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CARDIOVASCULAR MAGNETIC RESONANCE IMAGING IN DELIVERING AND EVALUATING THE EFFICACY OF HEPATOCYTE GROWTH FACTOR GENE IN CHRONIC INFARCT SCAR

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Abstract

Background—In open-chest model of acute infarct, epicardial delivery of hepatocyte growth factor (HGF) gene improved LV function. This study was designed to test 1) the efficacy of HGF gene in infarct scar delivered under MR guidance and 2) the potential of multiple MR sequences in assessing the effects of pCK-HGF (treatment) and pCK-LacZ (control) genes on myocardial structure and function.

Methods and Materials—Swine (6 per group) were subjected to myocardial infarct, under X-ray fluoroscopy, developed LV remodelling at 5 weeks. Multiple clinical MR imaging sequences were performed before delivery of gene (at 5 weeks after infarction) and 5 weeks after delivery of gene. Under MR-guidance, the active endovascular catheter was introduced into LV to transendocardially deliver 3.96×10^{11} viral copies of pCK-HGF or pCK-LacZ in the border and core of the infarct scar. Histological evaluation of the infarct scar was performed 5 weeks after delivery of gene.

Results—At 5 weeks after infarction, there was no significant difference in measured cardiovascular MR parameters between the groups. PCK-HGF gene caused significant improvement in the following parameters ($P < 0.05$ for these parameters): 3D strain (radial, circumferential, and longitudinal), perfusion (maximum upslope, peak signal intensity, and time to peak) compared with control pCK-LacZ at 5 weeks after delivery of the genes. The ejection fraction was higher in pCK-HGF treated ($43 \pm 1\%$) than pCK-LacZ control ($37 \pm 1\%$, $P < 0.05$). These changes are associated with a decrease in infarct scar size ($11.3 \pm 2.0\%$ in pCK-LacZ control and $6.7 \pm 1.3\%$, in pCK-HGF treated, $P < 0.01$) and transmuralities in 4 out of 5 infarct scar segments ($P < 0.05$) on DE-MR imaging. Microscopic study confirmed the increase in capillary ($P < 0.05$), and arteriole ($P < 0.05$) density of infarct scar in pCK-HGF treated compared with pCK-LacZ control animals.

Conclusions—HGF gene delivered under MR-guidance into infarct scar ameliorated global function, 3D strain, increased regional perfusion, infarct resorption and enhanced angiogenesis/

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arteriogenesis. This feasibility study provides novel approach and analysis methods and instrumentation for delivering and evaluating new locally delivered therapies.

Keywords

Angiogenesis; gene therapy; myocardial infarction; magnetic resonance imaging; interventional cardiology

Congestive heart failure has become a widespread public health concern; there are approximately 5 million patients in the United States.¹ Following infarction, the left ventricle (LV) undergoes structural remodeling resulting in functional deterioration². Despite significant advances in pharmaceutical, surgical and interventional therapies, positive results in angiogenesis and cardiac repair are limited. Growing evidence from preclinical³⁻⁵ and clinical⁶⁻⁹ studies suggest that gene therapy has the potential to enhance angiogenesis and improve LV function. Assessment of LV function after gene therapy has hinged on invasive analysis and most of the focus was on the cellular and molecular changes^{3,4,10-14}. For example investigators established the fundamental mechanisms of action of hepatocyte growth factor (HGF) that include angiogenesis, arteriogenesis, myogenesis, reduction of collagen deposition and apoptosis^{3,4,11-14}.

Our group and others demonstrated in open-chest model the improved LV function and perfusion at 8 weeks after delivering pCK-HGF gene into the epicardium of acute infarct^{5,15,16}. More recently, a minimally invasive approach under MR-guidance has been introduced for delivering vascular endothelial growth factor gene in acute infarct¹⁷. In routine clinic, a minimally invasive approach is critical for rapid recovery, reduction of morbidity and mortality and cost savings to the health care system. To our knowledge the efficacy of pCK-HGF and pCK-LacZ genes delivered transendocardially into the border and core of infarct scar, under MR-guidance, has not been demonstrated. Therefore, this study was designed to test 1) the efficacy of the effects of pCK-HGF and pCK-LacZ genes on infarct scar delivered under MR guidance and 2) the potential of multiple MR sequences in assessing the effects of these genes on myocardial structure and function.

MATERIALS AND METHODS

Therapeutic gene and delivery system

The phosphoenolpyruvate carboxykinase (pCK) vector, with hybrid hepatocyte growth factor (HGF) gene (VM202; ViroMed, Seoul, S-Korea) was used as a therapeutic agent and pCK- β -galactosidase (LacZ) gene as a control. The components of HGF gene and their functions have been previously described^{5,15}.

Six steerable, deflectable guiding catheters with a retractable needle were used for transendocardial delivery of the genes (SurgiVision Inc. Baltimore, MD)¹⁷. The endovascular catheter had a nitinol needle for puncturing the border and core of the infarct scar. The catheters were fitted with multiple MR radiofrequency receiver coils and were actively tracked to the target (infarct scar) under MR imaging (Figure 1).

Experimental protocol

The study conformed to the Guide for the Care and Use of Laboratory Animals (NIH Publication No 85-23, revised 1996) and approval was obtained from IACUC. Pigs (n=18, 30-32kg) were premedicated using acepromazine/ketamine and anesthetized using isoflurane/oxygen. A percutaneous balloon catheter (Boston Scientific, Natick, MA) was advanced distal to the 2nd diagonal branch under X-ray guidance and the artery was

occluded for 90min followed by reperfusion¹⁸. Coronary angiograms were repeatedly acquired to ensure complete occlusion/reperfusion.

A total of 18 animals were studied, where 2 animals died during the coronary occlusion/reperfusion and 4 died during the navigation of the endovascular catheter in LV at 5 weeks after infarction. The infarct was allowed to evolve for 5 weeks and the remaining animals were divided in a blinded fashion into 2 groups: pCK-HGF gene treated group (n=6) and pCK-LacZ control group (n=6). These 12 animals completed the study and follow-up. The specially designed endovascular catheter was used to deliver 3.96×10^{11} viral copies of the pCK-HGF (therapy, n=6) or pCK-LacZ (control, n=6). The number of injected viral copies in the border and core of infarct scar was 50% greater compared to those injected in acute infarct and the genes were delivered in over a larger infarct area by doubling the number of injection sites from 4 to 8 (0.3-0.4ml/site) at a rate of ~20s/site.^{5, 15}. All procedures were performed under sterile conditions. All animals received 3.3mg/kg lidocaine (IV, during occlusion/reperfusion and gene injection), 15 mg/kg trimethyl sulfa (PO, for 3 days after intervention).

Non-invasive clinical MR imaging sequences were performed at 5 weeks after infarction before delivery of gene and 5 weeks after delivery of the genes. The first MR imaging session was performed to establish a baseline. MR contrast media was delivered via the left ear vein for first pass perfusion (0.1mmol/kg Gd-DTPA, 3ml/s) using power injector. An additional dose of 0.05mmol/kg Gd-DTPA was delivered for delayed contrast enhanced MR imaging (DE-MR imaging).

Cardiovascular MR imaging

The hybrid XMR suite (Philips, Best, The Netherlands) featuring an X-ray catheterization lab and a 1.5T short-bore cardiovascular MR scanner with an in-room display monitor and console for viewing and interactive scanning enables LV catheterization under real-time imaging and for delivery of gene and its efficacy in infarct scar. Multiple MR sequences were simultaneously used in each session:

1. Cine MR imaging for measuring LV volumes, cardiac output, stroke volume, mass, wall thickness and % systolic wall thickening (radial strain);
2. Tagged MR imaging for measuring circumferential strain;
3. Phase-contrast velocity-encoded MR imaging for measuring longitudinal strain;
4. First-pass perfusion MR imaging for measuring regional myocardial perfusion;
5. DE-MR imaging for measuring infarct size and infarct transmural thickness.
6. MR-fluoroscopy for navigating the endovascular active catheter to the target.

Image analysis

Segment software was used by investigators blinded to treatment to determine LV volumes, ejection fraction, mass, perfusion, infarct size and transmural thickness and for measuring, longitudinal and systolic wall thickening (radial strain)^{5, 17, 19}. Regional wall thickness at end systole and end diastole of infarct scar (the target) and remote segments (LV free wall) were obtained, using DE-MR imaging as guidance. A 0° starting point at the postero-septal groove was used in the above parameters for each individual slice in the set (clockwise direction). The software calculated the mean centerline of 8 LV segments (45° per segment). Percent systolic thickening at all time points were determined on three slices (8 segments per slice). A total of 576 segments was measured for segmental systolic wall thickening covering 5 weeks after infarction and 5 weeks after delivery of genes time points using

Segment software, while *HARP* software was used blinded to treatment for measurement of peak systolic circumferential strain²⁰. For image registration of the circumferential strain the LV and the antero-septal groove were used as the anatomic landmark^{5, 19, 21}. The radial, circumferential and longitudinal strain were assessed in the same apical slices containing the infarct, shown on DE-MR images. Automatic signal intensity threshold +2SD above remote myocardium was used to define the infarct and infarct transmuralty²².

Histopathology

After the last imaging session at 5 weeks after delivery of genes, the animals were euthanized and the freshly excised hearts were sliced and stained with triphenyltetrazolium chloride (TTC) to delineate the infarct scar. MR images were registered with TTC slices in both groups. Large tissue samples that include infarct scar (the target) and remote myocardium (free LV wall) were obtained. Following fixation and paraffin-embedding, 5 μ m thick tissue sections were stained with hematoxylin and eosin, Masson trichrome and biotinylated *Bandeiria simplicifolia* isolectin B₄ (Vector Lab, Burlingame, Ca)²³. Standard immunoperoxidase method, biotinylated isolectin B₄ localized vascular endothelial cells. Vascular density and myocyte diameter were determined in a blinded fashion using previously described methods^{24, 25}.

Statistical analysis

Differences in data obtained at 5 weeks after infarction and 5 weeks after delivery of gene in both pCK-HGF gene treated and pCK-LacZ control groups were compared using one-way ANOVA for repeated measurements followed by post-hoc test when an overall significance was detected. The following parameters were compared using two-way ANOVA, followed by a Scheffe post-hoc analysis: 1) body weight, heart rate and arterial blood pressure, 2) LV function (ejection fraction, LV volumes, stroke volumes, cardiac output, 3D strain), 3) first pass perfusion parameters (maximum upslope, peak signal intensity and time to peak), 4) infarct transmuralty and size and 5) capillary and arteriole densities as well as myocyte diameters. Data were expressed as mean \pm SEM. $P < 0.05$ was considered statistically significant.

RESULTS

Cardiac interventions

Cardiac interventions were performed 1) under X-ray for coronary artery occlusion/ reperfusion and 2) MR-guidance for transendocardial delivery of gene. Animals (n=16) showed myocardial infarct 5 weeks prior to delivery of gene and (n=12) 5 weeks after delivery of gene on DE-MR imaging. The pCK-HGF and pCK-LacZ animals showed no significant difference in body weight, heart rate, and mean arterial blood pressure at any time point (Table 1). The advancement of endovascular active catheter from the aorta into the LV chamber then to the target, is shown in figure 1. The brightness of endovascular catheter in the dark background LV chamber facilitates the navigation of the catheter to the target. The injection was performed after turning off the active coils to eliminate the heating produced by coil activation.

LV mass and volumes

There was no significant difference in LV mass or wall structure (diastolic and systolic LV wall thickness) of remote myocardial segments at any time point between the groups. The segments with infarct scar were significantly thinner compared to remote myocardial segments in both groups at 5 weeks after infarction (Figure 2). Five weeks after delivery of pCK-HGF gene, there was a significant increase in wall thickness of segments with infarct

scar at both diastole (from 5.7 ± 0.5 mm at 5 weeks after infarction to 6.6 ± 0.3 mm at 5 weeks after therapy, $P<0.05$) and systole (5.7 ± 0.4 mm to 8.1 ± 0.5 , $P<0.05$) compared with pCK-LacZ (from 5.7 ± 0.4 mm to 5.4 ± 0.6 mm at diastole and from 5.5 ± 0.5 mm to 5.4 ± 0.5 mm at systole).

Furthermore, end-diastolic, end-systolic, stroke volumes, cardiac output and ejection fraction were also not significantly different between the groups 5 weeks after infarction (Table 2). Animals treated with pCK-HGF gene also showed significant decrease in end systolic volume (from 1.27 ± 0.05 at 5 weeks after infarction to 1.10 ± 0.05 ml/kg body weight at 5 weeks after delivery of gene, $P<0.05$) and an average increase of 5%, in ejection fraction (from 38 ± 1 at 5 weeks after infarction to $43\pm 1\%$ at 5 weeks after delivery of gene, $P<0.05$). PCK-LacZ animals showed no significant change in end systolic volume (1.31 ± 0.06 at 5 weeks after infarction to 1.28 ± 0.05 ml/kg body weight at 5 weeks after delivery of gene, $P=ns$) or ejection fraction (38 ± 2 at 5 weeks after infarction to $37\pm 1\%$ at 5 weeks after delivery of gene, $P=ns$), respectively. Furthermore, both end systolic volume and ejection fraction in pCK-HGF treated animals were significantly different from control pCK-LacZ at 5 weeks after gene delivery (Table 2).

3D strain

At 5 weeks after infarction, quantitative analysis of cine MR images revealed no significant difference between the groups in regional % systolic wall thickening (radial strain) (Figure 3). The segments with infarct scar were dyskinetic in pCK-LacZ control and akinetic in pCK-HGF animals at 5 weeks after delivery of the gene. However, there was significant improvement in systolic wall thickening of remote myocardial segments in pCK-HGF treated animals. In contrast, control animals showed a trend of further dysfunction in radial strain of remote myocardium at 5 weeks after pCK-LacZ delivery.

Data obtained from tagged and phase-contrast velocity-encoded MR images showed severe dysfunction in segments with infarct scar at 5 weeks after infarction in both groups (Figure 4). PCK-HGF, but not pCK-LacZ, treated animals showed significant improvement in both circumferential and longitudinal strain suggesting that these imaging sequences were sensitive enough to demonstrate the improvements at 5 weeks after delivery of gene. Figure 5 shows a pixel-by-pixel illustration of the difference in LV longitudinal strain between treated and control animals.

Myocardial perfusion

At 5 weeks after infarction, first pass perfusion MR imaging showed the infarcted segments as hypoenhanced, due to the delayed arrival of the contrast medium, compared to remote myocardium. The perfusion parameters measured in the infarcted and remote segments were not significantly different between the groups (Table 3). At 5 weeks after delivery of HGF gene, however, maximum upslope, $P<0.05$, peak signal intensity, $P<0.05$ and time to peak, $P<0.05$ were improved compared to 5 weeks after infarction in the same animals. Furthermore, treated animals at 5 weeks after delivery of pCK-HGF gene showed better perfusion compared with pCK-LacZ control animals (Table 3).

Infarct size and infarct transmuralty

DE-MR imaging highlighted infarct scar (the target) in short and long axis views allowing precise delivery of the genes (Figure 2). At 5 weeks after infarction the infarct size was not significantly different between the groups (Table 2). PCK-HGF treated animals showed significantly smaller infarct size ($6.7\pm 1.3\%$) compared to pCK-LacZ control ($11.3\pm 2.0\%$) at 5 weeks after delivery ($P<0.01$). These changes are associated with a significant decrease in infarct transmuralty in 4 of 5 segments with infarct scar ($P<0.05$) on DE-MR imaging

(Figure 6). TTC infarct size at autopsy was not significantly different from MR infarct size (Table 2). MR images and TTC histochemical staining shows the non-transmural infarct at 5 weeks in pCK-HGF, but not in pCK-LacZ, group (Figure 2). There was no significant change in infarct transmuralities in all segments with infarct scar in pCK-LacZ control animals.

Histopathology

Masson trichrome stain showed homogeneous chronic infarct scar at 5 weeks after pCK-LacZ delivery. However, pCK-HGF treated animals showed different infarct architecture compared to controls. For example, Masson trichrome stain showed few viable islands/peninsulas at the border of segments with infarct scar, while isolectin B₅ showed homogeneous increase in vascularity of infarct scar in pCK-HGF treated animals but disarray of vessels in control animals (Figure 7). The number of vessels in border and core of chronic infarct was greater in pCK-HGF treated (206 ± 10 capillaries/mm²) compared with pCK-LacZ control animals (78 ± 25 capillaries/mm², $P < 0.05$). A significant difference was also observed in the number of arterioles within the infarct scar in pCK-HGF (11.7 ± 1.9 arterioles/mm²) and pCK-LacZ control (3.4 ± 0.5 arterioles/mm², $P < 0.05$) animals. On the other hand, there was no significant difference in capillary and arterial densities in remote myocardium between treated (269 ± 30 capillaries/mm², 10.9 ± 2.1 arterioles/mm², respectively) and control animals (267 ± 54 capillaries/mm² and 10.7 ± 1.1 arterioles/mm², respectively). Myocyte diameters of remote myocardium in pCK-HGF ($16.4 \pm 0.8 \mu\text{m}$) and pCK-LacZ control animals ($18.3 \pm 0.2 \mu\text{m}$) were not significantly different, respectively. There was no evidence of inflammation in the segments with infarct scar in pCK-LacZ control and pCK-HGF treated animals.

DISCUSSION

The major findings of this study were that pCK-HGF, but not pCK-LacZ, gene showed beneficial effects on myocardial structure, perfusion and function when delivered under MR-guidance into the border and core of infarct scar. The used multi MR sequences have the potential in discriminating the effects of pCK-HGF gene (VM202) from plasmid-LacZ control. Cine, tagged and phase-contrast velocity-encoded, and first pass perfusion MR imaging showed the improvement in radial, circumferential and longitudinal strain and perfusion parameters (max upslope, peak signal intensity and time to the peak) after delivery of plasmid pCK-HGF gene compared with plasmid-LacZ control. Furthermore, pCK-HGF gene reduced infarct transmuralities and size as demonstrated in vivo on MR imaging and postmortem on TTC stain. At the microscopic level, pCK-HGF gene enhanced angiogenesis and arteriogenesis in the border and infarct core, thereby provided indirect evidence on the gene expression. The effects of pCK-HGF were demonstrated in this study at 5 weeks compared to 8 weeks in previous studies^{5, 15}, which may have clinical value in end-stage patients.

MR-guidance

The success of the pCK-HGF delivery of the genes in the border zone and core of the infarction under MR-guidance was evident from: 1) the improvement in global LV function, 2) increase in perfusion of the infarcted segments, 3) reduction in infarct transmuralities, 4) homogeneous increase in vascular density in the border zone and core of the infarction and 5) presence of few viable islands/peninsulas in the border zone.

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. This study is the first to use endovascular MR-guided catheters for transendocardial delivery of the pCK-HGF gene and pCK-LacZ in multi-site targets. Prior to the clinical use of endovascular active catheter the following refinements are needed: 1) determining the safety issues, 2) reduction in catheter diameter to 6F, 3) increased flexibility of the catheter shaft and tip, and 4) addition of guide-wire to the catheter to avoid vascular or ventricular perforation.

Gene therapy

Unlike previous invasive pathophysiological studies^{3, 4, 10-14}, the current study used noninvasive MR imaging in validating global and regional (3D strain) LV function perfusion and viability. The novel aspects of this study are the use of: 1) HGF gene in arresting fibrosis model (chronic infarct scar) with established remodeled LV, 2) percutaneous MR-guided delivery approach and multiple injections and 3) serial MR evaluation of 3D strain analysis, perfusion and viability of chronic infarct scar. The scope of this MR study was not to define the mechanism(s) of action of HGF gene due to the current limitation of MR imaging. Furthermore, multiple laboratories established antifibrotic, antiapoptotic and cardioprotective properties of HGF^{3, 4, 10-14}. Others found that HGF also increases the recruitment of bone marrow-derived endothelial progenitor cells and/or enhances the effect of these cells in myocardial infarct^{12, 26}. It is likely that preservation of myocardial function, perfusion and viability seen in the current study is derived from the multi-mechanism mentioned above.

3D strain

Echocardiographic studies in swine models (ischemic cardiomyopathy and LV dysfunction caused by doxorubicin) illustrated significant improvement in LV function after delivering HGF genes^{3, 10}. The used multiple MR sequences have the potential to comprehensively assess the changes in load-dependent parameters (such as ejection fraction, stroke volume and cardiac output) and regional 3D strain. PCK-HGF treatment reversed LV remodeling by increasing wall strain and infarct thickness. Tagged and phase-contrast velocity-encoded MR images were sensitive enough to demonstrate the improvements in circumferential and longitudinal strain after pCK-HGF, but not pCK-LacZ, injection. Figure 5 illustrates the clear difference in longitudinal strain at the pixel-by-pixel level between the groups. A previous study showed that the change in regional strain is linearly related to ejection fraction²⁷. The improvement in LV function cannot be attributed to differences in heart rate, blood pressure or LV mass, as these parameters were not significantly different between the groups (Table 1).

Myocardial perfusion

First pass parameters (maximum upslope, peak signal intensity and time to peak) were used to assess whether pCK-HGF gene increased regional perfusion. We found that pCK-HGF gene significantly improved perfusion in chronic infarct scar 5 weeks after delivery. The increase in perfusion is related to the increases in capillary and arteriole densities seen on histology and confirms the functionality of the new vessels. This study is consistent with a recent study that showed angiogenesis induced by VEGF or stem cell-based VEGF prevents demand-induced ischemia and improves LV ejection fraction²⁸.

Chronic infarct scar

MR imaging showed the decrease in transmural infarct extent and the increase in wall thickness in pCK-HGF gene treated animals compared with control. Hayakawa et al found that inhibition of apoptosis by HGF alters infarct tissue dynamics, making infarct scar thicker and rich in preserved cellular components¹¹. The increase in wall thickness in

chronic infarct scar is important because wall stress is directly proportional to the diameter of LV chamber and is inversely related to wall thickening of remote myocardium²⁹. These changes could also be related to the inhibition of collagen I, III and TGF-beta expression¹³.

This non-invasive MR study is the first to demonstrate spared epicardium on DE-MR imaging after delivery of pCK-HGF gene, a finding confirmed on postmortem. The presence of few islands/peninsula in the border zone in infarct has been previously documented after administration of HGF protein^{4, 12, 30} and VEGF gene in mice and swine^{25, 31}.

Study limitations

This study comprised of a small number of animals because our institutional animal care and use committee imposed a restriction on all investigators, requiring the use of a minimum number of animals necessary to achieve significance. Thus we did not perform a power calculation; instead we based the number of animals in our study on the number of animals in previous studies^{17, 32, 33}. The number of animals in our study, however, was greater than the number of animals in recently published reports^{32, 33}.

We had 25% procedural failure due to arrhythmia, during the navigation of the catheter in the LV chamber. Other investigators also found in swine that electro-mapping and intramyocardial delivery of plasmid HGF using the NOGA is associated with transient ventricular ectopic activity³. Other limitations include lack of: 1) measurement of HGF gene expression in myocardium or plasma because it has been documented in clinical³⁴ and experimental studies^{3, 4}, therefore, this study provides indirect evidence of gene expression 2) knowledge as to the origin of few islands/peninsulas at the border of chronic infarct scar, possibly by HGF mobilization of progenitor cells¹⁴. The presence of islands/peninsulas at the border of infarct scar warrant additional electrophysiologic studies. Previous study indicated that such islands/peninsulas may create ventricular arrhythmia³⁵. In addition, the safety of using endovascular active catheters must also be addressed prior to clinical use.

Clinical implications

The used of noninvasive multiple imaging sequences and image analysis may provide new insights for future studies designed to characterize the arrest of ongoing fibrosis and cardirotrophic activity in remodelled LV. The combination of this minimally invasive MR-guided procedure and gene therapy may be useful in end stage patients. MR-guidance of local 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.

Conclusion

MR-guided transendocardial delivery of HGF gene in infarct scar ameliorated global function and 3D strain, increased perfusion, and reduced infarct size. At the microscopic level, pCK-HGF gene enhanced angiogenesis and arteriogenesis. This study provides novel imaging and analysis methods and instrumentation for delivering and evaluating locally delivered therapies.

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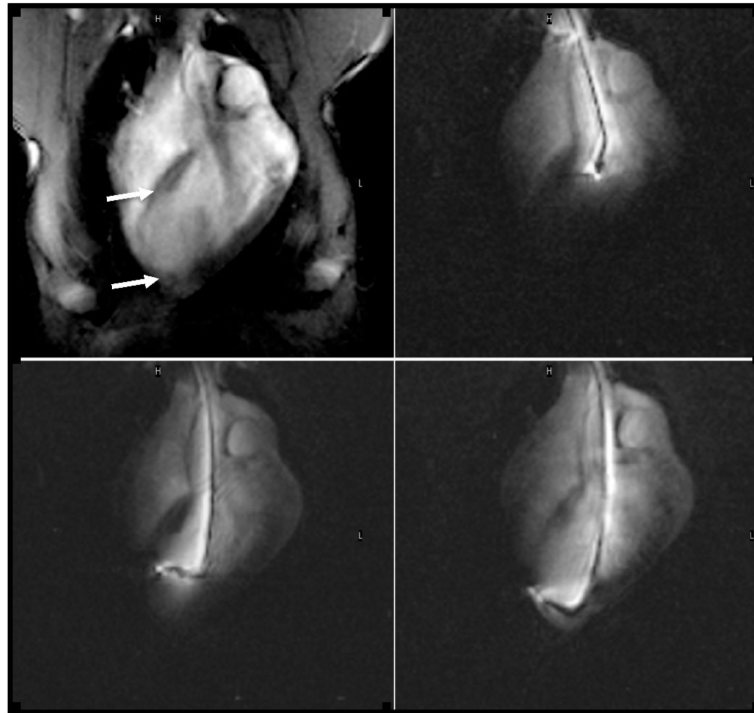


Figure 1. MR fluoroscopic images show the navigation of the endovascular catheter in the ascending aorta (top left), LV chamber (top right) and the injections of a gene at two different infarct sites in this plan 5 weeks after infarction. The first image was acquired with external imaging coils showing the entire chest and the infarcted thin wall (arrows), while the latter three images were created with signal from the catheter showing the catheter shaft and tip.

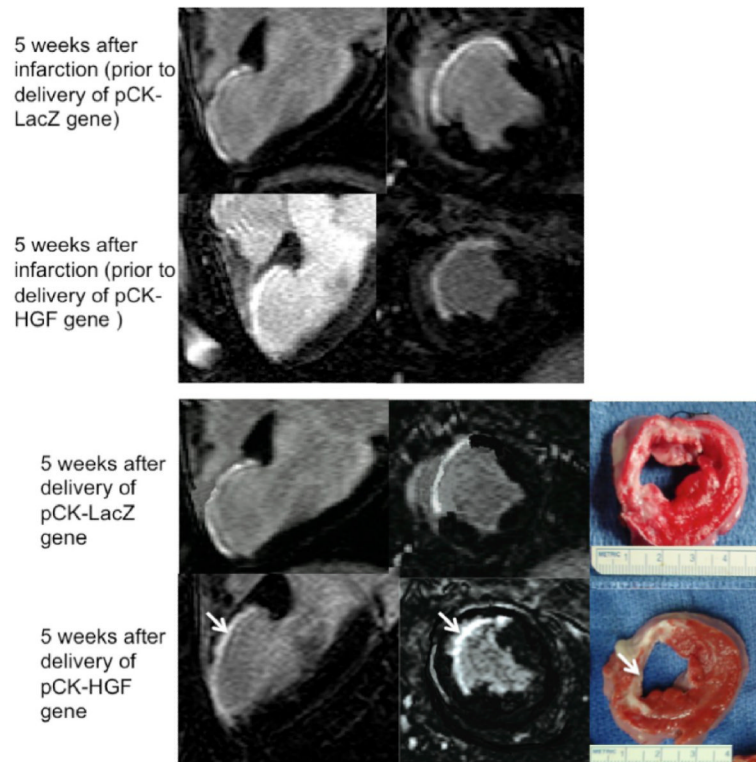


Figure 2. Long and short axis views of DE-MR images illustrate the hyperenhanced infarct at 5 weeks after infarction of two representative animals (top block). The bottom block shows the effects of pCK-LacZ (top row) and pCK-HGF genes (bottom row) on infarct transmurality (arrow) and size 5 weeks after treatment. Histochemical TTC staining (right LV slices) confirms the smaller and non-transmural infarct (arrow) in the pCK-HGF treated animal compared with pCK-LacZ control animal.

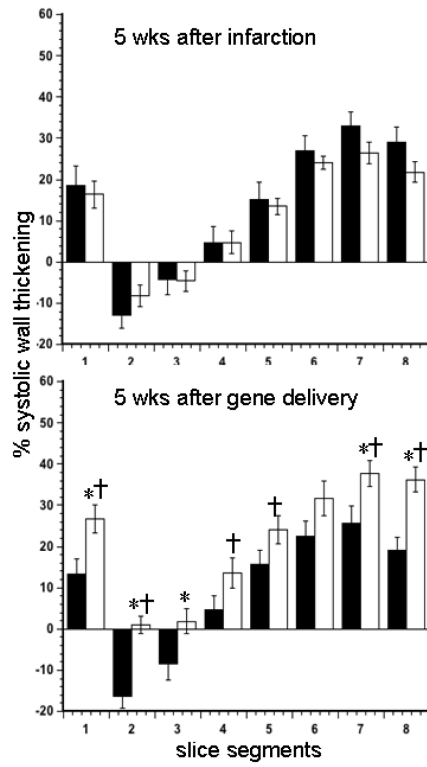


Figure 3.

Percent systolic wall thickening (radial strain) from 8 circumferential segments (average of 3 slices) of LV from pCK-LacZ control (black bars) and pCK-HGF treated (white bars) animals. The data were obtained at 5 weeks after infarction (top) and 5 weeks after delivery of gene (bottom). There was no significant difference between the groups at 5 weeks. However, the systolic wall thickening was markedly increased in treated compared with control animals. * $P < 0.05$ measurements after pCK-HGF compared with pCK-LacZ at 5 weeks after delivery of gene and † $P < 0.05$ 5 weeks after delivery of gene compared with 5 week after infarction of the same animals.

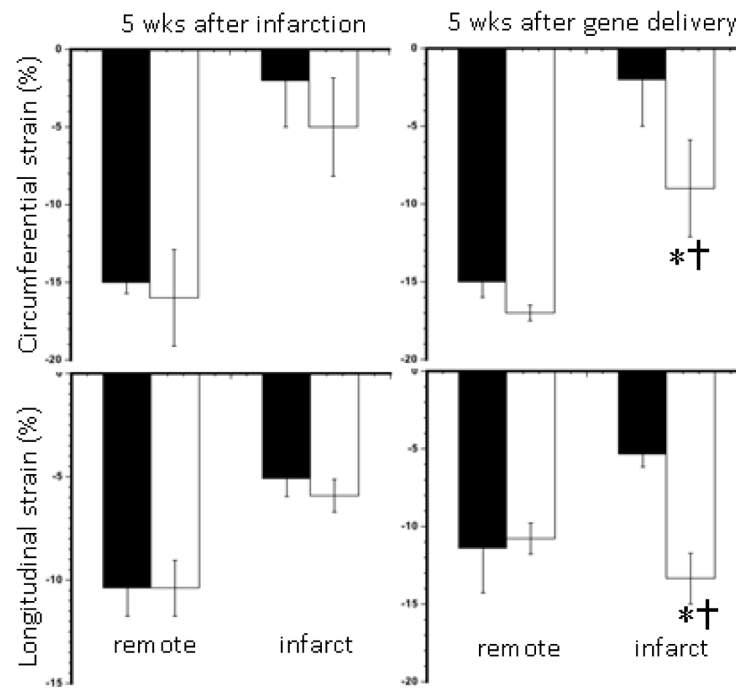


Figure 4.

Percent peak circumferential (top) and longitudinal (bottom) strain measurements obtained from remote myocardium and chronic infarct scar containing the infarct shown on DE-MR images for guidance. The circumferential and longitudinal strain were markedly increased in animals treated with pCK-HGF gene compared with pCK-LacZ gene. The data were obtained at 5 weeks after infarction (left blocks) and 5 weeks after delivery of gene (right blocks). Black bars represent animals injected with pCK-LacZ gene and white bars represent animals treated with pCK-HGF gene. * $P < 0.05-0.01$ measurements compared with pCK-LacZ control animal data and † $P < 0.05$ weeks prior to therapy.

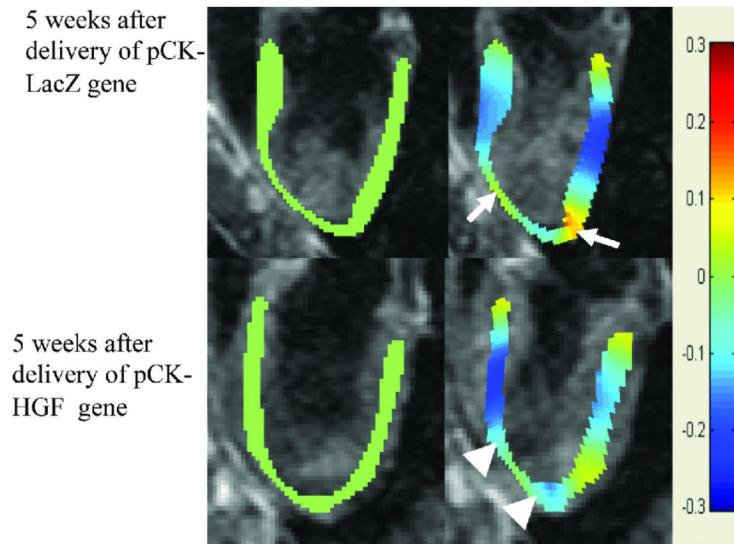


Figure 5. Longitudinal strain in pixel-by-pixel obtained from phase contrast velocity encoded MR images in animals treated with pCK-LacZ and pCK-HGF gene. Longitudinal strain at diastole is shown in green (left images) and at systole in blue. Top block demonstrates longitudinal strain at diastole (left images) and systole (right images) at 5 weeks after infarction. At 5 weeks after infarction, a marked decrease in longitudinal strain was noted in the apex of both animals (arrow) compared with remote myocardium. An improvement in strain was noticed at 5 weeks after delivery of pCK-HGF (arrowhead, right image in the bottom block), but not pCK-LacZ (arrow) gene.

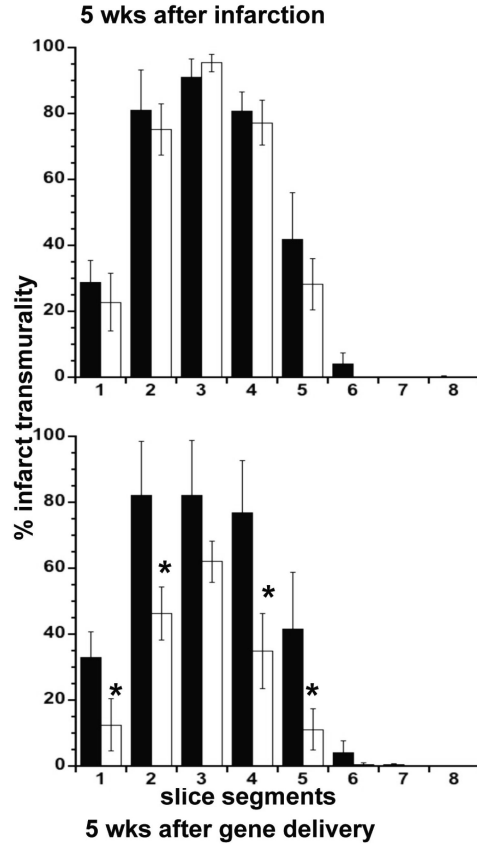


Figure 6. Percent infarct transmuralities before (upper block) and after (lower block) delivery of pCK-LacZ (black bars) and pCK-HGF (white bars). Prior to therapy, there was no significant difference in infarct transmuralities between the groups. pCK-HGF significantly reduced infarct transmuralities, but not pCK-LacZ. Infarct transmuralities did not change over the course of 5 week after delivery of pCK-LacZ. * $P < 0.05$ compared with pCK-LacZ group and to 5 weeks prior to delivery of genes.

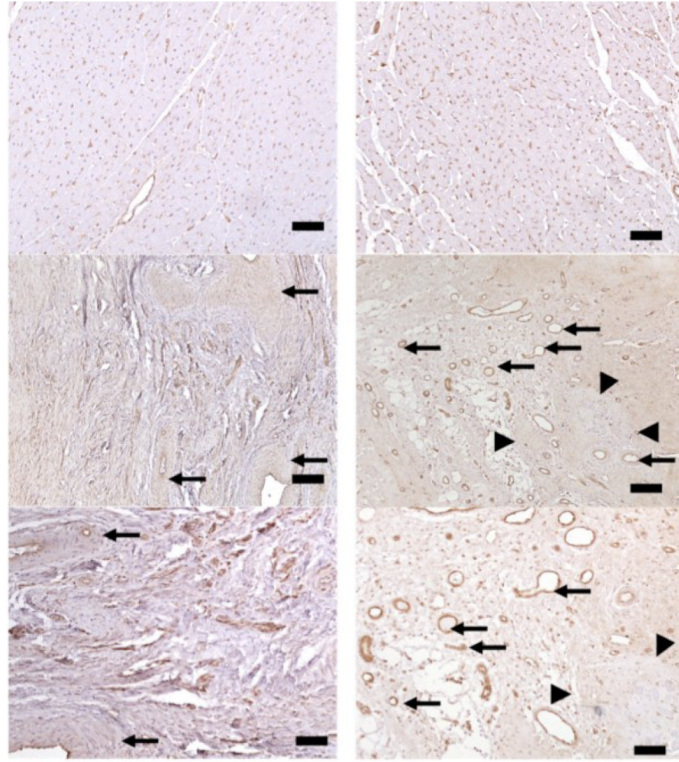


Figure 7. Histopathological heart sections stained with isolectin B₅ obtained from two animals 5 weeks after delivery of pCK-LacZ (left) and pCK-HGF (right). Remote myocardium obtained from both animals (top row) showed no difference in vascular density or myocyte diameters. The pCK-LacZ control animal showed few old thick-walled blood vessels (left-center, arrows) embedded in the dense scar tissue (left-bottom), whereas pCK-HGF treated animals showed numerous brown thin-walled new blood vessels (right-center and bottom, arrows) surrounding the few islands/peninsulas of viable myocyte at the border zone (arrowheads). The data supports the notion that pCK-HGF has angiogenic and may be myogenic properties. Calibration bars=200 microns (top panels), 50 microns (center panels), 25 microns (bottom panels).

TABLE 1

Body weight, heart rate and mean blood pressure measurements during the 10 weeks observation period.

Parameters	5 weeks after infarction		5 weeks after therapy	
	pCK-LacZ	pCK-HGF	pCK-LacZ	pCK-HGF
Body-weight (kg)	43±2	41±1	54±1	52±1
Heart rate (bpm)	92±3	98±7	86±3	88±3
Mean blood pressure (mmHg)	69±5	67±4	70±4	69±3

Serial MR measurements of global LV function and mass before and after delivery of the genes. Note that the genes were derived 5 weeks after infarction.

TABLE 2

Parameters	5 weeks after infarction		5 weeks after therapy	
	pCK-LacZ	pCK-HGF	pCK-LacZ	pCK-HGF
EDV (ml/kg)	2.10±0.05	2.03±0.08	2.03±0.06	1.99±0.09
ESV (ml/kg)	1.31±0.06	1.27±0.05	1.28±0.05	1.10±0.05 ^{**+}
Stroke volume (ml)	36±2	33±2	40±1	47±2 ^{**+}
Ejection fraction (%)	38±2	38±1	37±1	43±1 ^{**+}
Cardiac output (L)	3.3±0.2	3.2±0.3	3.4±0.2	4.2±0.3
LV mass (g)	96±3	102±5	117±4	123±4
MR infarct scar (%LV)	12.4±1.1	11.3±2.0	12.7±0.2	6.7±1.3 ^{**++}
TTC (%LV)			12.1±0.7	7.2±0.9 ^{**++}

* $P < 0.05$

** $P < 0.01$ compared to pCK-LacZ control animals at 5 weeks after treatment (unpaired t-test).

⁺ $P < 0.05$

⁺⁺ $P < 0.01$ compared to 5 weeks prior treatment (paired t-test)

TABLE 3

Regional perfusion parameters obtained from first pass MR imaging.

Groups	5 weeks after infarction		5 weeks after therapy	
	pCK-LacZ	pCK-HGF	pCK-LacZ	pCK-HGF
LV Blood				
Max upslope (s-1)	3075±408	2959±682	2507±241	3233±365
Peak signal intensity (au)	6295±361	6021±503	6342±269	6081±282
Time to the peak (s)	5.2±0.3	4.5±0.1	4.4±0.3	5.2±1.3
Remote myocardium				
Max upslope (s-1)	376±42	318±34	260±43	290±46
Peak signal intensity (au)	1533±81	1291±129	1688±150	1375±142
Time to the peak (s)	12.3±1.0	13.5±0.8	7.6±0.4	7.9±0.9
Infarct scar				
Max upslope (s-1)	134±24	127±21	130±29	367±58 ^{*‡}
Peak signal intensity (au)	1058±58	1067±33	1249±141	1482±126 ^{*‡}
Time to the peak (s)	15.0±1.0	15.4±1.1	14.1±0.5	10.6±1.1 ^{*‡}

^{*‡} P<0.05 compared to pCK-LacZ control animals at 5 weeks after delivery of the genes and 5 weeks after infarction data point, respectively.