Gene Therapy for Myocardial Angiogenesis
Initial Clinical Results With Direct Myocardial Injection of phVEGF_{165} as Sole Therapy for Myocardial Ischemia

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Background—We initiated a phase 1 clinical study to determine the safety and bioactivity of direct myocardial gene transfer of vascular endothelial growth factor (VEGF) as sole therapy for patients with symptomatic myocardial ischemia.

Methods and Results—VEGF gene transfer (GTx) was performed in 5 patients (all male, ages 53 to 71) who had failed conventional therapy; these men had angina (determined by angiographically documented coronary artery disease). Naked plasmid DNA encoding VEGF (phVEGF_{165}) was injected directly into the ischemic myocardium via a mini left anterior thoracotomy. Injections caused no changes in heart rate (pre-GTx 575 ± 615/min versus post-GTx 580 ± 616/min, \( P = \text{NS} \)), systolic BP (114 ± 7 versus 118 ± 7 mm Hg, \( P = \text{NS} \)), or diastolic BP (57 ± 2 versus 59 ± 2 mm Hg, \( P = \text{NS} \)). Ventricular arrhythmias were limited to single unifocal premature beats at the moment of injection. Serial ECGs showed no evidence of new myocardial infarction in any patient. Intraoperative blood loss was 0 to 50 cm³, and total chest tube drainage was 110 to 395 cm³. Postoperative cardiac output fell transiently but increased within 24 hours (preanesthesia 54.8 ± 0.4 versus postanesthesia 56.3 ± 0.8, \( P = 0.02 \)). Time to extubation after closure was 18.4 ± 1.4 minutes; average postoperative hospital stay was 3.8 days. All patients had significant reduction in angina (nitroglycerin [NTG] use 553.9 ± 10.0/wk pre-GTx versus 9.8 ± 6.9/wk post-GTx, \( P < 0.03 \)). Postoperative left ventricular ejection fraction (LVEF) was either unchanged (n = 3) or improved (n = 2, mean increase in LVEF 5%). Objective evidence of reduced ischemia was documented using dobutamine single photon emission computed tomography (SPECT)-sestamibi imaging in all patients. Coronary angiography showed improved Rentrop score in 5 of 5 patients.

Conclusions—This initial experience with naked gene transfer as sole therapy for myocardial ischemia suggests that direct myocardial injection of naked plasmid DNA, via a minimally invasive chest wall incision, is safe and may lead to reduced symptoms and improved myocardial perfusion in selected patients with chronic myocardial ischemia.

Key Words: angiogenesis ■ ischemia ■ myocardium

Intramuscular transfection of genes encoding angiogenic cytokines may constitute an alternative treatment strategy for patients with severe myocardial ischemia. This strategy is designed to promote the development of supplemental collateral blood vessels that will constitute endogenous bypass conduits around occluded native arteries, a strategy termed “therapeutic angiogenesis.”

This study describes the initial clinical experience with myocardial gene transfer as sole therapy for refractory angina pectoris. Five patients with chronic, severe angina underwent direct myocardial gene transfer of naked DNA encoding vascular endothelial growth factor (VEGF). There were no operative complications. All patients experienced marked symptomatic improvement and/or objective evidence of improved myocardial perfusion. This preliminary clinical experience suggests that therapeutic angiogenesis represents a potentially useful strategy for patients with coronary artery disease.

Patients

Patients were eligible for intramyocardial gene therapy if they had functional class 3 or 4 exertional angina, refractory to maximum medical therapy, areas of viable but underperfused myocardium, and multivessel occlusive coronary artery disease. Subjects were excluded if they had any of the following: a successful revascularization-

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tion within the previous 6 months, cancer, retinopathy, or an ejection fraction (EF) <20%.

Plasmid DNA (phVEGF165)
All patients received eukaryotic expression vector encoding the 165-amino acid isoform of the human VEGF gene (previously described)\(^3,4\) transcriptionally regulated by the cytomegalovirus promoter/enhancer (phVEGF\(_{165}\)).\(^5,6\)

Myocardial phVEGF\(_{165}\) Transfer
Plasmid DNA (125 \(\mu\)g) was administered by direct myocardial injection in 4 aliquots of 2.0 mL each via a mini-thoracotomy to the anterolateral left ventricular free wall. Continuous transesophageal echocardiographic monitoring was performed throughout the procedure. Patients were extubated in the operating room and monitored according to the protocol used for minimally invasive CABG.

SPECT Myocardial Perfusion Study
Subjects underwent a dobutamine single photon emission computed tomography (SPECT)-sestamibi study 1 month before and 60 days after gene transfer, with the use of dobutamine infusion up to 40 \(\mu\)g \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\). The acquisition of the poststress SPECT image began 10 minutes after the end of the stress period. Redistribution images were recorded either before or at least 4 hours after stress with the subject at rest. Redistribution and reinjection data were reconstructed in short-axis, vertical, and longitudinal long-axis views for analysis. With the use of the 13-segment model, viability and perfusion scores were assigned to each segment on the basis of the results of the nuclear scan. Perfusion was recorded as normal or abnormal. Segments were visually characterized as fixed, partially reversible, or totally reversible. On days 30 and 60, subjects underwent repeat nuclear perfusion testing using the identical stress protocol and isotope used at baseline.

Coronary Angiography
Patients underwent diagnostic angiography <1 month before and 60 days after gene transfer. All angiograms were interpreted by a reviewer blinded to the patient’s name, date of study, and sequence of study (ie, pre- versus posttreatment). Collaterals were graded\(^7\) as absent (0); filling of side-branches of a target-occluded epicardial coronary artery via collaterals without visualization of the epicardial coronary artery itself (1+); partial filling of the epicardial segment via collateral arteries (2+); and complete filling of the epicardial segment (3+). Each pair of films (baseline and follow-up) was scored independently.

Statistical Analysis
Data are reported as mean±SEM. Comparisons between paired variables were performed using a Student \(t\) test with a significance level of \(P<0.05\).

Results

Patients
Demographic and clinical data for the 5 men (aged 63.8±3.4 years) treated with phVEGF\(_{165}\) are shown in Table 1.

Perioperative Course
All patients underwent successful myocardial gene transfer. Mean operative time was 101.6±8.9 minutes. Patients were extubated 18.4±1.4 minutes postoperatively. Injections caused no changes in heart rate (75±15/min versus 80±16/min), systolic blood pressure (114±7 versus 118±2 mm Hg), or diastolic BP (57±2 versus 59±2 mm Hg). Ventricular arrhythmias were limited to unifocal extrasystolic beats (maximum \(n=5\)) at the moment of injection. Postoperative Cardiac output fell transiently but increased within 24 hours (preanesthesia=4.8±0.4 versus postanesthesia=4.1±0.3 versus 24 hours postoperative=6.3±0.8, \(P=0.02\)). Serial ECGs showed no evidence of myocardial infarction in any patient; no patient had an increase in creatine kinase isoenzyme above normal limits. Intraoperative blood loss was 5 to 50 cm\(^3\), and total chest tube drainage was 110 to 395 cm\(^3\). There were no major perioperative complications. Postoperative LVEF was either unchanged (\(n=3\)) or improved (\(n=2\), mean increase in LVEF=5%). All patients were discharged on postoperative day 4 except patient 2 who was discharged on postoperative day 3.

Change in Clinical Status
All 5 patients experienced a decrease in anginal frequency and severity (Table 1). There was no change in the anginal

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>DM</th>
<th>Prior Revascularization</th>
<th>CCS FC</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>67</td>
<td>0</td>
<td>CABG 1988; PTCA 1997</td>
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<tr>
<td>4</td>
<td>71</td>
<td>0</td>
<td>CABG 1982, 1984; PTCA 1992</td>
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<tr>
<td>5</td>
<td>59</td>
<td>+</td>
<td>CABG 1994; PTCA 1996, 1997</td>
<td>4</td>
</tr>
</tbody>
</table>

Pre- and Post- refer, respectively, to status of gene therapy.

ACE indicates angiotensin converting enzyme inhibitor; ASA, aspirin; BB, beta-blocker; CABG, coronary artery bypass graft; CCB, calcium channel blocker; CCS, Canadian Cardiovascular Study; D, diuretic; DM, diabetes; FC, functional class; N, nitrates; PTCA, percutaneous coronary angioplasty including balloon angioplasty, stent, directional and rotational atherectomy.

### TABLE 1. Demographic and Clinical Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>DM</th>
<th>Prior Revascularization</th>
<th>Medications</th>
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<td>ASA, BB, N</td>
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<tr>
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<td>69</td>
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<td>ASA, BB, CCB, D, N</td>
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<td>3</td>
<td>53</td>
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<td>PTCA 1989; CABG 1991, 1992</td>
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<td>CABG 1982, 1984; PTCA 1992</td>
<td>ASA, BB, D, N</td>
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<tr>
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<td>59</td>
<td>+</td>
<td>CABG 1994; PTCA 1996, 1997</td>
<td>ACE, ASA, BB, D, N</td>
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</tbody>
</table>

### TABLE 2. Perfusion Scan Results

<table>
<thead>
<tr>
<th>Normal Perfusion</th>
<th>Reversible Defect</th>
<th>Fixed Defect</th>
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</table>

Base indicates baseline; D30, 30-day follow-up study; D60, 60-day follow-up study.
pattern in any patient up to 10 days post–gene transfer. All patients began to experience a reduction in angina between 10 and 30 days after gene transfer. Angina was completely abolished in 2 patients (patients 1 and 4); patient 5, who has previously experienced daily angina, had only 2 episodes of angina between the day 30 and day 60 follow-up visits. Patients 2 and 3 continued to experience occasional angina but with reduced frequency and at much higher levels of activity. Nitroglycerin (NTG) use for the group of 5 patients decreased from 7.7±1.4 to 1.4±1.0 tablets per day by 60 days post–gene transfer (P<0.05). Brief synopses of the clinical courses of these 5 patients are provided below.

Patient 1, a 67-year-old man, experienced daily angina induced by mild activity requiring an average of 8 tablets NTG/d. All native vessels and 3 of 4 bypass grafts were occluded. Several institutions had advised the patient that the small caliber of his remaining native vessels precluded repeat CABG. Beginning 21 days after gene transfer, the patient experienced a decrease in the frequency and severity of his angina. By postoperative day 60, the patient was no longer experiencing angina and was no longer requiring NTG. He was able to engage in activities, such as swimming, which were previously impossible because of anginal pain.

Patient 2, a 69-year-old man, experienced daily angina precipitated by activity such as walking 10 yards; for several months he had been taking 12 tablets NTG/d. A vein graft to the left obtuse marginal (LOM) was occluded, and a diffusely diseased vein graft to a diagonal branch of the left anterior descending (LAD) coronary artery was not amenable to percutaneous revascularization. Additional surgery was not feasible because of poor target vessels. For 3 weeks after gene transfer, his symptoms remained unchanged. The patient then began to notice a decrease in NTG consumption accompanied by the ability to increase his level of activity. By day 60, the patient was able to exercise on the bicycle at his local gymnasium for up to 30 minutes. The patient’s NTG requirement decreased to a maximum of 2 tablets/d for occasional episodes of mild angina.

Patient 3, a 53-year-old man, experienced daily angina induced by walking ≤50 yards and used 6 NTG tablets/d. All native vessels were occluded; grafts to the LAD and right coronary artery (RCA) were patent, whereas an LOM graft was occluded. Percutaneous revascularization was not possible and a third bypass operation for single vessel bypass to a small-caliber target vessel was not feasible. The patient

<table>
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<tr>
<th>Patient</th>
<th>Extramural Vessel</th>
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<th>Post-GTx</th>
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<tr>
<td>2</td>
<td>OMB via SVG</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>RCA via LCX</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Diag via LAD via LIMA</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Diag via LAD via LIMA</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

RCA indicates right coronary artery; LAD, left anterior descending; Diag, diagonal; SVG, saphenous vein graft; OMB, obtuse marginal branch; PDA, posterior descending artery; LIMA, left internal mammary artery; and LCX, left circumflex.

Figure 1. SPECT-sestamibi perfusion imaging. Top, Example of improvement in a “fixed” defect (perfusion abnormality on resting image). In patient (Pt.) 4, a moderate area of decreased perfusion is seen in the infero-lateral wall (arrow) before gene therapy. After gene therapy, perfusion is improved. Bottom, Example of improvement in an area of ischemia. In Pt. 2, a small zone of decreased perfusion is seen in the inferior wall (arrow) before treatment. After treatment, the matching scan shows no evidence of this perfusion defect while a zone of ischemia on the anterior (opposite) wall persists.
Patient 4, a 71-year-old man, complained of daily angina precipitated by walking 10 to 20 yards, also required continuous oxygen use of supplemental oxygen had decreased. At day 60 follow-up, he reported a total of 2 anginal episodes in the previous month, each of which was resolved with a single NTG tablet.

**SPECT-Sestamibi Perfusion Imaging**

All patients had improvement in myocardial perfusion, revealed by comparison between pre- and posttreatment (Figure 1) SPECT-sestamibi imaging (Table 2). The mean number of normally perfused segments per patient increased from $6.0 \pm 1.1$ before gene transfer to $8.0 \pm 0.7$ ($P<0.05$) at day 60 after gene transfer (Figure 2). This was accompanied by a decrease in the mean number of irreversibly ischemic segments from $2.4 \pm 0.2$ to $1.2 \pm 0.4$ ($P<0.05$) at day 60 follow-up examination (Figure 2).

**Coronary Angiography**

Selective coronary angiography was performed before and 59.8±1.5 days after gene transfer (Table 3). Angiographic evidence for improved collateral flow into ischemic areas of the myocardium was observed in all 5 patients. The evidence of new collateral vessels consisted of improved filling of 4 previously identified vessels as well as the development of collaterals to 3 vessels which previously had no collateral filling. In 2 patients, there was improvement by a single Rentrop grade in one vessel territory; the other 3 patients demonstrated improvement in 2 territories by 1 to 3 Rentrop grades.

**Discussion**

The finding that VEGF could be used to achieve angiogenesis that was therapeutic was first demonstrated by Takeshita et al, who administered rhVEGF as a single intra-arterial bolus to rabbits with unilateral hindlimb ischemia. Similar findings with rhVEGF administration in canine and porcine models of myocardial ischemia were published shortly thereafter.

Gene transfer constitutes an alternative strategy for accomplishing therapeutic angiogenesis in patients with limb and myocardial ischemia. In VEGF, this is a particularly appealing strategy because the VEGF gene encodes a signal sequence which permits the protein to be naturally secreted from intact cells. Previous studies from our laboratory indicated that arterial gene transfer of cDNA encoding for a secreted protein could yield meaningful biological outcomes despite a low transfection efficiency. Indeed, preclinical animal studies established the feasibility of achieving therapeutic angiogenesis after site-specific gene transfer of naked DNA encoding VEGF121, VEGF165, and VEGF189. Subsequent clinical experience documented histological and angiographic evidence of phVEGF165-induced neovascularization in patients with critical limb ischemia. These findings established proof of principle for the concept that the angiogenic activity of VEGF is sufficiently potent to achieve therapeutic benefit.
The present study provides the first evidence for a favorable clinical effect of direct myocardial injection of naked plasmid DNA encoding for VEGF. Each patient experienced a reduction in anginal symptoms and nitrate use, and there is objective evidence for reduced ischemia by perfusion imaging. Because each patient enrolled in this study had long-standing, stable, severe angina, the change in clinical status observed for these 5 patients is unlikely to represent random chance. In contrast to work recently reported by Schumacher et al, in which administration of fibroblast growth factor-1 (FGF-1) was combined with conventional surgical revascularization, the present study used VEGF gene transfer as the sole therapeutic intervention.

This early experience, although encouraging from the standpoint of therapeutic angiogenesis and gene therapy, leaves several issues unresolved. Optimizing the anatomic site, number, and dose of intramyocardial injections will require further investigation. The FDA, Recombinant Advisory Committee of the NIH, and St. Elizabeth Medical Center Human Investigation Research and Institutional Biosafety Committees all concurred that the strategy of gene therapy alone administered via a mini-thoracotomy would not permit randomization against placebo (untreated controls). We anticipate that incorporation of a placebo group and clinical testing of alternative dosing regimens, including multiple treatments, will be addressed on availability of a catheter-based system for reliable percutaneous myocardial gene delivery; this is currently under preclinical investigation.

Furthermore, the choice of appropriate formulation or vector in the case of VEGF remains to be determined. As indicated above, rhVEGF protein has been shown to be efficacious for treatment of limb and myocardial ischemia in preclinical studies, and preliminary clinical investigation of rhVEGF together with the aforementioned studies of Schumacher et al. have suggested the potential usefulness of recombinant protein for therapeutic angiogenesis. The use of an adenoviral vector expressing VEGF121 has been shown to improve myocardial perfusion and function in a swine model of myocardial ischemia and is now being tested in human subjects. Likewise, alternatives to VEGF, including FGF-1, FGF-2, and FGF-5 are or will be investigated as genes or recombinant proteins in clinical trials of therapeutic angiogenesis.

References

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