Randomized, Single-Blind, Placebo-Controlled Pilot Study of Catheter-Based Myocardial Gene Transfer for Therapeutic Angiogenesis Using Left Ventricular Electromechanical Mapping in Patients With Chronic Myocardial Ischemia

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Background—Catheter-based myocardial gene transfer (GTx) has not been previously tested in human subjects. Accordingly, we performed a pilot study to investigate the feasibility and safety of catheter-based myocardial GTx of naked plasmid DNA encoding vascular endothelial growth factor-2 (phVEGF-2) in patients with chronic myocardial ischemia.

Methods and Results—A steerable, deflectable 8F catheter incorporating a 27-gauge needle was advanced percutaneously to the left ventricular myocardium of 6 patients with chronic myocardial ischemia. Patients were randomized (1:1) to receive phVEGF-2 (total dose, 200 μg), which was administered as 6 injections into ischemic myocardium (total, 6.0 mL), or placebo (mock procedure). Injections were guided by NOGA left ventricular electromechanical mapping. Patients initially randomized to placebo became eligible for phVEGF-2 GTx if they had no clinical improvement 90 days after their initial procedure. Catheter injections (n = 36) caused no changes in heart rate or blood pressure. No sustained ventricular arrhythmias, ECG evidence of infarction, or ventricular perforations were observed. phVEGF-2–transfected patients experienced reduced angina (before versus after GTx, 36.2 ± 2.3 versus 3.5 ± 1.2 episodes/week) and reduced nitroglycerin consumption (33.8 ± 2.3 versus 4.1 ± 1.5 tablets/week) for up to 360 days after GTx; reduced ischemia by electromechanical mapping (mean area of ischemia, 10.2 ± 3.5 versus 2.8 ± 1.6 cm²; P = 0.04); and improved myocardial perfusion by SPECT-sestamibi scanning for up to 90 days after GTx when compared with images obtained after control procedure.

Conclusions—This randomized trial of catheter-based phVEGF-2 myocardial GTx provides preliminary indications regarding the feasibility, safety, and potential efficacy of percutaneous myocardial GTx to human left ventricular myocardium. (Circulation. 2001;103:2138-2143.)

Key Words: gene therapy ▪ mapping ▪ catheters

Site-specific gene transfer (GTx) of naked DNA encoding vascular endothelial growth factor (VEGF) has been shown to induce therapeutic angiogenesis in animal models of hindlimb and myocardial ischemia. Subsequent clinical experience documented histological, angiographic, and/or physiological evidence of neovascularization in patients with critical limb ischemia after direct intramuscular GTx. More recently, this strategy has been applied to patients with chronic myocardial ischemia in whom conventional revascularization (PTCA and/or CABG) is not feasible. Because cardiac muscle is less accessible than the skeletal muscles of the lower extremity, myocardial GTx of naked DNA has, to date, required a minithoracotomy to expose myocardium for intramuscular injection. Studies to date have suggested that this approach is safe and may yield objective signs of improvement in myocardial perfusion.

Although the use of a minithoracotomy seems to be generally well tolerated, even in patients with advanced myocardial ischemia, nevertheless, the procedure has some risk associated with the administration of general anesthesia and some morbidity associated with surgical manipulation (particularly in patients with previous bypass surgery), and it limits the feasibility of repeat administrations. From a clinical trial perspective, GTx performed via thoracotomy carries the additional disadvantage of making randomization against placebo more difficult.
Accordingly, this pilot study was designed to assess the feasibility, safety, and potential efficacy of catheter-based, percutaneous myocardial GTxs of naked DNA encoding VEGF-2 administered via a novel needle-injection catheter versus a mock procedure.

**Methods**

**Patients**

Eligibility for catheter-based myocardial GTxs included stable Canadian Cardiovascular Society class 3 or 4 angina refractory to maximum medical therapy, multivessel occlusive coronary artery disease, and reversible ischemia on stress SPECT-sestamibi studies. Subjects were excluded if they had a reduced left ventricular (LV) systolic function (LV ejection fraction [EF] <20%, malignancy, or diabetic retinopathy). All patients continued their routine medical therapy after GTxs.

**NOGA LV Electromechanical Mapping**

Subjects underwent nonfluoroscopic LV electromechanical mapping (EMM) immediately before GTxs to guide injections of plasmid DNA to foci of ischemic myocardium. The NOGA system (Biosense-Webster) of catheter-based mapping and navigation has been previously described in detail. The editing of the raw data was performed by the NOGA system computer, and postprocessing analysis was performed by blinded observers.

Local functional analysis is based on linear local shortening (LLS), a parameter that calculates the fractional shortening of regional endocardial surfaces at end-systole and correlates with wall motion. Unipolar (UpV) and bipolar endocardial potentials recorded at the tip electrode, based on local intracardiac signal amplitudes, are used to map myocardial viability. LV combination of these 2 data sets permits an assessment of electromechanical function that can be used to identify foci of myocardial ischemia. For example, for a given myocardial segment of interest, an UpV ≥5 mV (thereby suggestive of viable myocardium) and normal local shortening ≥12% (thereby suggestive of normal contraction) would together imply normal myocardium. In contrast, an UpV <5 mV and abnormal local shortening <4% (signifying severe regional hypokinesis or akinesis) would denote a site of LV infarction. Alternatively, an UpV ≥5 mV and abnormal local shortening of 4% to 12% (indicating mild to moderate impairment of contractility) would suggest an area of ischemic, hibernating myocardium.

To quantify the area of ischemia, a 2D algorithm based on a standard reference frame was used to calculate both the area of ischemia and the total surface area in the 2D view depicting maximal ischemia.

**Plasmid DNA**

The phVEGF-2 plasmid containing the complementary DNA sequence encoding the 52-kDa human VEGF-2 (Vascular Genetics, Inc) was administered via the injection catheter. This expression plasmid is 5283-base pairs in length and was constructed by Human Genome Sciences. Preparation and purification from cultures of phVEGF-2–transformed *Escherichia coli* were performed by the Puresyn PolyFlo method and contained 1.22 mg/mL plasmid DNA in phosphate-buffered saline (20 mmol/L, pH 7.2; containing 0.01% [wt/vol] edetate disodium).

**Percutaneous Catheter-Based Myocardial GTxs**

After the completion of LV EMM, the mapping catheter was replaced by the injection catheter (Biosense-Webster), a modified SF mapping catheter, the distal tip of which incorporates a 27G needle that can be advanced or retracted by 4 to 6 mm. The catheter was flushed with sterile saline for 30 to 45 minutes before injections, thus prefilling the lumen before the introduction of the catheter into the circulation. The injection catheter was then advanced via a femoral arteriotomy across the aortic valve into the left ventricle, and it was manipulated to acquire stable points based on the parameters described above within the target region that had been superimposed on the previously acquired 3D map.

Once a stable point was attained, the needle was advanced 4 to 6 mm into the myocardium; the intracardiac electrogram detected transient myocardial injury and/or premature ventricular contractions as evidence of needle penetration into the myocardium. For patients randomized to GTxs (1:1 randomization with placebo), 6 injections were made into areas of ischemia (suggested by the combination of preserved voltage and abnormal wall motion). Each injection consisted of 1 mL of solution (total volume, 6 mL/patient) delivered from a 1-mL syringe, for a total dose of 200 μg of phVEGF-2. After completion of each injection, the needle was retracted and the catheter was moved to another endocardial site within the zone of ischemia. After the last injection and before needle retraction, the lumen was again flushed with 0.1 mL of sterile saline.

Because percutaneous myocardial GTxs had not been previously performed in human subjects, discussions with the US Food and Drug Administration and the Investigational Review Board of St Elizabeth’s Medical Center resulted in a procedural variation for patients randomized to placebo; in these patients, because no agent with the potential for benefit was to be administered, it was advised and agreed that the needle was not to be extended; the construction of this catheter does not permit injection of fluid if the needle is not extended. In every other respect, however, the procedure was reproduced, including advancing the catheter to 6 different areas and having the operators, as they located the appropriate ischemic sites, mimic the injection process, including instructions directed to the individual operating the work station and audible indications to the patient that an injection was “beginning” or “ending.”

Patients initially randomized to the control group were prospectively designated as eligible for crossover to the GTx arm after 90 days if they failed to demonstrate evidence of clinical improvement and showed no improvement in myocardial perfusion by SPECT-sestamibi scanning or LV NOGA EMM. All patients were blinded throughout the procedure by judicious use of conscious sedation, taped music played through headphones, and the aforementioned attempts by the operator to mimic GTxs in the control patients.

**SPECT Myocardial Perfusion Study**

In addition to LV EMM, subjects underwent a Persantine SPECT-sestamibi study before and after GTxs. The acquisition of the post-stress SPECT image began 10 minutes after the end of the stress period. Redistribution images were recorded before and at least 4 hours after stress with the subject at rest. Perfusion scores were calculated by a blinded observer for each patient based on the Cedars-Sinai 20-segment short-axis system. On day 90, subjects underwent repeat nuclear perfusion testing using the same stress protocol and isotope used at baseline.

**Statistical Analysis**

Data are reported as mean ± SEM. Comparisons between paired variables were performed using a Student’s *t* test with a significance level of *P* < 0.05.

**Results**

**Patients**

Demographic and clinical data for the 6 patients (aged 67 ± 3 years) are shown in the Table.

**LV Mapping Procedure**

Areas of electrically viable myocardium (UpV ≥5 mV) associated with abnormal/impaired wall motion (LLS <12%), ie, electromechanical uncoupling diagnostic of ischemia by the NOGA system, were detected in all patients before GTxs. Foci of ischemia involved the anterior (n = 2), lateral (n = 2), posterolateral (n = 1), and posterior (n = 1) LV walls.
Percutaneous LV GTx

Six patients underwent a total of 36 percutaneous catheter-based myocardial injections; this included 3 patients who crossed over to GTx and 3 who were initially randomized to phVEGF-2 GTx and 3 who based myocardial injections; this included 3 patients who performed in all patients at 90, 180, and 360 days after GTx. Of phVEGF-2–transfected patients, 4 of 6 demonstrated improved exercise duration for up to 360 days after GTx; the increase in exercise duration ranged from 7 to 127 seconds (mean, 72 ± 25 seconds). In the 2 patients in whom exercise duration was not improved, the test was terminated in one because of angina and in the other because of claudication. Of the 3 original control patients, 2 were not improved at 90 days after control assignment; after crossover to phVEGF-2 GTx, both were improved for up to 180 days after GTx. The one original control patient whose exercise test was improved 90 days after control assignment was permitted to crossover to

Continuous ECG monitoring for 24 hours after GTx disclosed no sustained ventricular or atrial arrhythmias. ECGs recorded after GTx showed no evidence of acute myocardial infarction or ischemia in any patient. Creatine kinase-MB levels were not elevated above normal limits in any patient after GTx. There were no major complications, including no echocardiographic evidence of pericardial effusion and/or cardiac tamponade.

Clinical Outcome

Clinically, phVEGF-2–transfected patients reported a reduction in anginal episodes per week (36.2 ± 2.3 versus 3.5 ± 1.2 episodes/week, \(P=0.002\)) and the weekly consumption of nitroglycerin tablets (33.8 ± 2.3 versus 4.1 ± 1.5, \(P=0.002\)) for up to 360 days after GTx. In contrast, although blinded patients randomized to the control group reported an initial reduction in weekly anginal episodes and nitroglycerin consumption, this changed clinical profile was not sustained past 30 days (Figures 1A and 1B). Indeed, by 90 days after treatment assignment, patients in the control group had regressed to values that were not statistically different from baseline values.

Modified Bruce protocol exercise tolerance testing was performed in all patients at 90, 180, and 360 days after GTx. Of phVEGF-2–transfected patients, 4 of 6 demonstrated improved exercise duration for up to 360 days after GTx; the increase in exercise duration ranged from 7 to 127 seconds (mean, 72 ± 25 seconds). In the 2 patients in whom exercise duration was not improved, the test was terminated in one because of angina and in the other because of claudication. Of the 3 original control patients, 2 were not improved at 90 days after control assignment; after crossover to phVEGF-2 GTx, both were improved for up to 180 days after GTx. The one original control patient whose exercise test was improved 90 days after control assignment was permitted to crossover to

No sustained injury pattern was observed during the injections as recorded by the endocardial electrogram.

Figure 1. A, Angina episodes per week at each follow-up time point for phVEGF-2 (n = 6) and control patients (n = 3). Patients initially randomized to control group were eligible for crossover to active agent after 90 days. *\(P<0.05\) vs baseline; †\(P=0.002\) vs baseline. B, Nitroglycerin tablets consumed per week at each follow-up time point for phVEGF-2 (n = 6) and control patients (n = 3). *\(P<0.05\) vs baseline; †\(P=0.002\) vs baseline. C, Area of ischemic myocardium, as based on NOGA LV EMM before and 90 days after myocardial injections of phVEGF-2 or sham procedure (see text for details). *\(P<0.04\) vs baseline; †\(P<0.05\) vs control. D, Perfusion scores calculated for each patient at rest and after pharmacological stress. VEGF-2 GTx resulted in significant improvement in both stress (\(P=0.03\) and rest scores (\(P=0.01\)) after 90 days. In contrast, patients randomized to the mock procedure showed no change in either rest or stress scores.
GTx due to continued angina and persistent ischemia on SPECT-sestamibi scanning and LV NOGA EMM. LVEF was not significantly altered for up to 360 days after GTx. For phVEGF-2–transfected patients, mean LVEF before GTx was 44\pm 6\%; it was 49\pm 7\% after GTx (P=0.07). For control patients, mean LVEF before and after instrumentation was 43\pm 4\% and 47\pm 7\%, respectively (P=0.423).

NOGA Electromechanical Assessment
Mean UpV and bipolar voltage recordings \( \geq 5 \) mV and \( \geq 2 \) mV, respectively, which defined myocardial viability in the ischemic segments, did not change significantly after GTx. Mean LLS in segments of myocardial ischemia, however, improved significantly from 5.3\pm 1.4\% to 12.5\pm 1.4\% (P=0.002) in patients transfected with phVEGF-2 (Figure 2). The area of ischemic myocardium was consequently reduced from 10.2\pm 3.5 cm\(^2\) before GTx to 2.8\pm 1.6 cm\(^2\) after GTx (P=0.04; Figure 1C) in these patients.

In contrast, patients in the control group demonstrated no change in the area of ischemia at 90 days after control assignment (9.9\pm 6.7 versus 9.6\pm 6.3 cm\(^2\) before versus after control), nor was the mean LLS significantly different (ie, LLS remained in the ischemic range) after sham procedures (6.7\pm 2.4\% and 8.1\pm 3.2\%, respectively, before and after control; P=0.342). After crossover to phVEGF-2, these patients demonstrated normalization of LLS in ischemic segments (6.7\pm 2.4\% versus 12.9\pm 1.7\%, P=0.04) and a reduction in ischemic area (9.9\pm 6.7 versus 2.2\pm 0.4 cm\(^2\), P=0.05) after 90 days compared with baseline (Figure 3).

SPECT Myocardial Perfusion Study
The results of EMM corresponded to improved perfusion scores calculated from SPECT-sestamibi myocardial perfusion scans recorded at rest (17.8\pm 2.1 versus 11.5\pm 2.6, before versus after GTx; P=0.01) and in pharmacological stress (22.3\pm 3.0 versus 16.5\pm 2.7, P=0.03) in phVEGF-2-transfected patients (Figures 1D and 4). There was no change in either stress (18.3\pm 5.4 versus 18.0\pm 3.5, P=0.893) or rest (16.3\pm 5.7 versus 15.0\pm 4.0, P=0.659) scores for patients in the control group before and after sham procedures (Figure 5).
Discussion

This is the first report to document percutaneous, catheter-based GTx to the myocardium of human subjects. Preclinical studies from our laboratory9 and from others13 investigated the safety and feasibility of this novel delivery catheter, which was used in conjunction with the Biosense-NOGA mapping system, for percutaneous myocardial GTx. These studies established that catheter-based GTx could be safely and successfully achieved in normal and ischemic porcine myocardium in a relatively site-specific fashion.

Catheter-based techniques for myocardial GTx in human subjects have thus far been restricted to intracoronary infusion of viral vectors encoding for angiogenic growth factors14 and are thus potentially limited by imprecise localization of delivery of the viral vector and possible systemic exposure. Moreover, intravascular delivery of naked DNA is not feasible because of the fact that naked DNA is degraded by circulating nucleases; lacking receptor-mediated uptake mechanisms characteristic of viral vectors, naked DNA GTx leads to insignificant tissue uptake of systemically administered transgene.

In addition, reports of improved clinical outcomes with surgical-based transmyocardial laser revascularization procedures have not been associated with any consistent change in exercise tolerance or improvement in myocardial perfusion, as assessed by radionuclide imaging.15-17 Furthermore, studies involving catheter-based laser revascularization procedures have not reported a sustained clinical response. Recently published data on a nonrandomized study of direct myocardial laser revascularization18 reported a modest increase in exercise duration and reduction in angina class at 6 months, but it failed to show any significant change in perfusion.18

Preclinical studies were also performed specifically to test the feasibility and safety of catheter-based delivery of naked plasmid DNA encoding for VEGF-1.19 Gene expression was documented by transient increase in serum levels of VEGF-1 monitored by ELISA assay (an ELISA assay is currently available for VEGF-1 but not for VEGF-2). Effective GTx was demonstrated by the presence of plasmid DNA in myocardial tissue by polymerase chain reaction. No VEGF protein or plasmid was identified in remote organs. Injections
caused no changes in heart rate, blood pressure, or O2 saturation. No sustained ventricular arrhythmias were observed. There was no ECG evidence of infarction. Objective evidence of reduced ischemia was documented in all VEGF-transfected animals. The mean area of ischemia decreased from 6.1 cm2 at baseline to 0.6 cm2 after GTx in phVEGF-1 animals and from 6.7 to 1.2 cm2 in phVEGF-2–transfected animals. No improvement was seen in the control animals. These findings therefore suggested that percutaneous myocardial injection of VEGF could be safely and reproducibly accomplished in the ischemic myocardium of swine and was associated with reduced evidence of ischemia by NOGA LV mapping.

The current pilot study was undertaken to determine if the encouraging experience with catheter-based myocardial GTx in animal models could be duplicated in patients, particularly with regard to safety. The absence of any adverse procedural outcomes, including ventricular arrhythmias, myocardial infarction, systemic embolization, or ventricular perforation, are encouraging in terms of the safety of the current device. These preliminary results were indeed judged sufficient to permit transition to a subsequent double-blind, placebo-controlled trial of catheter-based myocardial GTx of phVEGF-2; of the 19 patients thus far injected with phVEGF-2 or saline, there have again been no adverse procedural outcomes (unpublished data).

This preliminary experience thus suggests that it is feasible to replace currently employed operative approaches with minimally invasive techniques, whether using the catheter employed here or others under investigation, for applications of cardiovascular gene therapy designed to target myocardial function and perfusion. Such an approach may have at least 3 advantages compared with an operative approach. (1) It potentially allows more selective delivery of the transgene to targeted ischemic zones, including sites that are accessible from a minithoracotomy. (2) The catheter-based approach, because it obviates the need for general anesthesia and operative dissection through adhesions related to the placement of previous bypass conduits, facilitates placebo-controlled, double-blind testing of myocardial GTx. (3) The intervention can be performed as an outpatient procedure and repeated as necessary.

Although the clinical findings of this pilot trial concerning efficacy are similarly encouraging, the number of patients and the single-blinded design preclude firm conclusions in this regard. Subsequent double-blind studies in larger patient cohorts will ultimately resolve this issue.

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References


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