Angiogenesis Gene Therapy

Phase I Assessment of Direct Intramyocardial Administration of an Adenovirus Vector Expressing VEGF121 cDNA to Individuals With Clinically Significant Severe Coronary Artery Disease

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Background—Therapeutic angiogenesis, a new experimental strategy for the treatment of vascular insufficiency, uses the administration of mediators known to induce vascular development in embryogenesis to induce neovascularization of ischemic adult tissues. This report summarizes a phase I clinical experience with a gene-therapy strategy that used an E1−E3− adenovirus (Ad) gene-transfer vector expressing human vascular endothelial growth factor (VEGF) 121 cDNA (AdGVVEGF121.10) to induce therapeutic angiogenesis in the myocardium of individuals with clinically significant coronary artery disease.

Methods and Results—AdGVVEGF121.10 was administered to 21 individuals by direct myocardial injection into an area of reversible ischemia either as an adjunct to conventional coronary artery bypass grafting (group A, n = 15) or as sole therapy via a minithoracotomy (group B, n = 6). There was no evidence of systemic or cardiac-related adverse events related to vector administration. In both groups, coronary angiography and stress sestamibi scan assessment of wall motion 30 days after therapy suggested improvement in the area of vector administration. All patients reported improvement in angina class after therapy. In group B, in which gene transfer was the only therapy, treadmill exercise assessment suggested improvement in most individuals.

Conclusions—The data are consistent with the concept that direct myocardial administration of AdGVVEGF121.10 to individuals with clinically significant coronary artery disease appears to be well tolerated, and initiation of phase II evaluation of this therapy is warranted. (Circulation. 1999;100:468-474.)

Key Words: angiogenesis ■ gene therapy ■ genetics ■ coronary disease ■ ischemia

A new experimental strategy for treating myocardial ischemia is to induce neovascularization of the heart by use of “angiogens,” mediators that induce the formation of blood vessels.1,2 This approach is based on the knowledge that in the adult heart, the genes coding for angiogens and their receptors are expressed in low levels, apparently insufficient in most individuals to provide robust formation of collaterals in response to chronic ischemia.3,4

Vascular endothelial growth factor (VEGF), a protein coded by a 7-exon gene localized on chromosome 6, serves as a major angiogen in normal cardiac development.5 The VEGF gene is normally spliced into 4 different forms; of these, VEGF121 (containing 121 amino acids) and VEGF165 (165 amino acids) appear to be the most important. The VEGF proteins function by interacting with specific receptors on endothelial cells, which initiates a cascade of events culminating in endothelial cell migration, proliferation, aggregation into tubelike structures, and networking of the arterial and venous systems.5,5–8

Gene transfer represents one approach to delivering an angiogen to the heart in which the cDNA coding for VEGF is delivered to the myocardium, with the myocardial cells used to secrete the VEGF.8–10 Studies in experimental animals have shown that replication-deficient, recombinant adenovi-
TABLE 1. Demographics and Intraoperative and Postoperative Characteristics of the Study Population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A</th>
<th>Group B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjunct to CABG</td>
<td>Sole Therapy/Minithoracotomy</td>
</tr>
<tr>
<td>n</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>Male/female, n</td>
<td>11/4</td>
<td>4/2</td>
</tr>
<tr>
<td>Age, y</td>
<td>60±10 (45–83)</td>
<td>59±11 (40–73)</td>
</tr>
<tr>
<td>Ejection fraction, %†</td>
<td>45±10 (26–63)</td>
<td>34±12 (20–50)</td>
</tr>
<tr>
<td>Prior MI, %</td>
<td>60</td>
<td>83</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>60</td>
<td>50</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>93</td>
<td>66</td>
</tr>
<tr>
<td>History of CHF, %</td>
<td>27</td>
<td>33</td>
</tr>
<tr>
<td>Prior CABG, %</td>
<td>27</td>
<td>83</td>
</tr>
<tr>
<td>Prior PTCA, %</td>
<td>13</td>
<td>33</td>
</tr>
<tr>
<td>Hypercholesterolemia, %</td>
<td>27</td>
<td>67</td>
</tr>
<tr>
<td>CABG procedure, † %</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>×3</td>
<td>47</td>
<td>NA</td>
</tr>
<tr>
<td>×2</td>
<td>27</td>
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</tr>
<tr>
<td>×1</td>
<td>20</td>
<td>NA</td>
</tr>
<tr>
<td>None</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Site of vector administration, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAD</td>
<td>28</td>
<td>50</td>
</tr>
<tr>
<td>LAD/Cx</td>
<td>6</td>
<td>34</td>
</tr>
<tr>
<td>Cx</td>
<td>34</td>
<td>NA</td>
</tr>
<tr>
<td>Cx/right</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>Right</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Ramus</td>
<td>6</td>
<td>16</td>
</tr>
<tr>
<td>RCA</td>
<td>6</td>
<td>NA</td>
</tr>
<tr>
<td>Time for vector administration, min</td>
<td>3.9±2.4 (2–10)</td>
<td>6.0±1.0 (4–8)</td>
</tr>
<tr>
<td>Posthospitalization, ‡ d</td>
<td>12.1±11.5 (5–40)</td>
<td>5.0±3.0 (3–11)</td>
</tr>
</tbody>
</table>

‡For group A, for the 13 patients other than A5 and A15, average postoperative hospitalization was 10.4±9.2 days (range 5 to 36 days).

Based on experimental animal models demonstrating the development of new blood vessels after in vivo administration of an Ad vector expressing human VEGF121 cDNA, including anatomic and functional correction of ischemia in a pig model of coronary obstruction, the present study was directed toward evaluation of the administration of an E1 E3 Ad vector (AdGV VEGF121.10) expressing the 121-amino-acid form of human VEGF to individuals with clinically significant coronary artery disease. The AdGV VEGF121.10 vector was administered directly to an ischemic area of the myocardium as an adjunct to conventional CABG surgery in a region that could not be bypassed (group A) or through a minithoracotomy as sole therapy (group B).

Methods

AdGV VEGF121.10

The Ad gene-transfer vector AdGV VEGF121.10 (GenVec, Inc) is based on the genome of the Ad5 serotype, with deletions in the E1 and E3 regions. The expression cassette is in the E1 region and contains (right to left) the cytomegalovirus early/immediate enhancer/promoter, an artificial splice sequence, human VEGF121 cDNA, and the SV40 polyA/stop signal. AdGV VEGF121.10 was propagated in 293 cells, purified by CsCl density gradients, dialyzed, and stored at −70°C. The vector met all safety criteria established by the Food and Drug Administration’s (FDA) BB for clinical grade Ad vector preparations (FDA BB-IND 7381), including no detectable endotoxin or infectious agents and ≤1 replication-competent Ad for the total dose to be delivered. The vector was titered in plaque-forming units (pfu) and characterized as the unit to be used. Just before use, the vector was thawed, diluted in a 3% sucrose solution, drawn up as 100 μL in 1-mL-insulin syringes with a 27-gauge needle (Becton Dickinson), and transported to the operating room.

Study Design

The study, which was approved by the local Institutional Review Board and the NIH DNA Recombinant Advisory Committee, was similar for groups A and B and included men and women aged 18 to 85 years with demonstrable reversible left ventricular ischemia as assessed by dobutamine stress echocardiography, rest and stress 99m Tc-sestamibi nuclear medicine studies, and exercise tolerance testing. Twenty-four-hour Holter monitoring was used to exclude individuals with life-threatening arrhythmias. Other organ-specific inclusion criteria included room air PO2 >60 mm Hg, PCO2 <50 mm Hg, FEV1 >1.2 L, hematocrit >30%, white blood cell count <10,000, serum urea nitrogen <40 U/L, and creatinine <2.5 g/dL. Group A (adjunct) had a requirement for an ejection fraction of ≥25% and ≥1 bypassable vessel, with the vector to be administered in a viable, ischemic region not amenable to bypass grafting. Group B (sole therapy) had a requirement of an ejection fraction ≥30% and included patients in whom CABG could not be performed due to lack of suitable bypass graft targets.

AdGV VEGF121.10 was administered by direct myocardial injection to both group A and B patients in a myocardial territory, irrespective of size, that demonstrated reversible ischemia by 99m Tc-sestamibi perfusion scan with or without adenosine stress. The injections (100 μL/injection; 10 sites/patient; each site 1 to 1.5 cm apart) were administered to a region that extended from normal to ischemic territories. The vector (total dose 4×108 pu/patient) was then injected by direct visualization in the beating heart into a region of reversible ischemia.
**General Safety Parameters**

Blood parameters, including aspartate aminotransferase, alanine aminotransferase, bilirubin (total, direct, and indirect), alkaline phosphatase, albumin, white blood count, hemoglobin, hematocrit, platelet count, electrolytes, creatinine, serum urea nitrogen, lactate dehydrogenase, and creatine kinase (CK; CK-MB if the total CK was abnormal), were measured through the perioperative period and at days 14, 21, and 30 postoperatively.

Anti-Ad5 neutralizing antibody titers were assayed as previously described. Plasma levels of VEGF were determined by standard ELISA; the assay detects all forms of human VEGF. The samples were obtained in citrate tubes (Vacutainer L10278-00 2.7 mL; Becton Dickinson) to avoid contamination with platelet-derived VEGF. Nose, throat, urine, and blood samples (before therapy and on days 2, 4, and 7) were evaluated for both E1 adenovirus vector and wild-type Ad.

**Cardiac-Specific Parameters**

The degree of angina (on a scale of 1 to 4) was assessed preoperatively and 30 days after surgery by use of a questionnaire describing the Canadian Cardiovascular Society classification. Serial ECG was used to assess myocardial ischemia, infarction, or arrhythmia. In the adjunct group, 24-hour Holter monitoring was performed before therapy and at 7 days after therapy.

Biplanar contrast angiography was performed preoperatively within 2 months of the surgical procedure and at day 30 after therapy. The angiograms were reviewed by 3 interventional cardiologists blinded to treatment group and evaluated in the area of vector administration on the basis of Rentrop score (0 indicates no filling of collateral; 1, partial filling of branches of epicardial vessel; 2, partial filling of epicardial vessel; and 3, complete filling of epicardial vessel) and collateral score (number of distinct collateral vessels contributing to the filling of an epicardial vessel in the region of vector administration). All studies were read in random sequence, and samples from before and after the study were randomly presented to observers.

A 2-day combined rest-stress 99mTc-sestamibi study to assess myocardial viability was performed preoperatively within 2 weeks of the surgery and at 1 month after surgery. One hour after intravenous administration of 99mTc-sestamibi (25 to 30 mCi), ECG-gated single-photon emission computerized tomography (SPECT) imaging was performed with or without pharmacological stress with adenosine (140 μg · kg⁻¹ · min⁻¹ IV over 6 minutes). Semiquantitative analyses of perfusion were assessed by use of a 20-segment analysis (18 short axis and 2 long axis) in a blinded fashion by 2 nuclear cardiologists and scored in the region of vector administration on a scale of 0 to 4+; where 0 indicates no perfusion, 1 is severe hypoperfusion, 2 is moderate hypoperfusion, 3 is mild hypoperfusion, and 4 is normal perfusion. Using CEqual software (ADAC), we generated “bull’s-eye” images for rest scans, stress scans, and their differences (“reversibility” of stress-induced ischemia) quantified as a percentage of the entire myocardium compared with a sex-matched normal database.

Serial 2D echocardiography was used to determine the presence of pericardial effusion at baseline (within 2 weeks of operative procedure) and on days 2, 4, 7, 14, 21, and 30 postoperatively by a 0 to 3+ scale (0 indicates no effusion, 1 is mild effusion, 2 is moderate effusion, and 3 is large effusion). Regional wall motion at rest was assessed in the region of vector administration at baseline and on day 30 by an observer blinded to treatment groups, using a scale from 0 to 4+; where 0 indicates dyskinesis/akinesia, 1 is severe hypokinesis, 2 is moderate hypokinesis, 3 is mild hypokinesis, and 4 is normal.

Exercise tolerance testing was performed preoperatively and at day 30 according to a modified Bruce protocol. Peak heart rate, peak heart rate x peak systolic blood pressure, and ST-segment/heart rate (ST/HR) slope (from peak exercise regression of ST depression expressed as a positive value referenced to heart rate) were determined.

**Statistical Analyses**

Given that this is a phase I clinical trial, the number of patients at each dose in group A (n=3) and the total number of patients in group B (n=6) are too few to provide sufficient statistical power to discriminate within the variability of the various parameters that were assessed. Therefore, lack of statistical significance may not necessarily be interpreted as “no difference.” The results are presented without formal error estimates and in the context of trends suggested by the data.

**Results**

**Patient Demographics**

The AdGV121.10 vector was administered to the myocardium as an adjunct to CABG (group A) in 15 patients (Table 1). The patients who were undergoing sole gene therapy (group B) were of similar age and had similar risk factors to those in group A but had a trend to a greater degree of cardiac disease, including an average lower ejection fraction and a higher proportion having undergone a prior CABG or angioplasty procedure.

**Vector Administration**

In group A, the region of injection was in the distribution of the left anterior descending (LAD) or circumflex coronary artery in the majority of patients, with the remainder in other sites (Table 1). One individual in group A (A10, vector dose 4×10⁹ pu) underwent cardiopulmonary bypass, but no bypass graft was placed because of the severity of distal disease as assessed intraoperatively. In group B, 5 of 6 individuals were injected in either the LAD or circumflex territories. The average time required for injection was 3.9 minutes in group A and 6.0 minutes in group B; the time was longer in group B because of technical constraints imposed by the minimally invasive approach. Minimal extravasation of injectant was noted in both groups. Occasional premature ventricular beats were observed with insertion of the needle into the myocardium but were self-limited in all cases.

**General Outcome**

In group A, there were 2 perioperative (within 40 days of operative procedure) deaths. One, on postoperative day 40 in a 61-year-old male (A5, vector dose 4×10⁸ pu) who was undergoing a third CABG operation, was related to a large anterior wall myocardial infarction secondary to occlusion of a graft to the LAD artery. Autopsy revealed a bacterial pneumonia and lung abscess; there were no abnormalities in the myocardial territory (posterior descending coronary artery) treated with the vector. The second death occurred on postoperative day 5 in an 85-year-old female (A15, vector dose 4×10⁹ pu) secondary to complications associated with an atheroembolic event in the ileocolic artery distribution. There was 1 additional sudden death of unknown cause (patient A14, dose 4×10⁸ pu, day 145 after therapy) in group A (mean±SD follow-up 286±76 days; range 175 to 414 days).

In group B, patients were extubated in the operating room, observed in the recovery room until awake, and transferred to the routine care floor until discharge. There were no perioperative or late deaths (mean±SD follow-up 170±17 days; range 149 to 196 days).
General Safety Parameters

There was no evidence of a dose-related trend toward abnormalities in any blood parameters in group A and no differences in any blood parameters for group B at day 3, 7, or 30 compared with before therapy. Plasma VEGF levels were evaluated over a 30-day period after therapy in the individuals who received $4 \times 10^9$ and $4 \times 10^{10}$ pu in the adjunct group and in all patients in the sole-therapy group. There were no trends to increases above baseline levels except at day 3, when the average value was 158 pg/mL. There was no evidence of acute or sustained hypotension or hemodynamic compromise associated with sole therapy (group B). In both groups, serum anti-Ad5 neutralizing antibody levels were increased in all individuals, although more so in patients with higher pretherapy anti-Ad5 neutralizing antibodies (not shown). No shedding of vector or wild-type Ad was detected in any sample from any site in any patient (group A total 234 samples; group B total 71 samples).

Cardiac-Related Parameters

In group A, there was no dose-related trend of an increase in CK related to vector administration (Table 2). In group B, there was no increase in CK after therapy (Table 2). In either group, daily ECG during hospitalization and at 14 and 30 days showed no new ST changes or Q waves (Table 3). In group A, 24-hour Holter monitoring performed before therapy and at day 7 demonstrated no average increase in supraventricular or ventricular arrhythmias after therapy. Serial echocardiographic studies in both groups demonstrated no evidence of significant ($>2$) pericardial effusions. In both groups, resting echocardiographic assessment at day 30 compared with before therapy showed no regional wall motion abnormalities in the territory where the vector was administered.

Assessment of angina class in group A showed improvement in all individuals evaluated, but this cannot be attributed specifically to the Ad$_{59}$VEGF121.10 therapy because of the effects of bypass. However, in all 6 of the individuals in group B, there was a decrease in angina classification at day 30 compared with before therapy (Figure 1).

Coronary angiograms obtained 30 days after vector administration demonstrated no hemangiomas or other pathological vascular structures. In group A, a majority of the blinded observations demonstrated an improvement in Rentrop scores in the area of vector administration after therapy compared with before therapy (Figure 2A). The collateral scores in group A demonstrated a similar trend (Figure 2B). Likewise, in group B, in which no CABG was performed that might provide a watershed effect in the area treated with the vector, a majority of the Rentrop and collateral score observations

### Table 2. CK Values of Patients Receiving Ad$_{59}$ VEGF121.10 (10$^9$ pu) as Sole Therapy

<table>
<thead>
<tr>
<th>Patients</th>
<th>CK, U/L</th>
<th>CK-MB</th>
<th>CK, U/L</th>
<th>CK-MB</th>
<th>CK, U/L</th>
<th>CK-MB</th>
<th>CK, U/L</th>
<th>CK-MB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>85±47</td>
<td>5.7±0.0</td>
<td>479±547*</td>
<td>6.1±4.9†</td>
<td>107±71*</td>
<td>2.3±0.4†</td>
<td>65±46*</td>
<td>4.2±0.0†</td>
</tr>
<tr>
<td>Group B</td>
<td>153±115</td>
<td>…‡</td>
<td>339±305</td>
<td>…‡</td>
<td>190±91</td>
<td>…‡</td>
<td>103±52</td>
<td>…‡</td>
</tr>
</tbody>
</table>

*Patients A-5 and A-10 were excluded from analysis; A-5 had a large perioperative myocardial infarction, and A-10 had a compartment syndrome of the right lower leg.
†Patient A-5 was excluded from analysis because of a large perioperative myocardial infarction.
‡CK-MB was not assessed if total CK was within normal range.

### Table 3. Comparison of ECG at Baseline and Postvector Day 30 for Patients Receiving Ad$_{59}$ VEGF121.10

<table>
<thead>
<tr>
<th>Group and Patient</th>
<th>Vector Dose, pu</th>
<th>Atrial Fibrillation</th>
<th>New MI</th>
<th>New Ischemia</th>
<th>Any New Abnormality</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A1, A2, A4, A6–A14</td>
<td>4×10$^8$–4×10$^{10}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A3</td>
<td>4×10$^8$</td>
<td>0</td>
<td>Yes†</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>A5</td>
<td>4×10$^{15}$</td>
<td>0</td>
<td>Yes‡</td>
<td>Yes‡</td>
<td>Yes‡</td>
</tr>
<tr>
<td>A15</td>
<td>4×10$^{10}$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Yes‡</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1, B4–B6</td>
<td>4×10$^9$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B2</td>
<td>4×10$^9$</td>
<td>0</td>
<td>0§</td>
<td>0§</td>
<td>0§</td>
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<tr>
<td>B3</td>
<td>4×10$^9$</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Yes∥</td>
</tr>
</tbody>
</table>

MI indicates myocardial infarction.
*Possible anteroseptal MI, although this change could be attributed to change in lead placement.
†Anterolateral MI.
‡Loss of previously seen repolarization abnormality.
§Loss of preoperative anterior MI pattern.
∥Anterolateral ischemia.
showed a similar trend of improvement after therapy compared with before therapy (Figure 2C).

For group A assessed as a single cohort, semiquantitative analysis of the $^{99}$Tc-sestamibi images in the area of vector administration showed no changes in relative blood flow at 30 days after vector administration compared with pretherapy at rest or after adenosine-induced stress. Likewise, for group B evaluated as a cohort, analysis of the sestamibi images demonstrated no differences in relative blood flow in the area of vector administration at rest or after adenosine-induced stress. Interestingly, analysis of the sestamibi images for wall motion at stress in the region of vector administration showed an improvement at 30 days after vector administration in the majority of patients, both in group A (66% [8/12] improved) and in group B (66% [4/6] improved). For group B, in which bull’s-eye analyses of the sestamibi scans could be performed with assessment of vector administration as the only variable, 4 of 6 individuals showed an improvement 30 days after therapy in the proportion of myocardium that showed reversibility (reversible stress-induced ischemia) before therapy (70±31%) versus 30 days after therapy (54±36%).

Assessment of treadmill exercise in group A showed no differences (30 days after vector compared with before therapy) in exercise duration, heart rate×blood pressure, or ST/HR slope. For group B, in which vector administration was the only therapy, assessment of treadmill exercise showed an improvement in exercise duration in 50% (3/6), in peak heart rate×blood pressure in 50% (3/6), and in ST/HR slope (ie, lower values) in 75% (3/4; data not available in 2 secondary to right bundle-branch block precluding analysis; Figure 3).

**Discussion**

The development of strategies to deliver angiogens to revascularize the ischemic myocardium without the need for mechanical manipulation of atherosclerotic vessels is potentially of profound importance in the treatment of coronary artery disease. The present study demonstrates that it is feasible to safely use an adenovirus gene-transfer vector to deliver the coding sequence of the 121-amino-acid form of the human VEGF angiogen to the myocardium of individuals with clinically significant coronary artery disease. Based on the knowledge that VEGF plays a critical role in embryonic cardiac angiogenesis and on preclinical studies that demonstrate that the Ad$_{ov}$VEGF121.10 gene-transfer vector will induce functional angiogenesis in the ischemic myocardium of experimental animals, the results of the present study provide encouragement that this strategy may be useful in revascularizing the ischemic heart in humans.
angiographic studies showed increased coronary artery filling and/or number of collaterals in the coronary angiograms and no evidence of myocardial edema or pericardial effusions. These observations are consistent with the possibility of CABG-related watershed perfusion affecting the region of vector administration. Despite these constraints, the trends of several of the efficacy-related parameters assessed 30 days after therapy are encouraging. First, all participants had improvement in their angina classification. The ability to biologically revascularize tissues, if proven to be safe and efficacious in large, controlled trials, will be an invaluable treatment for patients with diffuse disease not amenable to conventional CABG or PTCA and may be useful as initial therapy in some individuals in place of routine CABG or PTCA therapy. In the present study, we used an Ad vector to deliver the VEGF121 cDNA. As an alternative, Losordo et al.9 used myocardial administration of a VEGF165 plasmid as sole therapy for myocardial ischemia and demonstrated a safety profile and trends in efficacy parameters similar to our study. If one assumes that the neovascularization induced by angiogenic therapy is persistent and physiologically relevant, the small-caliber vessels generated by this therapy may furthermore be relatively spared from the effects of atherosclerosis, which primarily affects larger vessels. Finally, given the decreased survival overall and decreased angina-free survival noted in patients in whom incomplete revascularization is accomplished, the advantages of providing “complete” revascularization in patients undergoing standard CABG or PTCA may also prove to be a significant benefit of this new therapy.

**Systemic Parameters**

Assessment of blood and urine parameters suggested no systemic abnormalities related to the vector, consistent with the general clinical experience with E1- Ad vector transfer to humans. Importantly, there was no evidence of liver function abnormalities as a function of vector dose; this is important because the liver is a major site of Ad vector–induced inflammation at high doses in some studies in experimental animals. One explanation for this lack of systemic toxicity in the human studies is that the vector preparations used in clinical studies are highly purified (<1 replication-competent Ad [RCA] per total dose), in contrast to laboratory-grade vectors, which are often contaminated with RCAs.

Myocardial administration of Ad0VEGF121.10 induced an increase in anti-Ad neutralizing antibodies in most of the study population. This was more pronounced in individuals with detectable serum anti-Ad neutralizing antibodies before therapy, consistent with the vector inducing a memory immune response against subgroup C Ad. Despite this, there was no evidence of systemic immune-related toxicity in any patient, including no immediate anaphylactic-type reactions, vasculitis, or renal damage.

Finally, it is known that systemic administration of the VEGF protein at high doses results in systemic hypotension in experimental animals and humans. However, the present study demonstrated no large increases in VEGF levels in the systemic circulation after myocardial administration of Ad0VEGF121.10 and no hypotension attributable to the vector, consistent with experimental animal studies using Ad0VEGF121.10.

**Future Role of Angiogenic Gene Therapy**

The ability to biologically revascularize tissues, if proven to be safe and efficacious in large, controlled trials, will be an invaluable treatment for patients with diffuse disease not amenable to conventional CABG or PTCA and may be useful as initial therapy in some individuals in place of routine CABG or PTCA therapy. In the present study, we used an Ad vector to deliver the VEGF121 cDNA. As an alternative, Losordo et al. used myocardial administration of a VEGF165 plasmid as sole therapy for myocardial ischemia and demonstrated a safety profile and trends in efficacy parameters similar to our study. If one assumes that the neovascularization induced by angiogenic therapy is persistent and physiologically relevant, the small-caliber vessels generated by this therapy may furthermore be relatively spared from the effects of atherosclerosis, which primarily affects larger vessels.

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