose, NY) ($n = 15$; weight range, 28–32 kg) were anesthetized by using isoflurane and oxygen (1.5%–2.0% and 2 L/min, respectively), and the left anterior descending artery was permanently occluded by using an open-chest surgical procedure (18) (M.S., A.J.). Three dogs were excluded because of the absence of infarction at contrast-enhanced MR imaging. The 12 others with infarction were randomly divided prior to treatment into treated ($n = 6$) and placebo control groups ($n = 6$). Three days after infarction, a percutaneous catheter fitted with multiple radiofrequency receiver coils was advanced from the right carotid artery into the LV chamber with MR fluoroscopic guidance (8,14,18). A full description of the catheter design has previously been published (8,14,18).

MR Imaging

Dogs ($n = 12$) with permanent coronary artery occlusion were imaged twice—namely, prior to treatment at 3 days after infarction (acute infarction) and after treatment at a mean of 50 days after infarction ± 3 (standard error of the mean) (chronic infarction). Our interventional 1.5-T MR imaging

unit (Intera; Philips Medical Systems, Best, the Netherlands) features a short bore to enable percutaneous catheterization and is equipped with an in-room display monitor and console for viewing and interactive imaging (3). The following MR imaging protocol was used (A.M.): (a) cine MR imaging for measuring LV volume, ejection fraction, and mass (19); (b) first-pass perfusion imaging for measuring relative myocardial perfusion (16); (c) delayed contrast-enhanced MR imaging for measuring infarction size (20); and (d) MR fluoroscopy for navigating the percutaneous endovascular catheter (by using passive tracking) and localizing the target tissue (by using active tracking) (8,21). Detailed information on imaging parameters has been previously published (8,16,19–21).

Quality control of injections (M.S.) required (a) that the catheter's tip be perpendicular ($\pm 30^\circ$) to the target site in two planes, (b) a few ectopic extraventricular beats to appear at the moment of needle penetration into myocardium, and (c) injection of spirodium into sample locations of myocardium to ensure functionality of the catheter and spatial distribution of the injectate (8). LV volumes and mass were measured as previously described by using software (ImageJ, version 1.36, 2005; <http://rsb.info.nih.gov/ij/>) (22) (M.S., A.M., M.B., L.D.). The coordinates of the regions of interest (35–45 pixels) for first-pass perfusion imaging were placed in the center between epicardium and endocardium and the LV chamber to avoid the volume averaging effect (M.S., A.M., M.B., L.D.). Changes in regional signal intensity during first-pass perfusion imaging were recorded in arbitrary units. Myocardial infarcts were defined on delayed contrast-enhanced MR images when signal intensity was greater than +2.0 standard deviations of that of the remote myocardium (mean signal intensity of entire free LV wall); this was a constant reference in all animals with regard to size and placement of regions of interest. Myocardial infarction (as a

percentage of LV mass) on MR images and that on TTC-stained slices were visually matched on the basis of anatomic landmarks, including LV shape, papillary muscle shape and location, and insertion point of the right ventricle onto the left (23). The investigators performed the analyses by consensus in a blinded fashion.

Postmortem and Histopathologic Evaluation

After final imaging (50 days ± 3), the LV was isolated, weighed, sliced, and stained with TTC to delineate scar tissue. Papillary muscle and insertion of the right ventricle were used for registration between MR images, TTC-stained slices, and histologic slices (P.U., M.S., L.D.). Following adequate fixation (in 10% formalin), tissue slices (5 μm) were stained with hematoxylin-eosin, Masson trichrome, and biotinylated *Bandeiria simplicifolia* isolectin B4 (Vector Lab, Burlingame, Calif) (24). Masson trichrome stain was used to define scar tissue. Biotinylated isolectin B4 delineated vascular endothelial cells with brown reaction product. Vascular density was determined in 10 fields of each of the following regions: the remote myocardium and the border zone and core of the occlusive infarction in both treated and control animals (P.U., M.S., L.D.).

Statistical Analysis

Data were expressed as means $\pm$ standard errors of the mean. $P < .05$ was considered to indicate a statistically significant difference. Changes over time within each group were compared by using one-way analysis of variance for repeated measurements followed by post-hoc testing when an overall significance was detected. The following parameters were compared within and between the groups by using two-way analysis of variance followed by a Scheffe post-hoc analysis: (a) first-pass perfusion, (b) LV function (ejection fraction and LV volume), (c) infarction size, and (d) capillary and arteriole densities. Bland-Altman analysis was used to determine the agreement in the mea-

measurements of infarction size at MR imaging at 50 days $\pm$ 3 and at TTC staining in both groups (M.S., L.D., M.B.).

Results

MR Fluoroscopy

MR fluoroscopy was performed after acute infarction and was successfully used for transendocardial delivery of genes in both groups ($n = 12$). The signal derived from the catheter could either be activated or suppressed, depending on visualization needs. Positioning the catheter in the LV was

made possible by rotating and deflecting the distal tip. The active coil at the tip of the needle, as well as short- and long-axis views of the LV, were valuable in guiding the catheter to the target. Occlusive infarction (the target) was visualized for at least 30 minutes as a bright region at delayed contrast-enhanced MR imaging and could also be appreciated on the MR fluoroscopic images (Figs 1–4). After the catheter tip was placed into the target, the needle was advanced (4 mm) into the myocardium. Figure 1 shows the delivery of plasmid *LacZ* and spodiamide in a control animal, while Figure

2 shows the four injection sites of *VEGF* genes and plasmid *LacZ* into the target in a treated animal. Spodiamide caused a signal void, which was observed in 16 phases in remote and infarcted myocardium at cine MR imaging, indicating successful transendocardial delivery (8) (Fig 1).

Myocardial Perfusion

Data of the first-pass perfusion parameters (maximum upslope and peak signal intensity) in acute and chronic infarctions of both control and treated animals are summarized in Table 1. Both maximum upslope and peak signal intensity were significantly greater in *VEGF* gene-treated animals than in control animals. MR images demonstrated occlusive infarction as a hypoenhanced region during first-pass perfusion in control and treated animals (Figs 3 and 4). Figure 5 shows the first-pass perfusion-time curves in the infarcted and remote myocardium of control and treated animals. The MR imaging estimate of perfusion showed significant improvement in the perfusion of infarcted myocardium in treated animals compared with that in control animals at 50 days $\pm$ 3.

LV Function

MR measurements of LV function were not significantly different between the groups in acute infarction (Fig 6). LV function in the treated group was greater than that in the control group. For example, treated animals showed increased ejection fraction (from $27.9\% \pm 1.2$ to $35.3\% \pm 1.6$, $P = .001$) and decreased end-systolic volume (from $63.4 \text{ mL} \pm 4.8$ to $52.7 \text{ mL} \pm 2.2$, $P = .05$) in chronic infarction (Fig 6). The treatment caused no significant reduction in end-diastolic volume (from $87.5 \text{ mL} \pm 5.5$ to $84.0 \text{ mL} \pm 3.5$, $P = .94$). The ejection fraction, end-systolic volume, and end-diastolic volume were not significantly changed between acute and chronic infarction in control animals ($27.5\% \pm 1.8$ to $27.9\% \pm 1.2$ [$P = 0.74$], $60.8 \text{ mL} \pm 4.4$ to $59.8 \text{ mL} \pm 1.6$ [$P = 0.83$], and $83.7 \text{ mL} \pm 5.5$ to $82.9 \text{ mL} \pm 1.4$ [$P = 0.89$], respectively); these findings are in line with those of a

Figure 1

Figure 1: MR images show, A, occlusion site on left anterior descending artery (black arrow) and the two sites of spodiamide injections (white arrows), as well as, B, myocardial infarction as a differentially enhanced region (arrowheads). The enhanced region served as a target for delivery of plasmid *LacZ* and spodiamide mixture in a control animal. C–F, Multisection images show the mixture in the core of infarction 5 minutes after delivery (arrowheads) at, C, E, diastole and, D, F, systole.

previous canine study by Jugdutt (25). LV mass in treated animals ($106.9 \text{ g} \pm 7.6$) was not significantly different from that in control animals in acute ($104.9 \text{ g} \pm 6.1$, $P = .84$) or chronic ($102.9 \text{ g} \pm 6.5$ vs $97.7 \text{ g} \pm 4.4$ [$P = .53$], respectively) infarction. At both the acute and chronic phases, heart rate, arterial blood pressure, and percentage oxygen saturation were comparable between treated and control animals (Table 2).

Myocardial Viability

In the acute phase, the extent of the contrast-enhanced region in treated animals (23.3% of LV mass ± 2.3) was comparable with that in control animals (24.6% of LV mass ± 3.0 , $P = .74$). At the chronic phase, both groups showed a significant decrease in the extent of the contrast-enhanced region as compared with that in the acute phase (treated animals, 8.5% of LV mass ± 0.9 , $P = .001$; and control animals, 11.3% of LV mass ± 0.9 , $P = .003$) (Fig 7). However, animals treated with plasmid *VEGF* showed a greater reduction in the extent of the contrast-enhanced region compared with control animals ($P = .048$). Infarction size at TTC staining in treated animals was also significantly smaller (9.4% of LV mass ± 1.5 , $P = .05$) than in control animals (12.7% of LV mass ± 0.4) but was not significantly different from the contrast-enhanced region at MR imaging ($P = .96$ in treated animals and $P = .25$ in control animals). Furthermore, Bland-Altman analysis showed close agreement between contrast-enhanced MR imaging and histochemical TTC staining for measuring infarction size (Fig 7).

Histopathologic Findings

The Masson trichrome stain highlighted the fibrosis and disclosed “tongues” of collagen at the boundary extending into viable myocardium. Infarcted myocardium from treated and control hearts contained very few scattered thick-walled arteries. In addition, infarcts from treated animals showed numerous small-caliber thin-walled vessels in the border zone and core of scar tissue (Fig 8). Lectin-stained (brownish) capillaries were greater in the border zone

Figure 2

Figure 2: A, Long-axis contrast-enhanced MR image shows the target infarction (arrows) as a region that differentially enhances after the delivery of the mixture of plasmid *VEGF* and *LacZ* in treated animals or plasmid *LacZ* and spirodianide in control animals. B–E, Selected MR fluoroscopic images show injection catheter in LV chamber (arrowhead) and the four sites of transendocardial injection in a treated animal (arrows).

(430 per square millimeter ± 117 , $P = .0001$) and the core of infarction (307 ± 112 , $P = .0001$) in the treated group than in the control group (268 ± 19 and 108 ± 17 , respectively). Larger vessels showed a similar tendency in the border zone (9.7 per square millimeter ± 1.8 , $P = .0003$) and the core of infarction (12.2 ± 3.8 , $P = .0006$) of treated compared with control animals (3.6 ± 0.8 and 2.9 ± 1.1 , respectively). There was no significant difference in capillary and arterial density in remote myocardium between treated (626 per square millimeter ± 65 [$P = .48$] and 2.5 ± 0.3 [$P = .87$], respectively) and control groups (679 ± 74 and 2.4 ± 0.6 , respectively).

Discussion

The validity of plasmid *VEGF* genes delivered with MR fluoroscopic guidance into the border zone and core of occlusive infarction was confirmed by neovascularization and improvement in myocardial perfusion, LV function, myocardial viability, and infarct resorption. MR imaging methods provide a unique platform for

transendocardial delivery of therapies and comprehensive evaluation of LV function, myocardial perfusion and viability, and infarct resorption. They also afford the ability to track endovascular devices that have been customized to the MR imaging environment. The coupling of these capabilities permits simultaneous visualization of the delivery catheter and diseased tissue and, ultimately, the administration of therapy, which is an exceptional capability. Imaging techniques sensitive to T1 and to T2/T1 ratio were comprehensively used for delivery and assessment of gene therapy. The results of this study indicate that plasmid *VEGF* genes have the potential to improve revascularization of occlusive infarction. The transendocardial gene delivery approach has important therapeutic value in patients in whom revascularization is not possible, as well as in patients with infarction in the territory of permanently occluded coronary arteries.

Interventional Imaging Modalities

Interventional procedures are typically performed with x-ray fluoroscopic guid-

ance, which cannot enable the interventionist to distinguish viable from nonviable myocardium. Additional limitations include (a) two-dimensional plane projections through the body, resulting in information loss around the dimension parallel to the beam; (b) divergence of x-rays from a point source, which distorts the perceived sizes of anatomic features; and (c) the lack of tissue contrast. This makes it difficult to directly measure infarction size or determine distances by using x-ray-based images (26). NOGA and MR imaging-guided approaches provide three-dimensional coverage to all border zones of infarcted myocardium (4,8). The NOGA system comprises a catheter with three coils

and an endocardial potential-measuring electrode located at the distal tip. Three external magnets are placed on the chest at different locations. A three-dimensional and real-time dynamic reconstruction of the LV, with an assessment of the electrical and mechanical properties of the myocardium, is then created. Viable myocardium is differentiated from scar tissue by measuring the endocardial potential and motion at the distal tip of the catheter. Limitations of this technique are that it is time intensive, the image quality is operator dependent, it does not provide an anatomic image of the heart, and it requires x-ray fluoroscopic guidance to determine catheter position.

Effective clinical trials of local gene or cell therapy for myocardial infarction require minimally invasive methods to replace open-chest surgery. Percutaneous endovascular catheter-based approaches have been recently introduced and used in MR imaging. The advances of our current reported nonsurgical method are the use of (a) MR fluoroscopy for active and passive catheter tracking; (b) a comprehensive MR imaging protocol to assess myocardial perfusion, LV function, and myocardial viability before and after therapy; (c) *VEGF* genes or the vehicle delivered precisely to the border zone and core of the infarction; and (d) spirodamide to ensure transendocardial delivery. In this study, we confirmed that MR fluoroscopy with active and passive tracking capabilities and other imaging techniques (first-pass perfusion, delayed contrast-enhanced, and cine MR imaging) could be successfully used in a single imaging session. Neovascularization in treated, but not control, animals suggests that the *VEGF* genes were appropriately delivered and adequately expressed in the targeted regions. We previously measured expressed *VEGF* protein in samples obtained from infarcted myocardium (27–29).

Gene Therapy

Gene therapy is customarily administered in a single time point, which takes advantage of the sustained expression of cellular-derived angiogenic factors produced by transfected cells. The strategy and beneficial effects of injecting the *VEGF* gene in a single time point into the myocardium in a model of permanent left anterior descending coronary artery occlusion has been previously reported in mice, dogs, and sheep (18,28,30). Ferrarini et al (18) found that a single transmyocardial injection of *VEGF* gene exerts a marked beneficial action, enhancing both arteriogenesis and cardiomyocyte viability.

The delivered plasmid *VEGF* gene resulted in neovascularization in the border zone and core of infarcted myocardium in a similar fashion as adeno-associated viral (AAV) vector-encoding *VEGF* (AAV-*VEGF*) genes

Figure 3

Figure 3: A–E, MR images in a representative control animal show ischemic myocardium (arrowheads) as a hypoenhanced region on, A–E, first-pass perfusion images and infarcted myocardium as a differentially enhanced region on, E, delayed contrast-enhanced MR images in both acute (top) and chronic (bottom) infarction (arrows in E). Note that in acute, but not chronic, infarction, microvascular obstruction is present in the core. F, Corresponding TTC-stained color section shows the pale scar tissue in chronic infarction (arrows).

(16–18) administered epicardially in open-chest swine and dog models, indicating that transendocardial delivery with MR fluoroscopic guidance was successful. We have demonstrated in a more severe ischemic insult model (occlusive infarction) that plasmid *VEGF* delivered with MR fluoroscopic guidance is as good in treating occlusive and reperfused infarctions as in similar, more invasive, studies with AAV-*VEGF* (16,18). The reason for testing the plasmid *VEGF* gene instead of the hypoxia-inducible cardiac-specific AAV-*VEGF* gene in the current study was that plasmid *VEGF* gene can be expressed in less hypoxic environments. This novel feature may allow this therapy to be utilized in the treatment of chronically injured myocardium (27–29). Previous studies of gene therapy predominantly utilized plasmid DNA because of its ease and low toxicity. Additionally, clinical studies in which a placebo control group and plasmid *VEGF* genes were used demonstrated the safety of locally administered gene therapy and showed positive effects on secondary end points (31,32) but not on perfusion, LV function, or myocardial viability.

Myocardial Perfusion and LV Function

Muehling et al (33) indicated that perfusion imaging offers a novel noninvasive method to detect collateral dependent myocardium with high sensitivity and specificity. Treated animals showed significantly steeper maximum upslope and higher maximum signal intensity after bolus than control animals. The improvement in perfusion and LV function seen at first-pass perfusion and cine MR imaging indicated successful delivery and efficacy of the *VEGF* gene. The neovascularization in occlusive infarction of treated animals offers an explanation for differences in maximum upslope and peak signal intensity. It is not known whether the *VEGF* genes or a downstream effect improved LV function and perfusion in treated animals compared with control animals. The greater signal intensity and maximum upslope in treated animals is most likely related to a combination of several factors, including neovasculariza-

Figure 4

Figure 4: A–E, MR images in a representative *VEGF* gene–treated animal show ischemic myocardium (arrowheads) as a hypo-enhanced region on, A–D, first-pass perfusion images and infarcted myocardium as a differentially enhanced region on, E, delayed contrast-enhanced MR images in both acute (top) and chronic (bottom) infarction (arrows in E). Note in acute, but not chronic, infarction the presence of microvascular obstruction in the core. The hypo-enhanced and enhanced regions on first-pass and delayed contrast-enhanced images, respectively, were smaller at 50 days, but not at 3 days, compared with those in a control animal (Fig 3). F, Corresponding TTC-stained color section shows the smaller pale scar tissue in chronic infarction (arrows) compared with that in the control animal in Figure 3.

tion, perfusion, blood volume, and the wash-in/wash-out kinetics of the contrast medium into the interstitium (34). MR imaging at its current stage of development is unable to help separate microvascular permeability from perfusion on the basis of peak signal intensity because *VEGF* increases both vascular density and permeability (35).

Cine MR images showed the significant improvement in LV function between the two groups. The neovascularization in the border zone and core of infarcted myocardium could potentially prevent demand-induced ischemia and stunning and subsequently

improved LV ejection fraction in treated animals (36). At 50 days $\pm$ 3, the increase (27%) in ejection fraction in *VEGF*-treated animals compared with that in control animals was relatively less than previous results (40%) with bone marrow–derived multipotent progenitor cell transplantation in occlusive infarction (37).

Infarct Resorption

The mechanism of infarct size reduction by angiogenic gene therapies may be related to improvement in perfusion, which speeds up the infarct resorption (16,29,30). Infarct resorption was ob-

Table 1

First-Pass MR Perfusion Parameters in Control and *VEGF*-treated Animals in Acute and Chronic Infarction

Location and Parameter*	Acute Infarction			Chronic Infarction		
	Control Animals	Treated Animals	P Value	Control Animals	Treated Animals	P Value
Remote myocardium						
Maximum upslope (sec^{-1})	194 $\pm$ 41	217 $\pm$ 33	.66	175 $\pm$ 23	195 $\pm$ 43	.61
Peak signal intensity (au)	1555 $\pm$ 47	1542 $\pm$ 78	.88	1789 $\pm$ 196	1603 $\pm$ 125	.47
Ischemic myocardium						
Maximum upslope (sec^{-1})	94 $\pm$ 16	118 $\pm$ 22	.30	117 $\pm$ 15	200 $\pm$ 32	.02
Peak signal intensity (au)	843 $\pm$ 138	771 $\pm$ 128	.68	1132 $\pm$ 80	1667 $\pm$ 100	.002

Note.—Unless otherwise specified, data are means $\pm$ standard errors of the mean.

* au = Arbitrary units.

Figure 5

Figure 5: Graphs show first-pass perfusion of gadoterate meglumine (*Gd-DOTA*) in acute and chronic myocardial infarction in control (left, $n = 6$) and *VEGF* gene-treated (right, $n = 6$) animals. The signal intensity–time curves show a steeper upslope and higher peak signal intensity in chronic infarction in treated animals (bottom right, ●) compared with control animals (bottom left, ●). □ = Remote myocardium at acute phase, ■ = remote myocardium at chronic phase, ○ = infarcted myocardium at acute phase, ● = infarcted myocardium at chronic phase. Arrows = approximate time of gadoterate meglumine bolus injection. a.u. = Arbitrary units.

Figure 6

Figure 6: Graphs show temporal changes in ejection fraction (top), end-systolic volume (middle), and end-diastolic volume (bottom) over 50 days $\pm$ 3 in control and treated animals at cine MR imaging. Treated, but not control, animals showed significant increase in ejection fraction and decrease in end-systolic volume. $\dagger = P < .02$ for treated versus control animals at 50 days $\pm$ 3.

served in both groups, but it was more pronounced in treated animals. The natural infarct resorption observed in control animals (54%) was lower than that in a previous study (76%) (38) but was significantly greater than that in humans (30%) (39,40) or swine (30%) (16,17). The higher magnitude of infarct resorption in treated animals provides evidence that the gene was properly delivered and was effective in occlusive infarction. Investigators have attributed

Table 2

Hemodynamic Data in Acute and Chronic Infarction in Control and Treated Animals

Group and Time of Infarction	Heart Rate (beats/min)	Systolic Blood Pressure (mm Hg)	Diastolic Blood Pressure (mm Hg)	Mean Blood Pressure (mm Hg)	Oxygen Saturation (%)
Control animals (n = 6)					
Acute	99 $\pm$ 2	98 $\pm$ 10	60 $\pm$ 10	76 $\pm$ 9	99 $\pm$ 1
Chronic	97 $\pm$ 2	96 $\pm$ 5	64 $\pm$ 3	76 $\pm$ 5	99 $\pm$ 1
Treated animals (n = 6)					
Acute	106 $\pm$ 2	99 $\pm$ 7	58 $\pm$ 3	77 $\pm$ 5	99 $\pm$ 1
Chronic	102 $\pm$ 1	94 $\pm$ 6	63 $\pm$ 3	72 $\pm$ 5	99 $\pm$ 1

Note.—Data are means $\pm$ standard errors of the mean.

Figure 7

Figure 7: Graphs show results of MR imaging and TTC assessment of myocardial infarction in control and treated animals. (a) Bland-Altman analysis shows agreement between contrast-enhanced regions in chronic infarction and infarction at TTC staining in control (left) and treated (right) animals. (b) Bar graph shows infarct size, as percentage of LV mass, in control and treated (right) animals at contrast-enhanced MR imaging in acute (green bars) and chronic (teal bars) infarction. Red bars = infarct size at TTC staining. In chronic infarction, the contrast-enhanced regions were significantly smaller in both groups than in acute infarction. However, the decline in the extent of infarcted regions was significantly greater in treated than in control animals at both MR imaging and TTC staining. * = $P < .05$ at 50 days $\pm$ 3 compared with 3 days, $\dagger = P < .05$ in treated compared with control animals.

the reduction in infarction size to *VEGF* activation of endogenous cardiac stem cells and/or indirect paracrine activity of *VEGF* on myocytes (18,41,42). A more recent study (18) showed arteriogenesis and myocyte regeneration in infarcts after *VEGF* gene delivery.

Study Limitations

The major limitations of this study were as follows: (a) The use of a long conductive interventional catheter in the MR imaging environment induces a potential risk of focal radiofrequency-related heating, (b) the lack of measurement of perfusion with microspheres because of MR imaging safety issues, (c) the fact that *VEGF* gene expression was not determined because it was measured in our previous study using real-time polymerase chain reaction (29), (d) the fact

that spodiamide was not mixed with plasmid *VEGF* because of possible interactions between compounds, (e) a limited sample size ($n = 6$ per group), and (f) the inability to fully and evenly distribute the gene within the core and border zone of the infarct.

The small number of injections in dogs is related to the presence of extensive innate collateral vessels in dogs as compared with humans (40,43) or swine (44) because natural infarct resorption is substantially less than in dogs (38). Therefore, in our previous swine study, we used eight injections of gene therapy, and in clinical scenarios, it may be necessary to administer a greater number of injections as well. However, there are some adverse effects of multiple injections of the *VEGF* gene, such as hem-

angiomas, owing to the unregulated expression of the gene (45).

Clinical Implications

MR fluoroscopic guidance of *VEGF* gene therapy enables a substantially reduced level of invasiveness compared with open-chest surgery, potentially resulting in treatment on an outpatient basis, rapid patient recovery, and cost savings to the health care system. This study also showed that MR imaging enables a comprehensive evaluation of ischemic heart disease.

In conclusion, the therapeutic efficacy of the *VEGF* gene delivered with MR fluoroscopic guidance into the border zone and core of infarcted myocardium was confirmed by neovascularization and improvement in LV function, myocardial perfusion, and infarct heal-

Figure 8

Figure 8: Photomicrographs of representative infarction in (a–c) control and (d–f) *VEGF* gene–treated animals. a, b, d, and e were stained with Masson trichrome stain, and c and f were stained with isolectin B4. At (a, d) low magnification, chronic infarction (I) in both groups comprised homogeneous replacement fibrosis with a distinct boundary at the interface between scar and viable tissue (M). At high magnification, chronic infarction in (b) treated animals contained numerous vessels, while (e) control infarction contained very few vessels (arrows). Biotinylated isolectin B4 localized vessels with brown reaction product, accentuating the neovascularity in (f) treated infarcts as compared with (c) infarcts in control animals. Calibration bars = 80 μ m.

ing. To our knowledge, there is no previous report that demonstrates the utility of MR fluoroscopic guidance for transendocardial delivery of genes. The results of this study demonstrate that MR imaging-guided transendocardially delivered plasmid *VEGF* genes have the potential to neovascularize or to improve vascular recruitment in occlusive infarction.

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