Electromagnetic Guidance for Catheter-Based Transendocardial Injection: A Platform for Intramyocardial Angiogenesis Therapy

Results in Normal and Ischemic Porcine Models

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OBJECTIVES To test the feasibility of myocardial angiogenic gene expression using a novel catheter-based transendocardial injection system.

BACKGROUND Angiogenesis has been induced by direct injection of growth factors into ischemic myocardium during open-heart surgery. Catheter-based transendocardial injection of angiogenic factors may provide equivalent benefit without need of surgery.

METHODS A new guidance system for intramyocardial therapy utilizes magnetic fields and catheter-tip sensors to locate a position in space and reconstruct three-dimensional left ventricular (LV) electromechanical maps without using fluoroscopy. A retractable 27G needle was coupled with the guidance system for LV transendocardial injection. In 12 pigs, the catheter was used to inject 0.1 ml of methylene-blue (MB) dye and 8 pigs had myocardial injections of adenoviral vector (1 × 10^10 particles per site) containing the LacZ transgene. Ten pigs underwent catheter-based transendocardial injection and six pigs were injected using transepicardial approach with the gene encoding adenovirus vascular endothelial growth factor-121 (Ad.VEGF121; 1 × 10^10 viral particles × 6 sites) and sacrificed at 24 h. Injection sites were identified with ultraviolet light by coinjection of fluorescent beads.

RESULTS Overall, 138 of 152 attempted injection MB tracks (91%) were found after sacrifice. Tissue staining was 7.1 ± 2.1 mm in depth and 2.3 ± 1.8 mm in width. No animal had pericardial effusion or tamponade. In Ad.LacZ injected animals, gross pathology showed positive staining in injected zones, and histology confirmed positive myocyte staining. Adenovirus vascular endothelial growth factor-121 injected sites showed high levels of VEGF121 production that was of similar magnitude whether injected using the transendocardial (880.4 ± 412.2 pg VEGF121/mg protein) or transepicardial (838.3 ± 270 pg VEGF121/mg protein) delivery approach (p = 0.62).

CONCLUSIONS Using this magnetic guidance catheter-based navigational system, transgenes can effectively be transfected into designated myocardial sites. Thus, if it is determined that direct intramyocardial injection of angiogenic factors enhances collateral function in patients, this less invasive catheter-based system offers a similar gene delivery efficiency and, thus, may have clear advantages compared with the surgically-based transepicardial injection approach.

The use of recombinant genes or growth-factors to enhance myocardial collateral blood vessel function may represent a new approach to the treatment of cardiovascular disease (1). Proof of this concept has been demonstrated in animal models of myocardial ischemia (2–7), and clinical trials are underway (8–10). Most strategies for transcatheter delivery of angiogenic factors have employed an intracoronary route, which may have limitations due to imprecise localization of genes or proteins and systemic delivery to noncardiac tissue. Thus, it would be desirable to have the capacity for direct delivery of angiogenic factors or genes to precisely defined regions of the myocardium rather than to the entire heart and, thereby, to minimize the potential for systemic exposure (11). The effect of direct intraoperative intramyocardial
injection of angiogenic factors on collateral function has been studied in a porcine model of myocardial ischemia; open chest, transepicardial administration of an adenoviral vector containing a transgene encoding an angiogenic peptide resulted in enhanced collateral function (7). Angiogenesis was also reported to occur with direct intramyocardial injection of an angiogenic peptide (8) or a plasmid vector (9) during open heart surgery in patients. Ideally, a catheter-based intramyocardial injection system could provide accurate intramyocardial injection of angiogenic factors (genes or peptides) without the need for surgery and general anesthesia (12).

This report describes a new electromagnetic-based platform system developed for catheter-based intramyocardial injection that may be used to achieve therapeutic angiogenesis. The guidance system (NOGA, Biosense-Webster, Diamond Bar, California) utilizes low-intensity magnetic field energy and sensor tipped catheters to locate the catheter position in space (13–18). Three-dimensional (D) electromechanical maps of the left ventricle (LV) help to differentiate between healthy and infarcted myocardium and can be used to identify most ischemic target regions without using fluoroscopy (16,18). This system was integrated with an injecting catheter having a 27G needle for intramyocardial delivery of potentially therapeutic agents.

The purpose of this study was to:

1) evaluate the feasibility and safety of electromagnetic guidance for a catheter-based transendocardial injection system in normal and ischemic myocardium,
2) assess the accuracy of the system to locate, in real time, the sites of intramyocardial injection,
3) identify local diffusion of a fluid (dye tracer) from the injection site into myocardial tissue,
4) determine whether gene transfer into normal or ischemic myocardium can be achieved using this integrated catheter system that results in successful gene expression (as assessed by LacZ reporter gene activity), and
5) determine if a therapeutically relevant gene can be effectively delivered via the electromagnetic guidance and injection catheter and can be expressed at high levels.

The latter was achieved by injecting transendocardially an adenoviral vector carrying the gene encoding vascular endothelial growth factor-121 (Ad.VEGF121) and comparing the levels of Ad.VEGF121 expression to those obtained by a surgical based transepicardial injection approach.

**METHODS**

**Electromagnetic-guided LV mapping and injection system.** As described previously (13–18), the electromechanical mapping system uses:

1) a location pad containing three coils generating ultralow magnetic field energy,
2) a stationary reference catheter with a miniature magnetic field sensor located on the body surface,
3) a navigation sensor mapping catheter (7F) with a deflectable-tip and electrodes providing endocardial signals, and
4) a workstation for information processing and 3D LV reconstruction.

The mapping catheter was introduced retrograde across the aortic valve into the LV. The initial three points outlining the boundaries of the LV (apex, aortic outflow and mitral inflow) were acquired with fluoroscopic guidance. Subsequently, no fluoroscopy was needed to acquire additional sampled points within the LV chamber. The mapping process proceeded only when the catheter tip was stable on the endocardial surface as evidenced by local activation time, location, loop and cycle length stability parameters. The system used a triangulation algorithm to reconstruct the LV anatomy, which was presented in real-time on a workstation. Once all endocardial regions were represented on the map, the operator completed the reconstruction of the LV map, the mapping catheter was removed from the LV and replaced by an 8F injection catheter (Biosense; Webster, California). The injection catheter was a mapping catheter modified to integrate a retractable 27G needle for LV intramyocardial injection. The catheter “dead-space” was 0.1 ml and was flushed by 0.1 ml using the injection material before its endovascular insertion. The needle was controlled by a handle mechanism that was located proximal to the standard deflection handle. The injection had a Luer fitting for connection to a 1 cc or 3 cc syringe. Standard manual operation of the syringe attached to the injection handle delivered the fluid to the myocardium. The operator of the syringe controlled the dosage using the gradations on the syringe.

The exact catheter-tip location, orientation and the injection sites were indicated in real-time on the LV map, and local electrical and location signals were traced to assure catheter stability and optimal endocardial contact. Animals were monitored for systemic blood pressure and surface electrocardiograms throughout the study protocol and during the recovery period. Arrhythmia was evaluated from the LV mapping catheter data during mapping and by electrocardiographic recording after injection.
Fluid dispersion tests. After adequate anesthesia with ketamine (20 mg/kg) and xylazine (2 mg/kg) i.m. and sodium pentobarbital (10 mg/kg) IV, animals (weight 50 to 70 kg) were intubated and received supplemental oxygen at 2 L/min and isoflurane inhalation throughout the procedure. Left ventricular electromechanical mapping was performed followed by intramyocardial injection of 0.1 ml of methylene-blue (MB) dye (1:10 dilution in normal saline) in 12 healthy anesthetized pigs. Needles were extended into the myocardium to 3 to 4 mm in two myocardial regions (i.e., anterior, septum, lateral, inferior or posterior) per animal. After the injection procedure, all catheters were removed, and the animals were sacrificed acutely. Injection sites were localized by gross examination of the endocardial surface in conjunction with localization data obtained from the LV maps. The extent of intramyocardial staining (maximal depth and width) was measured to identify local diffusion of MB dye tracer from the injection site. In addition, the success of achieving intramyocardial injection was assessed based on the number of endomyocardial injection sites found compared with the number of attempted injection sites.

The needle length, both straight and deflected, at preinjection and postinjection was measured before and after each experiment using a hand caliper to verify potential deviations from prespecified needle length (3 to 4 mm) or changes in needle extension during the course of the experiment.

Evaluation of reporter gene transfer. We next tested the capacity of the electromagnetic guidance and injection catheter system to deliver transgenes to ischemic or normal myocardium. Replication-deficient recombinant Ad. containing the transgene encoding beta-galactosidase (beta-gal; Ad.LacZ, GenVec Inc., Rockville, Maryland) and containing a nuclear localization sequence was injected into eight pigs (weight 50 to 70 kg) in vivo. Four of the eight pigs underwent induction of myocardial ischemia (see following text), and four additional pigs had normal hearts. The replication-deficient recombinant Ad. has been previously described, and its efficient gene transfer into myocardial tissue by a direct epicardial injection approach has been previously demonstrated (11). Myocardial ischemia was induced in four animals by the application of an ameroid constrictor placed around the left circumflex artery. The ameroid constrictor resulted in gradual (14 to 21 days) coronary artery occlusion and, in pigs, usually produces chronic ischemia rather than infarction (6,7,18,19). Five weeks after ameroid placement, the four ischemic pigs underwent general anesthesia (using the same protocol specified above) and selective coronary angiography to document occlusion of the ameroid constrictor site. A left ventricular electromechanical map was obtained, followed by injection of Ad.LacZ into the ischemic lateral wall area using the injection catheter. The ischemic myocardial territory was identified as that region of the LV anatomical map showing impaired mechanical contractility signals (18). This was located in the lateral wall supplied by the occluded left circumflex coronary artery.

Each site was injected with $10^{10}$ viral particles diluted in 0.1 ml (n = 6) and 0.2 ml (n = 2) solution containing sucrose 3%. Different volumes were used to determine whether keeping the needle length constant (3 to 4 mm) but increasing the volume of delivery would provide for greater gene transfer throughout the thickness of the ventricular wall without causing perforation of the myocardium. Animals were sacrificed at 24 h and myocardial tissue was evaluated for gene expression. Hearts were excised and underwent ex vivo retrograde perfusion with 200 ml normal saline. Injection sites were localized according to comparative anatomical and mapping derived measurements (i.e., linear distances from anatomical markers such as the apex and aortic valve and from two additional reference endocardial points obtained by MB injection into the heart). Full thickness myocardial samples (5 mm in width) were obtained from the presumed injection sites. The samples were fixed in a solution containing 2% formaldehyde and 0.2% glutaraldehyde in phosphate buffered saline (PBS) at pH 7.4 and cut into sections approximately 2 mm thick. Sections were then washed three times in PBS and placed in X-gal histochemical solution containing 5 mmol/L K$_4$Fe(CN)$_6$, 5 mmol/L K$_3$Fe(CN)$_6$, 1 mmol/L MgCl$_2$ and 1 mg/ml X-gal (5-bromo-4-chloro-3-indoyl-beta-D-galactopyranoside) in PBS for 24 h. Samples were subsequently processed for gross and microscopic histological analysis.

Evaluation of VEGF$_{121}$ gene transfer. We tested whether adenoviral vector carrying the gene encoding Ad.VEGF$_{121}$ can be effectively delivered via the electromagnetic guidance and injection catheter and to determine whether the transgene was efficiently expressed. These were assessed by determining the levels and distribution of VEGF$_{121}$ protein expression 24 h later. The ability of the replication deficient Ad. containing an expression cassette encoding for VEGF$_{121}$ (GenVec, Inc., Rockville, Maryland) to enhance collateral perfusion has been previously described (7). In order to localize precisely the sites of injection, we used a technique in which fluorescent microspheres (Flouresbright, Polysciences Inc., Warrenton, Pennsylvania) are mixed with the adenoviral vector carrying the relevant transgene in a 1:10 dilution and injected into the myocardium. This method allows for precise localization of the transfected tissue.

After harvesting the treated tissue, the site of transfection can be localized using an ultraviolet (UV) lamp; protein levels at the precise site of transfection are then determined. Using this method we tested the capacity of the electromagnetic guidance and injection catheter system to deliver the adenoviral VEGF vector to the myocardium of 10 normal pigs (weight 50 to 70 kg) undergoing catheter-based Ad.VEGF$_{121}$ gene delivery. Those results were compared...
with transepicardial Ad.VEGF
121 gene delivery via open chest left thoracotomy performed in six additional pigs. Six injections were performed per animal. Each site was injected with 10^10 viral particles diluted in 0.1 ml PBS solution. Three injections were localized to the anterior-septal region and three others to the inferior or lateral regions. Animals were sacrificed at 24 h and myocardial tissue was evaluated for gene expression. Hearts were excised and underwent ex vivo retrograde perfusion with 200 ml normal saline. All hearts were sectioned horizontally into 0.5 cm slices from the apex to the base of the heart. Injection sites were visualized under UV light and for each bead injection region a1 cm^3 central portion (surrounding the beads) was harvested and two additional lateral adjacent portions were also harvested to assess spread of VEGF121 protein. Additional samples were taken from “remote,” presumably non-transfected, portions of the LV and right ventricle. All samples were immediately frozen using liquid nitrogen and kept in −80°C until VEGF protein analysis using enzyme-linked immunosorbent assay (ELISA).

VEGF
121 measurement. Vascular endothelial growth factor-121 levels were quantified as per the manufacturer’s instructions using the R&D Systems human VEGF ELISA kit (#DVE00, R&D Systems, Inc. Minneapolis, Minnesota) with the following modification: the VEGF
165 used to generate the standard curve for the assay was replaced with VEGF
121 (#298-VS-005, R&D Systems, Inc. Minneapolis, Minnesota) to ensure accurate quantification of the desired VEGF isoform.

Statistical analysis. Nominal values are presented as means ± 1 standard deviation. Repeated measurements (pre- versus post-procedure) were compared using repeated measures analysis of variance. A comparison between peak VEGF levels in the surgical versus the catheter-based group was performed using unpaired t test. p < 0.05 was considered as statistically significant.

RESULTS

Fluid dispersion tests. The injection site was indicated in real-time on the LV map (Fig. 1). Using the catheter system, we injected 0.1 ml of MB as dye tracer in 12 pigs and measured the extent of intramyocardial staining when the needle was extended 3 to 4 mm (152 injections) into the myocardium. Staining was 7.1 ± 2.1 mm (range 2–11 mm) in depth and 2.3 ± 1.8 mm (range 1 to 9 mm) in its maximal width. Dye staining of the epicardial surface was found in only 4 of 152 injections (2.6%). Three of those were found when the dye was injected into the apical area. The frequency with which we targeted specific anatomic injection sites was: anterior 58 (38%), inferior 24 (16%), posterior 20 (13%), lateral 47 (31%) and apex 3 (2%). Overall, 138 of 152 attempted injection tracks (91%) were found immediately after sacrifice, ranging from 78% to 100% per single animal experiment. No animal died during the injection procedure. No animal had pericardial effusion or tamponade, and no episodes of sustained ventricular arrhythmia were noted immediately after intramyocardial injection. The mean arterial blood pressure (83 ± 15 vs. 81 ± 17 mm Hg, p = 0.56) and mean heart rate (86 ± 7 vs. 83 ± 11 beats/min, p = 0.52) did not change significantly during the injection procedure. There was no significant change in needle length over the course of the procedure (3.6 ± 0.2 mm before the injection, 3.2 ± 0.3 mm after the injection, p = 0.38). The endomyocardial surface after injection of MB dye is shown in Figure 2.

Figure 1. A representative electromechanical left ventricle map (right and left oblique projections, A and B, respectively) after intramyocardial injection of methylene-blue into normal heart showing the ability of the injection device to reach different myocardial territories (A; anterior-septal zone, B; lateral zone). The voltage amplitudes are color coded, and the exact location of the injection sites is tagged (brown) on the map in real-time.
**Ad.LacZ gene expression.** All eight tested animals survived the Ad.LacZ injection procedure until sacrifice at 24 h. No episodes of sustained ventricular arrhythmia were noted immediately after intramyocardial injection or during the recovery period. After fixation and X-gal staining for 24 h, gross pathology assessment showed that hearts injected with Ad.LacZ had positive blue staining. The beta-gal staining was mainly confined to approximately one half of the myocardial thickness, and it had a flame-shaped appearance. The ability of the system to target the injections into the ischemic area was demonstrated in all four ischemic animals. Ischemic myocardium was identified by the presence of mechanical dysfunction and preserved electrical activity in the left circumflex arterial distribution (Fig. 3). Macroscopic and microscopic examples are shown in Figure 4. Similar myocardial X-gal staining pattern was observed with 0.1 ml compared with 0.2 ml injected volume. Gross and histologic examination of the hearts injected with Ad.LacZ showed positive staining in the nuclei and cytoplasm of cardiac myocytes (Fig. 4). Inflammatory mononuclear cells were often seen around transfected myocytes. Noninjected myocardial samples from the left and right ventricles did not show Ad.LacZ positive staining after exposure to X-gal solution.

**Catheter-based (transendocardial) VEGF<sub>121</sub> gene transfer.** Overall, 60 injections of Ad.VEGF<sub>121</sub> have been performed in 10 animals. All animals survived for 24 h until sacrifice. Using UV light, 59 of the 60 injection sites (98.3%) were clearly identified (Fig. 5). The transfection sites seemed discrete and manifested significant VEGF production in 57/59 sites (96.6%) at the central portion of injection (880.4 ± 412.2 pg VEGF/mg protein). In two sites, measurable VEGF<sub>121</sub> levels were not detectable in either central or adjacent pieces despite the existence of beads in those sites. A significant drop-off of VEGF production was noted even 1 cm to the left (21.4 ± 7.9 pg VEGF<sub>121</sub>/mg protein) or right (73.3 ± 63.3 pg VEGF<sub>121</sub>/mg protein) of the injection site (Fig. 6). No detectable levels of VEGF<sub>121</sub> were found in any of the right

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**Figure 2.** (A) The tip of the injection catheter equipped with recording electrodes, a location sensor (hosted within the catheter tip) and a 27 G needle extending for 3 to 4 cm length. (B) The endocardial surface is displayed after multiple methylene-blue dye injections (arrows). (C) Intramyocardial flame-shaped "signature" of methylene-blue staining in a cross-section.

**Figure 3.** The ability of the magnetic-guided system to target the injections into the ischemic region of the heart. A representative electrical map (A; unipolar voltage) and mechanical map (B; local endocardial shortening) after induction of ischemia in the lateral wall by occlusion of the circumflex artery (left oblique views). The color scale is set between 5 mV (red) to 15 mV (blue/purple) for the voltage map and between 6% (red) to 12% (blue/purple) for the mechanical map. Note a significant mechanical impairment exists in the lateral wall (local endocardial shortening <6%, red zone) secondary to chronic myocardial ischemia, with preserved (normal) voltage potentials (~15 mV). The sites of injection (dots within the dysfunctional "red" zone) are targeted to the ischemic regions and are shown in real time on the maps.
ventricular samples. There were detectable levels of VEGF_121 (39.5 pg VEGF_121/mg protein) in only 1 of 10 remote LV samples although no fluorescent beads were identified in this site. In all other remote sites, no detectable VEGF_121 levels were identified.

**Surgical (transepicardial) VEGF_121 gene transfer.** Overall, 36 injections of Ad.VEGF_121 were performed in six animals. All animals survived for 24 h until sacrifice. Using UV light, all 36 injection sites (100%) were identified. The transfection sites manifested significant VEGF_121 production in all 36 sites at the central portion of injection (838.3 ± 270 pg VEGF_121/mg protein) (Fig. 6). Importantly, VEGF_121 levels were comparable with those obtained by the transendocardial injections (p = 0.62 for comparison between the surgical and catheter-based groups). Adjacent VEGF_121 levels were 10.0 ± 3.0 pg/mg protein (left piece) and 13.0 ± 3.0 pg/mg protein (right piece). Using the surgical injection approach, VEGF_121 could be detected in two of six left ventricular “remote” samples (27.0 and 163.3 pg/mg protein) and in one of six right ventricular samples (52.4 pg/mg protein).

**DISCUSSION**

The main findings of this investigation were: 1) the guided LV mapping technology and injection system provides an accurate and reliable means of delivering an injectate transendocardially into the LV, 2) the injection
technique caused no serious sequella such as death, hemodynamically destabilizing arrhythmias or cardiac tamponade, and 3) this system allows for the injection of an adenoviral vector containing genes for LacZ or VEGF121 into designated ischemic myocardial sites and results in successful gene transfer and protein expression.
Importantly, the VEGF<sub>121</sub> results showed much greater expression at the site of injection, with a significant ‘drop-off’ of VEGF production even 1 cm from the injection site. Our study also shows that this less invasive catheter-based approach for gene delivery enables an equivalent gene transfection efficiency and gene expression compared with a surgical-based transepicardial injection approach. Thus, we believe that this strategy of gene delivery offers a clear advantage over a surgical-based approach for use in therapeutic myocardial angiogenesis.

Previous animal studies have proven the feasibility of enhancing collateral function by delivery of angiogenic factors to the myocardium. This has been achieved either by intracoronary injection of the angiogenic factor at catheterization or by direct intramyocardial injection after thoracotomy (2–7). Successful angiogenesis has resulted from delivery of either angiogenic proteins or plasmids or adenoviral vectors containing transgenes that encode angiogenic proteins. Currently, however, it is unknown which is the most effective and safe delivery strategy (i.e., intracoronary vs. intramyocardial injection) to induce clinically important therapeutic angiogenic responses in ischemic myocardium.

A theoretical advantage of direct intramyocardial injection of angiogenic factors compared with intracoronary delivery is that with intracoronary injection, during its first pass, a significant amount of the angiogenic factor will not be taken up from the vascular compartment by the heart and, therefore, will be delivered to other tissues (20). It would, thus, appear desirable, if possible, to deliver all of the angiogenic material directly into the target tissue. This approach has been successfully accomplished using an adenoviral vector containing the VEGF<sub>121</sub> transgene that was directly injected into ischemic myocardium of pig hearts during thoracotomy (7). Both myocardial perfusion and function in this model were significantly improved (7). The feasibility and safety of such direct VEGF<sub>121</sub> transgene delivery approach in patients has been demonstrated (10). The first clinical experience with intramyocardial injection of an angiogenic factor was recently reported. Recombinant human fibroblast growth factor-1 was directly injected into the LV myocardium. If the biologic and clinical reproducibility of the injection parameters. Ultimately, with proper physician training, this system should permit the routine safe transendocardial delivery of therapeutic agents into the myocardium. At present, catheter design is in a rapid stage of evolution to reduce potential tissue trauma and to ensure reproducibility of the injection parameters. Ultimately, with proper physician training, this system should permit the routine safe transendocardial delivery of therapeutic agents into the myocardium. If the biologic and clinical importance of angiogenic treatment is ultimately demonstrated, the system we describe in this report may constitute an important new strategy of treating patients with myocardial ischemic syndromes.

**Study limitations.** Our study is limited by the lack of evaluation of systemic transgene delivery and expression. Until such an experiment is completed, we cannot definitively conclude that our approach totally limits gene delivery to the target tissue. In this regard, in 5% to 10% of injections, no track was found. This raises the possibility of systemic delivery of the genetic material, if indeed the reason for the failure was not “obstruction” with no material ejected, but rather that material was injected into the LV and thereby delivered systemically. Another limitation was the lack of precise pressure control due to manual delivery. The pressure effect may account for the fact that the needle can create deeper tracks and staining zones than its actual extension. Our study lacked histopathology evaluation comparing MB staining versus the myocardial transduction noted after Ad. injections. Finally, our study was not designed to explore whether the minimal inflammatory tissue response noted at 24 h after Ad. injection may have been associated with viral transduction, the results of catheter injection itself or both.

**Conclusions.** Intramyocardial delivery of angiogenic factors via the transendocardial catheter system appears safe and suitable for delivering therapeutic agents into the myocardium. At present, catheter design is in a rapid stage of evolution to reduce potential tissue trauma and to ensure reproducibility of the injection parameters. Ultimately, with proper physician training, this system should permit the routine safe transendocardial delivery of therapeutic agents into the LV myocardium. If the biologic and clinical importance of angiogenic treatment is ultimately demonstrated, the system we describe in this report may constitute an important new strategy of treating patients with myocardial ischemic syndromes.

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**REFERENCES**


