Phase 1/2 Placebo-Controlled, Double-Blind, Dose-Escalating Trial of Myocardial Vascular Endothelial Growth Factor 2 Gene Transfer by Catheter Delivery in Patients With Chronic Myocardial Ischemia

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Background—This phase 1/2 study investigated the safety of percutaneous catheter-based gene transfer of naked plasmid DNA encoding for vascular endothelial growth factor 2 (phVEGF2) to left ventricular (LV) myocardium in a prospective, randomized, double-blind, placebo-controlled, dose-escalating study of inoperable patients with class III or IV angina.

Methods and Results—A steerable deflectable 8F catheter with a 27-gauge needle at its distal tip was advanced percutaneously to the endocardial surface of the LV in 19 patients (age, 61±2 years) with chronic myocardial ischemia who were not candidates for conventional revascularization. Patients were randomized in a double-blind fashion to receive 6 injections (total volume, 6.0 mL) of placebo or phVEGF2 in doses of 200 μg (n=9), 800 μg (n=9), or 2000 μg (n=1) guided by LV electromechanical (NOGA) mapping with a gene-to-placebo ratio of 2:1. A total of 114 LV injections were delivered and caused no hemodynamic alterations, sustained ventricular arrhythmias, ECG evidence of infarction, or ventricular perforation. End-point analysis at 12 weeks disclosed a statistically significant improvement in Canadian Cardiovascular Society (CCS) angina class in phVEGF2-treated versus placebo-treated patients (2.1.3 versus 2.0.1, \( P=0.04 \)). Remaining efficacy end points—including change in exercise duration (91.8 versus 3.9 seconds), functional improvement by ≥2 CCS classes (9 of 12 versus 1 of 6), and Seattle Angina Questionnaire data—all showed strong trends favoring efficacy of phVEGF2 versus placebo treatment.

Conclusions—This phase 1/2, double-blind, randomized trial provides preliminary data that support safety of phVEGF2 catheter-mediated myocardial gene transfer. The statistically significant reduction in anginal class and strong positive trends for remaining end points suggest that a larger phase 2/3 trial is warranted. (Circulation. 2002;105:r75-r81.)

Key Words: growth substances • gene therapy • ischemia • angina • angiogenesis

Phase 1/2 human clinical trials of myocardial gene transfer (GTx) for therapeutic angiogenesis have thus far involved direct intraoperative injection of plasmid DNA or adenoviral vectors encoding angiogenic cytokines directly into ischemic myocardium.1–5 Although these trials demonstrated safety and feasibility in patients with end-stage coronary artery disease, the use of operative thoracotomy precluded randomization against placebo. Nevertheless, objective evidence of improvement in myocardial perfusion after GTx was demonstrated by both single-photon emission CT (SPECT) radionuclide perfusion imaging and left ventricular (LV) electromechanical (NOGA) mapping (EMM).4 Although mini-thoracotomy proved to be generally well tolerated, the risks of general anesthesia and manipulation of preexisting bypass grafts, particularly the left internal mammary artery, limit its initial and repeat applications.

Nonoperative myocardial GTx in human subjects was initiated as catheter-based intracoronary infusion of viral vectors encoding for angiogenic growth factors.6 This method, however, is not effective for so-called naked plasmid DNA (DNA delivered without the use of viral vectors) because plasmid DNA is rapidly degraded in circulating blood. Accordingly, preclinical studies7,8 demonstrated that intramyocardial injections of plasmid DNA by a novel delivery catheter in conjunction with LV (NOGA) EMM could be used to safely perform percutaneous myocardial angiogenesis.
GTx to porcine myocardium. A human pilot study in 6 subjects with refractory myocardial ischemia has subsequently provided early feasibility and safety data for percutaneous myocardial GTx of naked plasmid DNA encoding vascular endothelial growth factor-2 (phVEGF2) to human LV myocardium.9

In the present report, we describe the results of a randomized, double-blind, placebo-controlled, dose-escalating clinical designed to further investigate the safety of percutaneous catheter-based GTx of naked plasmid DNA encoding for phVEGF2 into the LV myocardium of patients with class III or IV angina.

Methods

Patients
Eligibility for catheter-based myocardial GTx included Canadian Cardiovascular Society (CCS) class III or IV angina refractory to maximum medical therapy, multivessel coronary artery disease not suitable for bypass surgery or angioplasty, and reversible ischemia on stress SPECT Tc 99m sestamibi nuclear imaging. Subjects were excluded if they had a previous history or current evidence of malignancy, active diabetic retinopathy, or evidence of severe LV systolic dysfunction (LV ejection fraction [EF] <20% by transthoracic 2D echocardiography). All patients were managed on their maximized medical therapy as required after GTx.

All protocols were approved by the Institutional Review Board and Institutional Biosafety Committee of St Elizabeth’s Medical Center (Boston, Mass) and Scripps Clinic (La Jolla Calif), as well as by the US Food and Drug Administration (FDA, Center for Biologics Evaluation and Research).

LV Electromechanical Mapping
Subjects underwent nonfluoroscopic LV EMM immediately before GTx to guide injections of naked plasmid VEGF2 DNA to foci of ischemic myocardium. The NOGA system (Biosense, Johnson & Johnson) of catheter-based mapping and navigation has been previously described in detail.7,10–12 Follow-up EMM was performed at 12 weeks after GTx. Editing of the raw data was performed by blinded investigators using the NOGA system and postprocessing analysis package.

SPECT Myocardial Perfusion Study
SPECT Tc 99m sestamibi nuclear perfusion studies were performed in all patients before GTx; in 12 patients, including 7 who received phVEGF2, the study was repeated on day 90 after GTx using the identical stress and rest protocol and sequence as well as the same radiopharmaceutical. The remaining 7 patients did not undergo follow-up Tc 99m sestamibi scans after the protocol had been placed on clinical hold.

The poststress images were acquired ~10 minutes after the conclusion of the dipyridamole infusion. Rest imaging was acquired before the performance of the dipyridamole stress test. The unprocessed image data were submitted to a core laboratory, where the images were reconstructed and reoriented in a uniform fashion in the standard 3 projections (short-axis, vertical, and longitudinal long-axis) for analysis. The SPECT images were then visually interpreted, blinded to clinical data and timing of study, with the use of a semi-quantitative 20-segment model. Perfusion scores (0 to 4, normal to absent activity) were calculated for each patient on the basis of the Cedars-Sinai 20-segment short-axis system.13 Summed stress (SSS) summed rest (SRS) scores were determined by adding all segments scores for the stress and rest studies respectively. The sum difference score reflects the amount of myocardial ischemia and was determined by the subtraction of the SRS from the SSS.

Plasmid DNA
The phVEGF2 plasmid containing the complementary DNA sequence encoding the 52-kDa human VEGF2 (Vascular Genetics Inc) was administered via the injection catheter (vide infra). This expression plasmid is 5283 bp in length and was constructed by Human Genome Sciences. Preparation and purification from cultures of phVEGF2-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% [w/v] edetate disodium).

Randomization and Dose Allocation
Patients were randomized with a gene-to-placebo ratio of 2:1 (phVEGF2 or saline) in 3 escalating-dose groups: 200, 800, and 2000 µg. The study design prescribed 9 patients for each dose cohort (6 phVEGF2 and 3 placebo patients for each dose) and, thus, 18 phVEGF2 versus 9 placebo subjects by study completion.

Percutaneous Catheter-Based Myocardial Injection
After completion of LV EMM, the mapping catheter was replaced by the injection catheter (Biosense-Webster), a modified 8F mapping catheter, the distal tip of which incorporates a 27-gauge 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 introduction of the catheter into the circulation. The injection catheter was then advanced retrograde via an 8F femoral arterial introducer sheath, across the aortic valve into the LV, and 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 NOGA point was attained, the needle was advanced 4 to 6 mm into the myocardium; myocardial penetration was confirmed by transient myocardial injury by the intracardiac electrogram recorded from the catheter tip and/or premature ventricular contractions at the time of needle advancement. In each patient, a total of 6 injections were performed into foci of myocardial ischemia identified as areas of preserved unipolar voltage and abnormal wall motion via EMM (NOGA) mapping. Each injection consisted of 1 mL of solution (total volume, 6 mL/patient), delivered from a 1-mL syringe, of placebo (saline) or phVEGF2 in doses of 200, 800, or 2000 µg. After completion of each injection, the needle was retracted, the catheter manipulated to another endocardial site within the zone of ischemia, and a new syringe was used to perform an additional injection. After the final injection and before needle retraction, the lumen was again flushed with 0.1 mL of sterile saline.

Clinical Outcomes
The prespecified primary efficacy parameters were change from baseline in CCS angina classification and exercise tolerance at the 12-week follow-up visit.

All patients were observed for 24 hours after the procedure in the coronary care unit. Serial myocardial isoenzymes (CPK-MB) were measured for the first 24 hours. Serial ECGs and standard transthoracic echocardiograms were performed preoperatively, within 24 hours after the procedure, and at each follow-up visit (weeks 2, 4, 8, 12).

At each postoperative visit, Seattle Angina Questionnaires were completed, weekly number of angina episodes and nitroglycerin consumption was documented, and functional assessment based on CCS classification14 was performed by investigators blinded to treatment assignment. Patients were also evaluated specifically for evidence of peripheral edema and congestive heart failure. Exercise treadmill testing was performed at baseline and at week 12 using a modified Bruce protocol.15

Major complications were defined as acute myocardial infarction (CPK-MB >2× normal with or without associated electrocardiographic changes), ventricular perforation, sustained ventricular arrhythmias requiring institution of pharmacologic or mechanical therapy, stroke, or death.
Clinical Outcomes
The primary efficacy end point for this clinical trial was CCS angina class status. All patients were classified as CCS class 3 or 4 anginas before randomization. At 12-week follow-up, mean CCS class was decreased (ie, improved) significantly in phVEGF2-transfected patients (pre-GTx 3.5±0.2 versus post-GTx 2.2±0.4, P=0.012) (Figure 1A); 4 of 12 patients improved by ≥2 classes, 4 of 12 patients improved by ≥3 classes, and 1 of 12 reported elimination of angina (Figure 1B). In contrast, mean CCS class did not change significantly for placebo patients (preinjection 3.3±0.2 versus postinjection 3.1±0.3; P=0.6), whereas 1 of 7 reported elimination of angina; none of the remaining 6 placebo patients improved by >1 functional class. Comparison of functional class at 12 weeks disclosed a statistically significant mean change in CCS angina class for phVEGF2 compared with placebo (−1.3 versus 0.1, P=0.04) (Figure 1C).

Clinically, phVEGF2-transfected patients reported a reduction in anginal episodes per week (23.7±4.9 versus 10.3±5.7, P=0.04). In contrast, the reduction reported in placebo patients was not significant (20.4±4.7 versus 12.1±6.3, P=0.32). Weekly consumption of nitroglycerin tablets was reduced in both groups at 12 weeks post-GTxs, but failed to achieve statistical significance (16.6±5.4 versus 8.8±5.8, P=0.26, for phVEGF2-transfected patients; 18.1±6.4 versus 9.9±6.6, P=0.35, for placebo patients). Seattle Angina Questionnaire end points showed trends favoring efficacy of phVEGF2 versus placebo treatment (Figure 1D), although these findings did not reach statistical significance.

Comparison of clinical end points between phVEGF2-transfected patients (dose 200 µg, n=6; dose 800 µg, n=6) disclosed no significant differences for functional class
(P=0.71), weekly angina episodes (P=0.41), or nitrate tablets consumption (P=0.26).

LVEF, determined by transthoracic 2D echocardiography, was not significantly altered at week 12 follow-up. For phVEGF2-transfected patients, LVEF before GTx was 49.9 \pm 14\% versus 48.6 \pm 12\% for after GTx (mean \pm SD, P=NS); for control patients, mean LVEF before and after injection was 50.4 \pm 13\% versus 54 \pm 13\% (P=NS).

**Exercise Treadmill Testing**

Modified Bruce protocol exercise tolerance testing was performed in all patients. The mean duration of exercise increased significantly at 12-week follow-up in phVEGF2-transfected patients (479.0 \pm 51.5 versus 607.3 \pm 52.4 seconds, P=0.02) but was unchanged in patients randomized to placebo (426.4 \pm 103.4 versus 432.0 \pm 116.3 seconds, P=0.92) (Figure 2A). The mean increase in exercise duration for the phVEGF2 cohort after randomization (91.8 seconds) was nearly 1.5 minutes greater than that of the placebo group (3.9 seconds, P=0.26 for comparison of active versus placebo, Figure 2B).

**Electromechanical Mapping**

LV EMM (Figures 3 and 4) was available for analysis in 15 patients. Mean unipolar and bipolar voltage recordings $5 \text{mV}$ and $2 \text{mV}$, respectively, defining myocardial viability in the ischemic segments, did not change significantly after GTx. Mean LLS in segments of myocardial ischemia, however, improved significantly from 5.9 \pm 1.0\% to 13.2 \pm 1.3\% (P=0.004) in patients transfected with phVEGF2. The area of ischemic myocardium was consequently reduced from 6.4 \pm 2.2 cm$^2$ (before GTx) to 2.6 \pm 1.4 cm$^2$ (after GTx) (P=0.013 in these patients).

In contrast, patients in the control group demonstrated no change in the area of ischemia at 90 days after control assignment (5.7 \pm 2.1 cm$^2$ preinjection versus 5.4 \pm 1.8 cm$^2$ postcontrol, P=0.39), nor was the mean LLS significantly different (ie, LLS remained in the ischemic range) after saline injections (7.5 \pm 0.7 cm$^2$ postinjection) to 10.2 \pm 1.1\% (postinjection, P=0.11).

**SPECT Myocardial Perfusion Study**

SPECT myocardial perfusion imaging data at baseline and follow-up (Figures 3 and 4) were available for 11 patients,
including 7 who received phVEGF2; the remaining patients
did not undergo follow-up SPECT scans after the protocol
had been placed on clinical hold. Of the 7 patients who
received phVEGF2, 5 showed improvement in SSS and 1
showed no change. A trend for improvement (decrease) in
SSS was observed after GTx (baseline 11.4 ± 6.2 versus 4
weeks 8.8 ± 5.0, P = 0.074), representing a 23% improvement
in perfusion score. A similar trend was noted in the summed
difference score (baseline 6.8 ± 5.1 versus 4 weeks 4.0 ± 1.5,
P = 0.118), constituting a 42% improvement owing to im-
provement observed in 5 of 7 patients who received ph-
VEGF2. The placebo patients demonstrated a nonsignificant
increase in the SSS, with only 1 patient demonstrating a
decline in the score. Similar findings were apparent for the
placebo group when examining the summed difference score.
An analysis confined to only those segments that were
abnormally perfused at baseline demonstrated a significant
improvement in the SSS for the GTx patients (baseline
1.65 ± 0.44 versus 4 weeks 1.07 ± 0.50, P = 0.016); no change
was noted for the placebo group.

Complications
There were no acute complications from any of the 114
catheter injections performed in this study. During the
follow-up period after GTx, 1 patient who had received a
placebo injection suffered a cerebrovascular accident and
myocardial infarction 4 weeks after procedure. A second
patient who received placebo experienced worsening of his
anginal symptoms. Although the approved FDA Investiga-
tional New Drug application provided crossover to active
treatment in the event of clinical deterioration, this option was
precluded by the clinical hold and permission for compas-
sionate use could not be obtained; this patient consequently
underwent percutaneous transluminal coronary angioplasty
and stent treatment of a saphenous vein bypass graft to the
right coronary artery with no subsequent change in his
functional (CCS 3) status. A third placebo patient experienced a transient (cerebrovascular) ischemic attack 3 weeks after the procedure.

Among patients who received phVEGF2, 1 patient with a history of mild aortic stenosis, heart failure, and sleep apnea failed to improve after 800 μg phVEGF2; this patient ultimately underwent PTCA at another institution without success 1 year after GTx and was subsequently hospitalized on multiple occasions for unstable angina and pneumonia complicated by myocardial infarction. A second patient with underlying heart failure and an LVEF of 25% initially experienced improvement in his anginal symptoms after 200 μg phVEGF2 GTx, including an increase in exercise treadmill time from 3:54 to 5:27 minutes. Progression of the patient’s heart failure, however, led to cardiac transplantation 9 months after GTx.

**Discussion**

This randomized, double-blind, placebo-controlled, dose-escalating clinical trial was designed to further investigate the safety of percutaneous catheter-based GTx of naked plasmid DNA encoding for phVEGF2 into the LV myocardium of patients with class III or IV angina. A total of 114 LV injections were delivered and caused no hemodynamic alterations, sustained ventricular arrhythmias, ECG evidence of infarction, or ventricular perforation. Comparison of efficacy end points at 12 weeks disclosed a statistically significant change in CCS angina class, which decreased by 1.3 in phVEGF2-treated patients versus 0.1 in placebo-treated patients (P=0.04). Remaining efficacy end points—including change in exercise duration (91.8 versus 3.9 seconds), functional improvement by ≥2 CCS classes (9 of 12 versus 1 of 7), and Seattle Angina Questionnaire data—all showed strong trends favoring efficacy of phVEGF2 versus placebo treatment.

Intramyocardial injection is required in the case of naked plasmid DNA, because the plasmid would undergo prompt degradation were it to be delivered into circulating blood. This is in direct contrast to viral vectors, which are not subject to similar intravascular degradation and may thus be delivered via an intracoronary route. The strategy of direct intramyocardial injection is designed to shield naked DNA for effective GTx and to avoid potential toxicities that might result from the use of viral vectors. Moreover, because there is a lower risk of provoking an immune response to naked DNA than a viral vector, the combined use of naked DNA and catheter delivery preserves the option for repetitive administration, should that be proven effective. Although naked DNA GTx is less efficient than viral GTx, previous work has demonstrated that genes encoding proteins that are naturally secreted from intact cells, such as VEGF, can achieve meaningful biological outcomes despite low transfection efficiency. Our positive experience in the present study with 144 injections in 19 patients adds to previously reported positive experience involving 36 injections performed with the same catheter system in 6 patients and in other preclinical studies. The cumulative absence of any acute adverse events with this catheter system is encouraging for percutaneous myocardial injection as a safe vehicle for myocardial GTx.

Previous reports of subjective and objective improvement after VEGF GTx have been limited to dose-escalating trials involving consecutive-treated, nonrandomized patients with critical limb or myocardial ischemia. In each of these studies, interpretation of symptomatic outcome in particular was complicated by the lack of a placebo-treated control group. Inclusion of a control group was recently facilitated by the advent of catheter-based GTx, which minimized the ethical dilemma of blinded placebo controls whereas a previous 6-patient pilot trial using this approach showed reduction in anginal symptoms and nitroglycerin use in VEGF2 versus placebo patients. This trial was limited because it was a single-blind design in which patients randomized to the control arm underwent a mock procedure (the catheter was advanced to the LV, but no injection was performed).

In this regard, the current double-blind, placebo-controlled results are encouraging and consistent with the previous nonrandomized or single-blind results of myocardial GTx. Despite the small size of the patient cohort, it was nevertheless possible to demonstrate a statistically significant improvement in CCS anginal classification. Although the size of this initial cohort did not permit the changes observed in exercise testing to reach statistical significance, the increase in treadmill time after VEGF2 GTx (91.8 seconds) far exceeded that observed for control subjects (3.9 seconds) and was similar to or in excess of what has been previously described for patients receiving laser myocardial revascularization or continued medical therapy in 5 contemporary controlled studies.

The possibility that improvement in symptomatic status documented in the current and previous trials of myocardial VEGF2 GTx constitutes evidence of bioactivity is further supported by objective evidence in these trials of enhanced myocardial perfusion. In patients undergoing intraoperative, direct myocardial injection of VEGF naked DNA, for example, stress SPECT Tc 99m sestamibi myocardial imaging disclosed that mean perfusion-defect scores for both stress and rest images were significantly decreased (ie, improved) at day 60. It is particularly intriguing to note that not only defects observed in the perfusion scans with pharmacologic stress, but also those observed at rest, improved after GTx. This observation suggests that these pretest defects constitute foci of hibernating myocardium rather than myocardial scar and may have been successfully resuscitated as the result of therapeutic neovascularization.

Rescue of foci of hibernating myocardium after VEGF GTx was subsequently confirmed in a subset of 13 consecutive patients by the use of catheter-based EMM, shown to distinguish among infarcted, ischemic, and normal myocardium. Moreover, the identical LV anatomic site that was observed to be improved by EMM was consistently observed to be improved by SPECT Tc 99m sestamibi myocardial perfusion imaging as well. Initial experience with catheter-based GTx in the single-blind study referred to above confirmed these findings by demonstrating reproducibility of the SPECT and EMM maps from baseline to post-mock.
procedure, followed by site-specific improvement in both SPECT and EMM 90 days after the patient crossed over to VEGF2 GTx. The SPECT and EMM findings showing evidence of improved perfusion in the current trial are thus consistent with these previous studies of VEGF GTx.

This preliminary experience suggests that it is feasible to replace currently employed operative approaches with minimally invasive techniques for applications of cardiovascular gene therapy designed to target myocardial function and perfusion. Such an approach may have at least 3 advantages compared with those of an operative approach. First, it potentially allows more selective delivery of the transgene to targeted ischemic zones, including sites that are less accessible by a mini-thoracotomy. Second, the catheter-based approach, because it obviates the need for general anesthesia and operative dissection through adhesions related to placement of previous bypass conduits, facilitates placebo-controlled, double-blind testing of myocardial GTx. Third, the intervention can be performed as an outpatient procedure and repeated if necessary. These findings thus indicate that further similarly designed studies to establish definitive evidence of safety and efficacy of the approach used here in a larger patient cohort are merited.

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References

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