Cardiac Angiogenesis: Part 2

An Emerging Technology for the Treatment of CAD

Part 2 of this article looks at issues of study design, efficacy end points and potential complications of treatment with angiogenic agents. The authors also look at future research, which may provide feasible therapy in the future.

By Michael J.B. Kutryk, MD, PhD; and Duncan J. Stewart, MD

Introduction

This is the second of a two-part review examining angiogenesis as a potential, exciting new therapeutic strategy for the treatment of coronary artery disease (CAD).

This article will review the clinical data reported to date and issues related to the design of clinical trials, as well as the potential unwanted consequences of treatment with angiogenic agents.

About the author...

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E.M., a 64-year-old female, has coronary risk factors, including a strong family history of premature coronary artery disease, diabetes and hyperlipidemia. Her cardiac history dates back to early 1984 when she began experiencing crescendo symptoms of angina, which culminated in a myocardial infarction (MI). Following her MI, E.M. underwent three-vessel coronary artery bypass grafting (CABG) with saphenous vein grafts to the left anterior descending (LAD) artery, the posterior descending branch of the right coronary artery (RCA) and first diagonal branch of the LAD artery.

She remained symptom-free until 1988, when recurrence of her anginal symptoms prompted repeat CABG, with bypass of the second diagonal branch of the LAD artery with the left internal mammary artery, and repeat bypass of the posterior descending branch for the RCA, using a saphenous vein graft (SVG).

Her symptoms returned once again in 1992, and angiography revealed occlusion of the LAD distal to the first diagonal branch. Percutaneous dilatation of the LAD stenosis was successful and eliminated her pain. In 1996, unstable anginal symptoms prompted a repeat angiogram. A significant lesion in the RCA, distal to the anastomosis of the SVG, was identified and successfully dilated.

In 1997, E.M. returned with symptoms of unstable angina and she once again underwent coronary angiography. This revealed a significant stenosis of the proximal portion of the SVG to the RCA. The vein graft was successfully treated percutaneously with implantation of an intracoronary stent. She was feeling well until early in 2000, when she began having symptoms of unstable angina. Myocardial perfusion imaging revealed reversible ischemia in the inferolateral wall. Coronary angiography was performed and showed a long, non-critical lesion in the proximal circumflex coronary artery (<50% diameter stenosis). Her physicians felt percutaneous treatment would be of no benefit. Despite maximal medical therapy, her symptoms persisted. Her exercise tolerance was limited to less than one block at a slow pace and less than one flight of stairs. E.M. felt her symptoms were not tolerable and requested something more be done.

**Question:** What, if any, are therapeutic options for this patient?

*Discussion on page 55*
Clinical Trials

Until recently, human angiogenic experiments have been predominantly limited to small series in which vascular endothelial growth factor (VEGF) or fibroblast growth factor (FGF), protein or gene, have been administered. Preliminary human trials in peripheral vascular disease have demonstrated improvements in ankle-brachial index, enhancements of angiographically visible collaterals, improvements in rest pain and analgesic medication use, ulcer healing and diminished critical limb ischemia. Delivery strategies for myocardial angiogenesis have included intracoronary, epicardial or direct myocardial injection of either VEGF or basic fibroblast growth factor (bFGF) protein, or genetic material. The latter can be delivered as naked plasmid deoxyribonucleic acid (DNA) or in a viral vector.

Schumacher et al were the first to report on therapeutic angiogenesis in human myocardium. This randomized, blinded study enrolled 40 patients undergoing coronary artery bypass grafting (CABG) with a left internal mammary artery graft and a left anterior descending (LAD) artery stenosis distal to the anastomosis site. Patients were randomly assigned to direct intramyocardial injection of acidic fibroblast growth factor (aFGF) or denatured protein control near the distal non-grafted segment. At the three-month mark, researchers observed increased coronary blush, a surrogate measure (unvalidated) of collateral formation among FGF-injected patients, as compared to placebo. This effect was still in evidence three years later and was associated with improved echocardiographic ejection fraction and functional class.4

Sellke et al reported on a series of eight patients with ischemic heart disease who received bFGF as an adjunct to CABG. These patients had at least one major arterial distribution not amenable to revascularization, but were otherwise candidates for CABG. The growth factor was delivered by sustained-release microcapsules implanted around the ischemic territory during surgery. At the 12-week follow-up, three patients showed clear enhancement of perfusion to the unrevascularized myocardium, three patients had minimal overall change and one patient had a new fixed defect on stress nuclear perfusion imaging.

A double-blind, placebo-controlled study was performed by Laham et al in which patients undergoing CABG were randomly assigned to one of three treatment strategies groups:
• 10 µg bFGF (n = 8);
• 100 µg bFGF (n = 8); or
• Control (n = 8).
Surgery involved bypass to the LAD coronary artery and other diseased arteries, if necessary. At the time of surgery, if a small non-graftable coronary artery was found, heparin-alginate microcapsules, which release bFGF slowly over three to four weeks, were implanted.6 At the 16-month follow-up, there was no recurrent angina or repeat revascularizations in the 100 µg bFGF group, compared to the control group, which had three reports of recurrent angina and the need for two repeat revascularizations. Significant reduction in the size of nuclear perfusion defects and reduced target ischemic zones, as shown by magnetic resonance imaging (MRI), were observed in the group administered 100 µg bFGF. Although only 24 patients were studied, the trends suggested bFGF could induce functionally significant angiogenesis.

Intracoronary injection of bFGF protein was performed in patients with stable CAD by Unger et al.7 Doses greater than 30 µg/kg were associated with hypotension and bradycardia. Compared to placebo, patients receiving bFGF had similar exercise treadmill times at one month.

One of the largest phase one studies was an open label, interpatient dose escalation study with intracoronary or intravenous infusion of recombinant FGF-2 (bFGF).8,9 The study involved 66 patients with severe CAD who were not candidates for percutaneous revascularization or bypass surgery (no option patients). At the six-month follow-up, patients showed a significant improvement in exercise time of more than two minutes. Improvements in the quality-of-life parameters for frequency of angina and exertional capacity were also observed. MRI studies showed significant improvements in wall motion in the target regions and a reduction of ischemic area.

Protein therapy, performed with varying doses of intracoronary recombinant human VEGF, was studied by Henry et al in 15 patients who were suboptimal candidates for conventional revascularization techniques.10 Nuclear perfusion imaging was performed at 30 and 60 days and seven patients underwent angiographic assessment. Investigators reported an overall improvement in perfusion in seven patients and minimal changes in the patients who had received the lowest doses. Collateralization was improved in five of the seven patients who underwent angiography. Hendel et al also evaluated the intracoronary administration of VEGF protein.11 In the study, 14 patients received various doses of recombinant human VEGF protein. Investigators reported a significant improvement in resting (but not stress) perfusion, as assessed by nuclear scintigraphy.

Gene therapy, using either naked plasmid DNA or adenovirus as a vector for VEGF, has been tested in several early clinical trials.
naked plasmid DNA encoding for the 165-isoform of VEGF (VEGF_{165}) as sole therapy via mini-thoracotomy (no CABG).\(^{12}\) All patients were found to have improved perfusion scores as assessed by nuclear imaging at 30 and 60 days and had radiographic evidence of improved collateral flow into ischemic areas on angiography. In addition, patients reported improvements in anginal class and a reduction in nitroglycerin use.

Symes \textit{et al} reported a phase one clinical trial assessing the safety and bioactivity of intramyocardially administered naked plasmid DNA encoding phVEGF_{165} in 20 patients.\(^{13}\) Patients were included in the study if they were deemed to have inoperable CAD, Canadian Cardiovascular Society (CCS) class III or IV angina, and reversible ischemia on stress sestamibi scanning. phVEGF_{165} \((125 \, \mu g, \, n = 10), \, 250 \, \mu g \, [n = 10])\) was injected through a mini left anterior thoracotomy as sole therapy. All surgeries were uneventful, however, there was one late death (at four months). A reduction in ischemic defects on single photon emission computerized tomography (SPECT) sestamibi scans was observed in 13 of 17 patients at 60 days. A total of 16 patients were followed to 90 days, and all reported a reduction in angina.

Similar results were reported by Vale \textit{et al}.\(^{14}\) Their study employed three doses of naked plasmid DNA encoding the phVEGF_{165}, which was injected into the myocardium as sole therapy in patients with symptomatic myocardial ischemia. A total of 30 patients, who were not candidates for conventional revascularization, were treated with a total dose of either 125 \(\mu g \, (n = 10), \, 250 \, \mu g \, (n = 10), \, \text{or} \, 500 \, \mu g \, (n=10)\) of phVEGF_{165}. Twenty-six \((87\%)\) of the 30 patients reported clinical improvement. Exercise tolerance (Bruce protocol) increased significantly up to 360 days post-gene delivery. Stress SPECT-sestamibi myocardial imaging was performed in 29 patients, followed to 60 days. Mean perfusion-defect scores for both stress and rest images were significantly decreased (improved) at day 60. Left ventricular (LV) ejection fraction was either unchanged \((n = 16)\) or improved \((n = 14, \, \text{mean increase in LVEF} = 5\%)\) following gene therapy.

Hendel \textit{et al} and Fortuin \textit{et al} have reported results of a dose-ranging trial examining gene transfer of VEGF-C (VEGF-2).\(^{15,16}\) VEGF-C shows 30\% homology to VEGF_{165} and is a specific ligand for the endothelial receptor tyrosine
kinases VEGFR-2 and VEGFR-3. Three doses of VEGF-C were delivered via intra-myocardial injection after mini-thoracotomy in 30 patients. Stress SPECT-sestamibi myocardial imaging was performed at baseline and at four and 12 weeks after VEGF injection. Among the 27 patients available for follow-up perfusion imaging, improvement was seen in 15 of the rest scans and in 12 of the stress scans, with evidence of a dose-dependent effect. CCS decreased from 3.6 ± 0.5 at baseline to 1.3 ± 1.0 (P < 0.005) at the 12-week follow-up and average exercise times increased from 5:55 ± 3:20 minutes to 7:56 ± 3:24 minutes (P < 0.005). At 12 weeks after treatment, endocardial electromechanical mapping demonstrated a significant improvement in myocardial contractile function without an improvement in myocardial viability, suggesting rescue of hibernating myocardium.17

Rosengart et al injected adenovirus vector containing AdGV-VEGF121.10 (VEGF121) directly into the myocardium of 21 patients, either as an adjunct to CABG (15 patients) or as stand-alone therapy via mini-thoracotomy in 30 patients.18,19 Five VEGF121 dose groups were evaluated in the adjunct therapy group (n = three patients per dose group), with total doses as follows: 4 plaque forming units (PFU) x 10^8 pfu, 4 pfu x 10^8.5 pfu, 4 pfu x 10^9 pfu, 4 pfu x 10^9.5 pfu, and 4 pfu x 10^9 pfu per patient, injected by direct visualization in the beating heart in the region of reversible ischemia identified by 99mTc-sestamibi scanning. In all patients, stress nuclear perfusion scan assessment of wall motion 30 days after therapy suggested improvement in the area of gene administration. In addition, patients who received stand-alone therapy showed improvements in angina class, exercise duration, rate-pressure product and time to ST-segment depression. Trends toward improvement in angina class and exercise treadmill testing at the six-month follow-up in the sole therapy group suggest decreased myocardial ischemia at six months.19

Collectively, these studies describe the experience of 298 patients without blinded outcome assessment. Although data from these studies cannot be used to make conclusions concerning efficacy, they firmly established the feasibility and safety of different methods of gene transfer, and have set the stage for larger randomized trials.

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Only two relatively large, randomized, double-blind, placebo-controlled studies of angiogenic therapy have been performed in humans. The FGF-2 Initiating Revascularization Support Trial (FIRST) recruited 337 patients with angina who were considered sub-optimal for traditional revascularization. Participants were randomized to three doses of intracoronary recombinant bFGF protein (0.3 µg/kg, 3.0 µg/kg and 30 µg/kg). At 90 days, there was no difference between groups in the primary end point of exercise treadmill times, or in the secondary end
points of nuclear perfusion parameters ($P = 0.64$), and quality of life indices (Seattle Angina Questionnaire [SAQ]). On post-hoc analysis, a benefit was shown in older patients (> 63 yrs) which was statistically significant ($P = 0.025$), when compared to younger patients.

The VEGF in Ischemia for Vascular Angiogenesis (VIVA) trial involved a patient cohort similar to that of the FIRST Trial, with evidence of a reversible perfusion defect on nuclear scans. Patients ($n = 178$) were assigned randomly to two doses of VEGF (17 ng/kg or 50 ng/kg) or placebo. VEGF protein was administered as a 20-minute intracoronary infusion during coronary angiography, followed by three four-hour intravenous infusions on days three, six and nine. Although no improvement was seen in the primary end point of treadmill scores after 60 days, mean CCS anginal class was significantly lower for the high-dose group, as compared to the placebo group after 120 days ($1.6 \pm 0.1$ versus $2.1 \pm 0.1$, $P = 0.04$). No safety concerns were raised in either of these landmark trials.

Although both trials were unable to demonstrate efficacy by their primary end point, several factors may account for the lack of effect. In the two randomized human trials, growth factor delivery was accomplished via an intracoronary or intravenous route. It is unclear if this method provides adequate tissue levels to stimulate and maintain angiogenesis. This is particularly true for bFGF, given the poor specificity for target endothelium. In fact, dose-ranging studies for both FGF and VEGF suggest a graded effect at higher doses.\textsuperscript{7,11} It is possible that injection into myocardium or peri-cardial fat is necessary for clinically relevant dose delivery.\textsuperscript{6}

**Issues of Study Design**

In planning controlled trials to assess the effectiveness of gene therapies, investigators must consider a number of factors. These include:

- Selection of the appropriate means of delivery for therapeutic material;
- Determination of appropriate end points to be studied;
- Quantification and objectification of the results;
- Assurance of adequate controls;
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- Selection of patients to be included;
- Determination of the mechanisms of any observed clinical effects; and
- Assessment of complications — potential, actual, local, systemic, immediate and long-term.

Delivery Modalities and Strategies

Delivery of growth factors has been accomplished using two means:
- Through the use of single or multiple doses of recombinant protein; or
- By a gene transfer approach.

Injection of naked DNA into myocardium has been shown to result in growth factor expression for a considerable period of time, without incorporation into host DNA.

Each strategy, however, has its limitations. Potential advantages for the use of proteins include the ability to adjust their dose, thereby being able to define a therapeutic window between efficacy and toxicity. This would allow withdrawal of treatment if and when necessary. Factors against the use of protein for therapeutic angiogenesis are:
- The considerable cost involved in producing significant quantities of pyrogen-free materials;
- The appearance of secondary effects (prolonged administration of bFGF is associated with a decrease in arterial pressure, moderate thrombocytopenia and moderate anemia); and
- The requirement for repeated or prolonged administration of protein.

Local perivascular delivery via myocardial injection, pericardial fat implantation of coated microspheres or pericardial instillation, has been attempted in order to address the latter limitation.

Delivery strategies for protein have recently been studied more thoroughly in experimental models. In a pig model, intrapericardial and intramyocardial delivery has been shown to result in more favorable myocardial distribution of growth factor, as compared to intracoronary or intravenous delivery. Additional data from the study indicated intrapericardial delivery was limited to the epicardial layers and required a normal pericardium.

In contrast to protein delivery, gene therapy results in the prolonged secretion of growth product by host cells, offering sustained protein levels with a single administration. The potential for extraneous uptake of the gene or vector, as well as distant, unwanted effects in non-target tissues (related either to the vector or the gene product it encodes) is of concern.

There are many means of delivering gene-coding for angiogenic products. The simplest is through the delivery of naked plasmid DNA. Injection of naked DNA into myocardium has been shown to result in growth factor expression for a considerable period of time, without incorporation into host DNA. Many facilitated means of delivery have also been studied. Liposomal encapsulation has been tested, however, current techniques are associated with low transfection efficiencies. Retrovirus encapsulation and delivery allows for effective and long-term gene
Efficacy End Points

The choice of efficacy end points for clinical trials remains an area of controversy. The ideal end point in angiogenesis trials should have the following characteristics:

• It should address the primary hypothesis and represent a direct marker of efficacy;
• It should be clinically meaningful;
• It should be easily measured and not be prohibitively costly to perform or analyze;
• It should provide insight into mechanisms; and
• It should lend itself to statistical analysis.

The end points for trials of angiogenesis can be considered either clinical (angina status, functional capacity or quality of life) or physiologic (improved myocardial perfusion, improvement in vessel collateralization, improvement in global or regional wall motion).

Objective end points such as death, myocardial infarction and repeat revascularization, can provide solid data. Results from several trials in patients with no therapeutic option, however, indicate a 5% mortality rate over two years of follow-up. A prohibitively large study population, therefore, would be required to show a reduction in mortality. One of the surrogate clinical assessments which has been considered an end point for angiogenesis trials is exercise stress-testing. The advantages of exercise testing as a clinical end point for angiogenesis trials include the following:

• It is often used in phase one and two studies and is, therefore, a familiar end point;
• The results are quantitative and semi-objective (rate pressure product, time to ST depression); and
• The findings are fairly reproducible.

The disadvantages of exercise testing as
an end point are the following:
• Comorbidities (e.g., peripheral vascular disease, chronic obstructive lung disease, arthritis) may limit exercise performance;
• Day-to-day variability exists; and
• The reasons for test termination may still be subjective.

Changes in CCS score or response to the SAQ have also been used as clinical end points in angiogenesis trials. The advantages of these types of assessments are that they are highly relevant to patients, easy to interpret, sensitive to change, fairly reproducible, and familiar to most clinicians. The disadvantages of these assessments are that they are more subjective than exercise stress testing (double-blinding is necessary), the CCS score requires observer input (SAQ doesn’t), changes in SAQ are not easily interpreted (due to a lack of familiarity with this material by clinicians) and the placebo effects are substantial.

The advantages of using the Medical Outcome Study or Health Utility Index (HUI) are that they are both broadly applicable, sensitive to change and normal values have been established for various disease states. Disadvantages of these types of analyses as end points are that they are considered to be softer end points, they are more subjective and the changes are not easily interpreted (lack of familiarity by clinicians).

One of the problems common to all clinical end points is that they are prone to placebo effects. One solution for this problem is to look for objective end points that can explain the subjective outcomes, such as a reduction in CCS class. In this regard, “angiogenesis-specific” quality-of-life or symptom-assessment tools may be necessary. An additional problem with clinical end points is that small changes may be undetected, but still may be clinically meaningful (basement effect).

Although clinical end points are employed in trials of myocardial angiogenesis, physiologic assessments are preferred as primary end points. Several physiological end points have been considered, including SPECT myocardial perfusion imaging, MRI, and positron emission tomography (PET). The advantages of nuclear scintigraphy are that it is sensitive to changes after revascularization, it is reproducible and wall motion can be assessed. There are concerns, however, over the adequacy of spatial resolution obtainable with nuclear imaging. MRI has enormous potential, and is able to provide excellent spatial resolution and information on structure, function and flow. Although MRI is gaining greater acceptance with time, prohibitive cost and restrictive availability limits its use. PET scanning is more sensitive than SPECT in measuring coronary flow reserve. It remains the only way to measure absolute blood flow. The limitations of PET imaging are the poor spatial resolution, the lack of widespread availability and cost.

Potential Complications

Angiogenic agents are thought to have the potential to induce unintended neovascularization in non-targeted tissues. Mitigating this possibility are data suggesting that only under appropriate conditions will angiogenesis occur in response to a cytokine. Both FGF and VEGF receptors are upregulated when tissues become ischemic and it would be expected that ischemic tissue would respond more sensitively to the biological effects of FGF and VEGF, as compared to normal tissues.
This concept was supported by a study in which normal and ischemic canine myocardium was exposed to high local levels of aFGF protein, administered with an epicardial sponge over a prolonged time. In this study, only ischemic myocardium responded with an angiogenic response. Although the high threshold for neovascularization in normal tissue is reassuring, there is still concern about patients who have co-existent conditions in which cytokine receptors are abnormally upregulated, such as malignant tumors and diabetic retinopathy.

There is a potential for angiogenesis therapy to trigger the growth of existent, but unrecognized, tumors. In addition to the existence of direct effects of angiogenic agents on tumor cell proliferation, there is evidence to suggest solid tumors require an angiogenic stimulus to supply nutrients required for growth beyond a critical size. Induction of angiogenesis, therefore, indirectly may contribute to the growth of dormant tumors. Angiogenic factors also may contribute to de novo tumor development. Furthermore, the mechanisms by which FGF and VEGF have the potential to stimulate neoplastic growth are relevant to their reported proatherogenic effects.

The potent vascular permeability activity of VEGF can also have unwanted consequences. Transient peripheral edema was frequently observed in studies of VEGF administered to patients with lower extremity ischemia.

There may be several reasons for the disparate results of the reported clinical trials of angiogenesis and the results from animal models. In addition to issues of dose, mechanism of delivery and end point, the choice of patient cohort to study may have confounded the clinical trials, making positive results unattainable. Unlike animal populations, the patient population of interest has demonstrated an inability to form or recruit adequate collateral vessels prior to inclusion in the trials. In addition, the response to simple growth factor delivery may differ in the presence of diffuse atherosclerosis and endothelial dysfunction, compared with the response in experimental ischemic models. Various cardiac medications and health states may inhibit and negatively impact the angiogenic response, including:

- Acetylsalicylic acid;
- Captopril;
- Lovastatin;
- Furosemide;
- Hypercholesterolemia;
- Smoking;
- Diabetes; and
- Age.

Also affecting the general outcome of clinical trial results is the recognition that patients enrolled in clinical angiogenesis trials are specifically selected on the basis of anatomy, symptoms, LV function, concurrent disease and motivation.

**Current animal studies are focusing on the mechanisms of angiogenesis, examining in particular the roles of different compounds and the local and host factors that govern their effectiveness.**
Future Research

Current animal studies are focusing on the mechanisms of angiogenesis, examining in particular the roles of different compounds and the local and host factors that govern their effectiveness. The action of angiogenic factors in the milieu of CAD is also an area of active research. The results of animal studies and early results of clinical trials suggest the delivery of a cocktail of angiogenic factors might be more effective than a single agent, and may more closely mimic physiologic angiogenic response. Finally, stem or progenitor cell transplantation may allow for the development of all components required for new myocardium and a functioning vascular network and may provide feasible therapy in the future.

References


