VEGF Gene Delivery for Treatment of Ischemic Cardiovascular Disease

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There are increasing numbers of patients with ischemic myocardial disease not amenable to traditional methods of revascularization. These patients may benefit from new research into the use of naturally occurring angiogenic compounds, such as vascular endothelial growth factor (VEGF) for re-establishing blood flow into regions of hibernating myocardium. Animal studies and human clinical trials evaluating VEGF demonstrate increases in myocardial perfusion after treatment, with some patients reporting improvement in anginal symptoms. Further research into the ideal form of VEGF therapy (protein, plasmid, or adenoviral) and delivery method (intracoronary, intramyocardial, or epicardial) seems justified. (Trends Cardiovasc Med 2002;12:108–114) © 2002, Elsevier Science Inc.

Percutaneous transluminal angioplasty (PTCA) and stenting or operative coronary revascularization (coronary artery bypass grafting, CABG) are procedures that effectively re-establish blood flow to distal myocardial vascular beds and provide relief of angina for most patients with lesions from coronary artery disease. However, percutaneous interventions are plagued by high rates of restenosis and bypass grafts are subject to progressive atherosclerotic occlusion.

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Furthermore, because of progression of native myocardial disease and the finite number of useful conduits for subsequent operative revascularizations, many patients are not considered to be candidates for additional operative or percutaneous interventional procedures. A recent study at two institutions (Mukherjee et al. 1999) found that 12% of the patients referred for coronary angiograms were not candidates for an interventional approach based on their cardiac vascular pathology. Approximately 6,750,000 Americans suffer from angina and an additional 350,000 new patients will experience angina symptoms for the first time this year, thus suggesting that 100,000 patients per year could benefit from an alternate approach to revascularization.

The delivery of angiogenic factors to ischemic myocardial tissues, either by direct injection of protein or by application of gene transfer methods, is an attractive treatment strategy for patients with diffuse severe atherosclerotic coronary artery disease who have no other options available to them. The two angiogenic growth factors that have been utilized in human clinical trials are basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). These naturally occurring proteins are mitogenic for endothelial cells, and certain isoforms appear to stimulate the formation of capillary and small arteriole beds. This review focuses on the development and application of VEGF for the treatment of ischemic myocardial disease in humans.

• Molecular Studies

Folkman (Folkman 1971, Folkman et al. 1971) proposed the existence of a tumor angiogenesis factor (TAF) that he and his colleagues isolated from Walker tumor cells. This factor induced the growth of new capillaries in a dorsal air sac model in rats. A discussant of one of Folkman’s initial papers (Folkman 1971) highlighted the potential usefulness of this type of molecule in normal or ischemic tissues. Folkman responded that previous experiments in his laboratory following removal of TAF (Folkman 1971) that would limit the long-term utility of the compound. The group subsequently isolated an 18 kDa heparin-binding protein from tumor-derived capillary endothelial cells (Shing et al. 1984).

Ferrara and Henzel (1989) isolated and sequenced (Leung et al. 1989) a 45 kDa protein from bovine pituitary cells that also had a high affinity for heparin and was mitogenic for cultured endothelial cells. This protein, unlike bFGF and platelet-derived growth factor (PDGF), was not mitogenic for other cell types, including fibroblasts and smooth muscle cells. The group provisionally named the protein vascular endothelial growth factor. Using human cDNA libraries, they were able to isolate three homodimers of the VEGF DNA coding for 121, 165, and 189 amino acid moieties (Leung et al. 1989). These three gene products were subsequently demonstrated to be the result of alternate splicing of a parent mRNA (Tischer et al. 1991). Subsequent characterization of the VEGF isoforms have revealed four subtypes (VEGF<sub>121</sub>, VEGF<sub>165</sub>, VEGF<sub>189</sub>, and VEGF<sub>206</sub>; Houck et al. 1991); however, only VEGF<sub>121</sub> and VEGF<sub>165</sub> are diffusible after secretion, whereas the other two moieties remain cell-bound (Houck et al. 1992). Parallel work by Connolly and colleagues on a molecule designated vascular permeability factor (VPF) demonstrated that it had angiogenic properties in rat cornea and rabbit bone-healing models (Connelly et al. 1989). Both laboratories independently published identical amino acid sequences (Keck et al. 1989; Leung et al. 1989), thus confirming that VEGF and VPF were the same molecule.

Two subtypes of VEGF receptors, Flt-1 (VEGFR-1) and FLK-1/KDR (VEGFR-2) have been identified on endothelial cells (de Vries et al. 1992, Millauer et al. 1993, Terman et al. 1992) and are thought to mediate the mitogenic effects of VEGF, with the VEGFR-2 receptor thought to be the dominant mediator of endothelial division (Neufeld et al. 1999). Thus far these receptors have only been localized to macrophages and endothelial cells, which explains the specificity of the VEGF response to endothelial cells as opposed to other vascular cell types. By contrast, VEGF has been found to be secreted from a variety of cell types (Leung et al. 1989), as well as through the interaction between extracellular matrix-bound VEGF and macrophage secreted metalloproteinases (Bergers et al. 2000). VEGF exerts its response through a paracrine effect and is mediated by a typical signal sequence for secretion located at the NH<sub>2</sub> terminus. Thus small amounts of the biologically active molecule can have profound mitogenic effects.

Ischemia (hypoxia) appears to stimulate VEGF protein expression by inhibiting the destruction of a hypoxia inducible factor-1α (HIF-1α), thus allowing it to bind to a hypoxia response element (HRE) in the VEGF promoter and activate it (Neufeld et al. 1999). HIF-1α also increases expression of VEGF receptors, indicating a putative role in ischemia-induced myocardial collateralization (Banaï et al. 1994a; Banaï et al. 1994b). Key references on the regulation of hypoxia-induced angiogenesis can be found in Semenza (2001) (Figure 1). Once transcribed, under hypoxic conditions VEGF mRNA is stabilized by the HuR protein binding to its 3′-untranslated region (3′-UTR) as well as other proteins that inhibit its degradation (Brennan et al. 2000, Levy et al. 1998). The development of a stable and functional vessel is highly dependent on the surrounding cellular milieu and not just on the presence of VEGF. In the absence of pericytes recruited by platelet-derived growth factor-BB (PDGF-BB) the developing neovascularization remains completely VEGF-dependent and the vessels regress when its secretion ceases (Benjamin et al. 1999).

VEGF also appears to play a role in embryologic vascular development. An elegant series of experiments published simultaneously by two different groups demonstrated abnormal vascular development in embryos of VEGF knockout mice (Carmeliet et al. 1996, Ferrara et al. 1996; for a review, see Darland and D’Amore 1999). The importance of VEGF in vascular development is clearly shown by the fact that even in embryos that are heterozygous (+/−), and therefore lack only one of the two VEGF alleles, the mutation is lethal by early-gestation. VEGF appears to play an active role in the induction, maintenance, and growth of normal vascular endothelial cells as well as tumor vascular development.

• Preclinical Studies

Given the ability of VEGF to stimulate neovascularization in animal tissue models (Connolly et al. 1989), investigating its potential use in the treatment of ischemic muscle became the obvious next step. Isner’s group at St. Elizabeth’s
Hospital demonstrated its effectiveness in relieving ischemia in a rabbit hind-limb model with intraarterial and intramuscular VEGF\textsubscript{165} protein delivery as well as intramuscular plasmid administration (phVEGF\textsubscript{165}). Animals underwent surgical excision of the common femoral artery with subsequent VEGF\textsubscript{165} protein administration via intraarterial delivery (500 µg or 1000 µg; Takeshita et al. 1994b), intramuscular injection (200 µg, 500 µg, or 1000 µg; Takeshita et al. 1994a), intramuscular plasmid injection (500 µg; Tsurumi et al. 1996), or plasmid delivery via attachment to a hydrogel coating of an angioplasty balloon (phVEGF\textsubscript{121}, phVEGF\textsubscript{165}, phVEGF\textsubscript{189}, 400 µg each; Takeshita et al. 1996). There were significantly increased vascular collaterals in the treated limb 10 days following treatment compared to the untreated controls in all studies, with a dose-dependent increase in collateralization evident in the direct protein injection study (Takeshita et al. 1994b). Rabbit calf blood pressure and Doppler flow wire evaluations showed improved distal tissue flow. Animals had histologic evidence of increased capillary density and microbead distribution after treatment. No evidence of muscular edema, as might be expected from VEGF’s permeabilizing effects, was seen. They also demonstrated that three of the isoforms (VEGF\textsubscript{121}, VEGF\textsubscript{165}, and VEGF\textsubscript{189}) were equipotent in this model, although there was no dose-dependent effect seen with naked DNA administration (Takeshita et al. 1996).

VEGF delivery (as protein, plasmid, adenovirus, or cell-mediated) to the myocardium has also been explored in great detail. A variety of delivery methods have been employed, including intravenous, intracoronary, intramyocardial, and intrapericardial/epicardial. Banai and colleagues (Banai et al. 1994a), using VEGF\textsubscript{165} protein delivered by an implanted intracoronary catheter that allowed for daily dosing over a 4-week period in a canine model of ischemia, were able to demonstrate a 40% increased collateral blood flow at maximal vasodilatation by microsphere injection and an increase in the number of intramyocardial distribution vessels. Although these animals had a strong therapeutic response and appeared to tolerate the daily dosing regimen, several subsequent studies evaluating intracoronary VEGF protein delivery in swine models revealed profound and often acutely fatal hypotension (Hariawala et al. 1996, Lopez et al. 1997, Sato et al. 2001). This effect has important clinical relevance, as many clinicians are interested in pursuing a minimally invasive approach in these patients and percutaneous intracoronary delivery appears to be an ideal delivery modality. The hypotension appears to be in part mediated through release of nitric oxide (NO) and can be ameliorated by the concomitant administration of a competitive inhibitor of nitric oxide synthase (NOS; Hariawala et al. 1996, Lopez et al. 1997, Sato et al. 2001). Most importantly, however, this response appears to be dose-related. Hariawala and colleagues (1996) delivered a single, intracoronary 2 mg dose of human recombinant VEGF\textsubscript{165} to ischemic pigs. Four of the eight treatment...
animals, but none of the normal saline-treated animals, had an immediately fatal hypotensive arrest. The surviving animals did go on to develop significantly improved myocardial collateralization. Although the chronic effects of a lower VEGF dose were not reported, a dose of 500 μg also induced profound hypotension. Most recently, Sato et al. (2001) evaluated several dosing regimens and found that intracoronary doses as low as 0.25 μg/kg initiated angiogenesis and resulted in hypotension that could be ameliorated by a NO-synthase inhibitor. Animals receiving the NO-synthase inhibitor Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) had similar development of neovascularization as paired animals receiving intracoronary VEGF protein, indicating nitric oxide inhibition does not attenuate the angiogenic properties of VEGF protein.

Alternative delivery methods have been explored, including intrapericardial/epicardial application and direct gene transfer using attenuated (replication-deficient) adenovirus. Pearlman et al. (1995) utilized MRI imaging to show that epically delivered VEGF protein decreased gadodiamide contrast arrival times in ischemic porcine myocardium (correlating with enhanced tissue perfusion) and increased ejection fraction. Mack et al. (1998) delivered 10⁶ pfu injections of Ad-VEGF₁₂₁ directly into ischemic myocardium with similarly impressive results. Although the authors expressed concern over potential myocardial inflammation/myocarditis with an adenovirus vector, no such effect was observed. Direct myocardial injection of a viral vector appeared to be safe and effective with several important implications: (1) VEGF delivery was not associated with hypotension; (2) focal, therapeutic angiogenesis was seen; and (3) adenovirus expression was only associated with 2 to 3 weeks of protein expression. There may be a benefit to a short protein expression time as seen in this model and in evidence from our laboratory demonstrating that persistent delivery of VEGF protein leads to fatal hemangioma development in a mouse model where myoblasts transduced with the VEGF gene are injected into normal hearts (Lee et al. 2000). In this type of model, protein expression is constitutive and does not show the same attenuation as seen when plasmid DNA or adenoviral vectors are used. That hemangiomas developed after two weeks of continuous VEGF expression suggests there may be a dose-related response and that continuous VEGF exposure may be deleterious owing to excess stimulation of endothelial cells and subsequent vascular development. If dosage is regulated by delivery of fewer VEGF producing cells or by using delivery methods that can be controlled, such as regulatable vectors (Rossi et al. 2000), this problem may be overcome. Clearly, either an appropriate dosage of VEGF alone, which may vary among individuals, or co-delivery with one or more additional molecules, such as angiopoietin-1, are needed in order to prevent the leakiness often observed in the tortuous vessels typical of those induced by the VEGF secreted by tumors. Alternatively, molecules that recruit pericytes may also serve to stabilize vessels. Thus, attention must be paid to achieve the precise balance of cell-signaling molecules and cell types to achieve therapeutic angiogenesis.

- **Human Clinical Trials**

  **St. Elizabeth's Medical Center**

  Because of the success of VEGF therapy in human peripheral vascular disease trials (Baumgartner et al. 1998, Isner et al. 1998, Isner et al. 1996) and the intriguing animal myocardial data, Isner's group at St. Elizabeth's Medical Center initiated human trials in January of 1998 to evaluate the efficacy of VEGF treatment for chronic myocardial ischemia (see Table 1). A phase I clinical study using the VEGF₁₆₅ plasmid (phVEGF₁₆₅) was launched (Losordo et al. 1998). Entry criteria included functional class III or IV exertional angina refractory to maximal medical therapy, areas of viable but underperfused myocardium, and multivessel occlusive coronary artery disease. Patients were excluded if they had a successful revascularization within the previous 6 months, cancer, retinopathy, or an ejection fraction less than 20%. Five patients underwent anterolateral thoracotomies with direct exposure of the heart. Four 2-ml aliquots of phVEGF₁₆₅ (total dose of 100 μg DNA) were injected under direct vision into the myocardium. The patients all tolerated the procedure well. All 5 patients experienced dramatic improvements of subjective criteria (angina frequency and nitroglycerin tablet use) and objective myocardial evaluations (dobutamine SPECT imaging and coronary angiography). This was a dose-escalating study (250 μg and 500 μg) and similar encouraging results were achieved with minimal toxicity in these patients (Symes et al. 1999, Vale et al. 1999). The major limitations of this study were the small numbers of patients and the lack of placebo controls (the use of untreated controls was not warranted at this time given the relatively invasive nature of the treatment; Losordo et al. 1999). Nonetheless, a randomized double-blind controlled study at another site would help overcome all remaining doubts, by validating efficacy and safety.

  The authors were able to corroborate their evaluation of improvement of left ventricular viability using left ventricular electromechanical mapping (LVEMM) employing the NOGA system (Biosense-Webster) (Vale et al. 1999). The NOGA LVEMM evaluates localized ventricular viability and wall motion (fractional shortening) using electromagnetic field sensors combined with intracardiac electrograms. The technique allows percutaneous mapping of multiple segments of the myocardium and a correlation of viability with SPECT imaging. In this study, NOGA evaluation correlated well with SPECT imaging in 13 patients and demonstrated significant recovery of hibernating myocardium after phVEGF₁₆₅ therapy. Left ventricular ejection fraction improved from 31.3 ± 2.7% to 36.9 ± 2.3% 60 days after gene therapy (p = .023). There was also a statistically significant reduction in the area of ischemic myocardium (6.45 ± 1.37 cm² versus 0.95 ± 0.41 cm², p = .001). Although not explored here, the technique of NOGA LVEMM combined with the percutaneous delivery of intramyocardial VEGF (plasmid or protein) might allow for the precise localization of hibernating myocardium that would maximally benefit from angiogenic therapy. The St. Elizabeth's group has begun exploring the potential for percutaneous delivery of intramyocardial phVEGF₁₆₅ (Vale et al. 1999).

  **New York Presbyterian Hospital, Cornell University**

  Replication-deficient adenoviruses represent an alternative delivery vector for
therapeutic genes. As described by Mack et al. (1998), intramyocardial delivery of AdVEGF_{121} elicited significant angiogenesis in a porcine model with minimal inflammatory response. Based on the encouraging outcomes of this study, Rosengart et al. (1999) initiated a phase I clinical study at Cornell University investigating the feasibility and safety of using an adenovirus gene transfer method to transfect myocardium with VEGF_{121} DNA (AdVEGF_{121}). Patients were divided into two groups: Group A underwent at least one bypass graft at the time of surgery (n = 15) and Group B received virus injection as sole therapy. Patients were typical for these types of studies in that they had frequent, medically recalcitrant anginal episodes, were poor surgical/interventional total revascularization candidates, and had an ejection fraction of at least 25% (30% in Group B).

Group A patients (coronary artery bypass graft + virus) underwent traditional sternotomy and on-pump CABG. Patients were further subdivided into 5 escalating dosage groups (4 \times 10^6 pfu, 4 \times 10^8 pfu, 4 \times 10^9 pfu, 4 \times 10^9 pfu, n = 3 each group). Injections were performed during rewarming while the patient was on partial cardiopulmonary bypass. Group B patients (virus only) were injected though a small thoracotomy incision. Both groups were followed for approximately 6 months, although only 30-day data were reported. As in the plasmid trials, patients in both groups reported a marked reduction in the frequency and severity of angina by postoperative day 30. There appeared to be no evidence of myocardial inflammation as no cardiac enzyme leak, EKG changes, or arrhythmias were noted. Interestingly, unlike the plasmid studies that showed a large rise in plasma VEGF levels after treatment, no appreciable rise was seen in this study. Whether this relates to confined local release or less active cellular production is unknown. Combined rest/stress \textsuperscript{99m}Tc-setamibi studies did not reveal a difference in relative blood flow in the areas of vector administration, but did show a trend toward improved wall motion at stress. Although the study lacked the power to demonstrate significant improvements in patient outcomes and would have benefited from a randomized, double-blind strategy, the investigators concluded that an adenoviral vector appeared safe and feasible. Duplication of these results by other groups should follow to resolve these issues, and it remains premature to speculate on the ultimate utility of adenoviral vectors in this setting. Concerns remain regarding the overall safety of this technique and will, one hopes, be addressed in future studies.

The VIVA Trial

A large multi-center trial exploring the utility of intracoronary delivered recombinant VEGF_{165} protein (rhVEGF_{165}) was recently completed, although the final results have not yet been published. Seven institutions participated in a small dose escalating study in 14 patients (Hendel et al. 2000). Patients received either low dose (0.005 and 0.017 \mu g/kg) or high dose (0.05 and 0.167 \mu g/kg) intracoronary rhVEGF_{165}. SPECT studies revealed minimal change in stress images, but did show improvement in resting images. There was a trend toward improved SPECT scoring with high dose VEGF (5/6 versus 5/8). This small study validated the relative safety of the intracoronary delivery of VEGF protein without the need for anti-NOS therapy. In effect, this study allowed for the advent of the VEGF in Is-

### Table 1. Summary of human VEGF trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients</th>
<th>Delivery method</th>
<th>Factor</th>
<th>Dose</th>
<th>Primary outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Losordo et al. (1998)</td>
<td>5</td>
<td>Direct myocardal injection</td>
<td>pHVEGF\textsubscript{165}</td>
<td>125 \mu g</td>
<td>Angina frequency Dobutamine SPECT Coronary angiography</td>
</tr>
<tr>
<td>Syynes et al. (1999)</td>
<td>20</td>
<td>Direct myocardal injection</td>
<td>pHVEGF\textsubscript{165}</td>
<td>125 \mu g, 250 \mu g</td>
<td>Angina frequency Dobutamine SPECT Coronary angiography</td>
</tr>
<tr>
<td>Vale et al. (1999)</td>
<td>13</td>
<td>Direct myocardal injection</td>
<td>pHVEGF\textsubscript{165}</td>
<td>250 \mu g, 500 \mu g</td>
<td>Angina frequency Dobutamine SPECT Coronary angiography</td>
</tr>
<tr>
<td>Rosengart et al. (1999)</td>
<td>21</td>
<td>Direct myocardal injection</td>
<td>Ad\textsubscript{GV}VEGF\textsubscript{121.10}</td>
<td>4 \times 10^4 to 4 \times 10^{10} pu</td>
<td>Angina frequency Dobutamine SPECT Coronary angiography LVEMM (NOGA)</td>
</tr>
<tr>
<td>Hendel et al. (2000)</td>
<td>14</td>
<td>Selective coronary injection</td>
<td>rhVEGF\textsubscript{165}</td>
<td>0.005 \mu g/kg, 0.017 \mu g/kg, 0.05 \mu g/kg, 0.167 \mu g/kg</td>
<td>Dobutamine, exercise, or dipyridamole stress SPECT</td>
</tr>
<tr>
<td>VIVA Trial (Henry et al. 1999 and 2001)</td>
<td>178</td>
<td>Selective coronary injection, intravenous infusion</td>
<td>rhVEGF\textsubscript{165}</td>
<td>17 ng/kg/min, 50 ng/kg/min</td>
<td>Placebo</td>
</tr>
</tbody>
</table>
chemia for Vascular Angiogenesis (VIVA) trial, which is the first and only prospectively randomized, placebo-controlled study to evaluate VEGF's efficacy in myocardial disease. To date, 178 patients have been enrolled from 20 US centers for 20-min intracoronary rhVEGF165 infusion followed by 4-h intravenous infusions on days 3, 6, and 9. Patients were randomized to low dose (17 ng/kg/min), high dose (50 ng/kg/min), or placebo. The study endpoints were differences in angiina scores and treadmill exercise times. The initial results revealed no significant differences in treadmill exercise times or angiina classification at 60 days between groups (Henry et al. 1999). One-year follow-up data continued to show no significant improvement in exercise time (although there was a trend toward improvement in the VEGF group, \( p = 0.17 \)) and a worsening in all groups in anginal class (Henry et al. 2001). Patients appeared to tolerate the procedure well and there was a low incidence of death, myocardial infarction, and cancer in all three groups. Interestingly, the outcomes were somewhat surprising as the overall mortality for these patients was much lower than would have been expected. Although the VIVA trial's initial results are disappointing, it might be inferred that the modes of delivery (intracoronary and intravenous) provide tissue levels too low to incite an angiogenic response. Further work with placebo-controlled, gene, or viral direct myocardial delivery might be more encouraging.

**Summary**

The use of angiogenic factors is an attractive treatment modality for patients with diffuse coronary artery disease who are not candidates for traditional revascularization methods. The technique appears to augment the natural relationship between ischemic tissues and the development or recruitment of new and/or existing collateral vessels. Several angiogenic proteins (FGF, VEGF, HIF-1α and its regulators, as well as developmental endothelial locus–1) are currently being investigated in a variety of animal models and human trials. The need for balance and a harmonious interplay of molecules and cells with both angiogenic and remodeling functions is clearly required to ensure long-term production of fully functional “well-tempered” blood vessels (Blau and Banfi 2001). Whether a single compound can induce a multiplicity of factors in a cascade or lead to feedback loops, or whether delivery of a cocktail of factors will prove to be most efficacious, remains unknown. At present, there remain many important questions and considerations regarding the use of angiogenic growth factors for the treatment of diffuse coronary artery disease. It is unclear whether the perfusion to hibernating myocardium induced through angiogenesis will remain durable over the long term. Will the new vessels be subject to the same atherosclerotic changes that have ravished the native myocardial vessels, thus ultimately limiting the usefulness of the technique? Will percutaneous approaches allow for multiple re-interventions over time to maintain the patency of the vessels, and is there a maximal safe dose before the advent of hemangiomas? Finally, will the long-term outcomes in placebo-controlled trials eventually demonstrate a sufficiently significant benefit to patients in the current fiscally tight healthcare market to justify the continued use of these factors? Although all of these questions should be answered with time, the use of angiogenic growth factors in the treatment of medically recalcitrant angina is promising and part of our ever-expanding clinical armamentarium.

**References**


