Clinician Guide to Angiogenesis

Neil P. Fam, MD, MSc; Subodh Verma, MD, PhD; Michael Kutryk, MD, PhD; Duncan J. Stewart, MD

Case Presentation: J.A. is a 63-year-old man with type II diabetes and hypercholesterolemia. He has suffered 2 previous myocardial infarctions and has chronic angina. Previous angiography showed diffuse 3-vessel coronary artery disease not amenable to conventional revascularization. What are the current concepts surrounding the use of therapeutic coronary angiogenesis in this setting?

Since recognition of the central role of angiogenic factors in tumor growth over 30 years ago, physiological and pathological angiogenesis has been implicated in diverse conditions, including vascular insufficiency, inflammation, and diabetic retinopathy. Despite considerable advances in medical therapy and improvements in revascularization procedures for coronary artery disease, a substantial proportion of patients suffer from refractory angina or recurrent myocardial ischemia requiring hospitalization. In the past decade, a number of clinical trials have examined the role of therapeutic angiogenesis for myocardial ischemia. In this article, we focus on the fundamental mechanisms of angiogenesis and discuss current and future issues in therapeutic coronary angiogenesis.

Principles of Angiogenesis

Three distinct mechanisms of new blood vessel formation have been identified: Vascularization, angiogenesis, and arteriogenesis. Vascularization refers to the formation of blood vessels from endothelial progenitor cells, a process that was initially described as occurring during embryonic development, and more recently, in adult animals. Angiogenesis involves the sprouting of new capillaries from preexisting vessels, whereas arteriogenesis refers to remodeling of newly formed or preexisting vascular channels into larger and well-muscularized arterioles and collateral vessels. The generation of new vascular channels by angiogenesis and arteriogenesis has been shown in both animal models of myocardial ischemia and in patients with coronary disease. Importantly, the formation of collateral vessels in acute and chronic coronary occlusion may preserve perfusion to ischemic myocardium and thereby maintain myocardial function.

Angiogenesis is a dynamic process of endothelial proliferation and differentiation. The formation of a functioning vasculature requires the orchestrated interaction of endothelial cells, extracellular matrix, and surrounding cells. The major physiological stimuli for angiogenesis include tissue ischemia and hypoxia, inflammation, and shear stress. A number of specific factors are known to stimulate or inhibit angiogenesis, including vascular growth factors, inflammatory cytokines, adhesion molecules, and nitric oxide. Importantly, regulation of these factors in both spatial and temporal domains is critical to efficient neovascularization, and different biological activities are required in the different phases of angiogenesis from initiation to maturation (Figure).

Vascular Growth Factors

An ever-expanding arsenal of mediators has been implicated in the process of angiogenesis. The prototypical angiogenic factor, vascular endothelial growth factor (VEGF), is a circulating glycoprotein that promotes blood vessel growth in response to ischemia and other stimuli. The VEGF family includes VEGF-A (VEGF-1, 6 known isoforms), VEGF-B (VEGF-3), VEGF-C (VEGF-2), VEGF-D, VEGF-E, and placental growth factor (PIGF). VEGF-A and -C bind and activate VEGF receptor-2 (VEGFR-2 or flk-1/kdr), a receptor tyrosine kinase expressed by endothelial cells and endothelial progenitors. In addition to stimulating...
Regulation of angiogenesis. In response to stimuli such as hypoxia, VEGF induces vasculogenesis and endothelial cell proliferation. Ang1–Tie2 interactions mediate vessel maturation and maintain vessel integrity through the recruitment of peri-endothelial cells. Ang2 blocks Ang1–Tie2 signaling, loosening vascular structure and exposing the endothelium to inducers of angiogenesis such as VEGF. In the presence of VEGF, endothelial cells migrate and proliferate to form new capillary sprouts and blood vessels. Ang2 expression in the absence of VEGF stimulation leads to vessel regression and apoptosis. RTK indicates receptor tyrosine kinase. Modified from reference 6.

angiogenesis, VEGF increases the circulating pool of endothelial progenitor cells. The angiopoietins represent another major family of angiogenic factors. The 2 best characterized angiopoietins, Ang1 and Ang2, are ligands for the Tie2 receptor tyrosine kinase that is present on endothelial cells and endothelial progenitor cells. These growth factors work in concert with VEGF and other angiogenic molecules such as the ephrins to effect and modulate blood vessel formation.

On the basis of elegant mouse gene knockout experiments, a dynamic model of blood vessel formation has been proposed (Figure). In response to stimuli such as hypoxia and tissue injury, VEGF induces endothelial cell migration and proliferation and initiates blood vessel formation. In contrast, Ang1–Tie2 interactions mediate neovessel maturation into more complex and larger vascular structures and maintain vessel integrity through the recruitment of peri-endothelial cells and the reestablishment of basement membrane. However, in quiescent mature blood vessels, Ang1 signaling serves to inhibit endothelial cell activation and thus may impede the efficient initiation of the angiogenic response in the face of hypoxia or tissue injury. Ang2 (an endogenous antagonist of Tie2) functions to block this Ang1–Tie2 signaling, facilitating endothelial activation in response to inducers of angiogenesis, such as VEGF. Under certain conditions, Ang2 expression, especially in the absence of VEGF, may lead to vessel regression by interfering with tonic Ang1 endothelial cell survival signaling via Tie2. However, data from our group and others suggest that Ang2 may play a more complex role, as it can also acquire Tie2 agonist activity after extended periods of exposure or at higher concentrations. Thus, the local balance of growth factors and the temporal pattern of their expression can critically influence the vascular microenvironment toward either vessel stability and maturation or angiogenesis and new vessel growth. This concept is particularly relevant to clinical trials of vascular growth factors for therapeutic coronary angiogenesis, which have thus far used only single growth factors with arguably modest results.

**Nitric Oxide**

Nitric oxide (NO) is thought to mediate angiogenesis via multiple mechanisms. Using in vitro models of angiogenesis, Hood and colleagues established the importance of NO by showing that capillary formation is prevented by inhibitors of NO synthase, and that VEGF upregulates en-
endothelial NO synthase expression and NO production. Similarly, NO has been shown to be an important downstream mediator of other vascular factors including basic fibroblast growth factor (bFGF), transforming growth factor (TGF)-β, and angiopoietin-1. Moreover, NO stimulates endothelial proliferation and migration, functions as an endothelial survival factor, and may augment angiogenesis through vasodilation-induced increases in local blood flow. In addition, our group demonstrated an important paracrine role for endogenously produced NO in promoting angiogenesis. Conversely, impaired NO production decreases the angiogenic response, as illustrated by 2 studies. Matsunaga and colleagues demonstrated that the anti-angiogenic effects of the endogenous angiogenesis inhibitor angiotatin are augmented in conditions of impaired NO production. In a study by Verma and colleagues, the inflammatory marker C-reactive protein (CRP) was shown to inhibit endothelial nitric oxide synthase expression and NO production, as well as basal and VEGF-induced angiogenesis.

Endothelial Dysfunction and Angiogenesis

These findings emphasize the critical role of NO in angiogenesis. The concept of impaired NO production and endothelial dysfunction as a common link between traditional vascular risk factors (such as advanced age, hypercholesterolemia, diabetes, and smoking) and atherosclerosis is particularly important, as many of these risk factors may also impair or inhibit the angiogenic response. Indeed, angiogenesis may be viewed as another facet of normal endothelial function. Thus, patients who are candidates for current coronary angiogenesis trials may be the least likely to respond to treatment. Aggressive risk factor modification as a strategy to improve endothelial dysfunction and restore the angiogenic response should be advocated.

The commonly prescribed lipid-lowering medications hydroxymethylglutaryl coenzyme-A reductase inhibitors (statins) have been reported to be pro-angiogenic at lower therapeutic doses and anti-angiogenic at higher doses. Although to some extent the beneficial effects of statins can be attributed to an improved lipoprotein profile, there is new evidence that these agents may have direct effects on endothelial function that are independent of circulating lipids, resulting in differential actions on endothelial growth and apoptosis. Low-dose statin therapy may promote angiogenesis via multiple mechanisms, including enhanced NO production, augmented VEGF release, and activation of the Akt signaling pathway. In addition, statins also increase endothelial progenitor cell (EPC) mobilization and accelerate reendothelialization after vascular injury. Thus, statin therapy may be an effective means of improving endothelial function and enhancing the angiogenic response in patients undergoing therapeutic coronary angiogenesis.

Angiotensin-converting enzyme (ACE) inhibitors are another powerful class of medications with significant effects on endothelial function. ACE inhibitors block the generation of angiotensin II and prevent the breakdown of bradykinin, an important mediator of NO release. ACE inhibitors improve endothelial dysfunction in patients with coronary disease and have been shown to stimulate angiogenesis and collateral vessel formation in a rabbit ischemic hindlimb model.

Clinical Trials of Therapeutic Coronary Angiogenesis: An Overview

The goal of therapeutic angiogenesis is to stimulate new blood vessel growth in the adult heart and thereby improve myocardial perfusion and function. A growing number of angiogenic factors have been applied in human clinical trials, using a variety of delivery strategies, including intravenous, intracoronary, intrapericardial, and intramyocardial approaches. Four large randomized placebo-controlled trials of coronary angiogenesis have been reported to date (Table). The FIRST study (FGF-2 Initiating Revascularization Support Trial) recruited 337 patients with chronic angina who were ineligible for mechanical revascularization and randomized them to 3 doses of intracoronary bFGF protein versus placebo. There were no significant differences between the groups after 90 days for the primary end points of exercise time, nuclear perfusion findings or quality of life, although post hoc analysis suggested a significant improvement in exercise time in patients over 65 years of age with severe angina.

The VIVA trial (VEGF in Ischemia for Vascular Angiogenesis) randomized 178 patients with reversible nuclear perfusion defects to receive single dose intracoronary VEGF-1 protein, followed by 3 intravenous doses or placebo. After 60 days, there were no significant differences in exercise time or angina class between the groups. At 120 days, however, there was a significant improvement in angina class in the high dose VEGF group. Although patients experienced less angina, there was no objective improvement in nuclear perfusion or angiography.

The findings of the AGENT (Angiogenic GENE Therapy) trial were published in 2002. The investigators randomized 79 patients with chronic stable angina to receive a single intracoronary dose of replication-defective adenovirus containing the FGF4 gene (Ad5-FGF4). Single intracoronary injections of Ad5-FGF4 were well tolerated with no immediate adverse events, although transient asymptomatic elevations in liver enzymes occurred in 2 patients and fever of less than 1 day’s duration occurred in 3 patients. After 4 and 12 weeks, there were no significant differences in exercise treadmill testing (ETT) between the groups. However, a protocol-specified subgroup analysis showed a significant improvement in ETT in patients with a baseline ETT
### Large Randomized Placebo-Controlled Trials of Therapeutic Coronary Angiogenesis

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<th>Trial</th>
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<td>FIRST</td>
<td>337</td>
<td>Chronic angina, ineligible for revascularization</td>
<td>Intracoronary FGF-2 protein</td>
<td>90 days to 6 months</td>
<td>No significant difference in exercise time or nuclear perfusion. Post hoc analysis suggested significant improvement in patients &gt;65 with severe angina.</td>
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<tr>
<td>VIVA</td>
<td>178</td>
<td>Chronic angina, ineligible for revascularization, reversible perfusion defect</td>
<td>Intracoronary VEGF-1 protein</td>
<td>60 and 90 days</td>
<td>Significant improvement in angina class at day 120 in high dose VEGF group. No difference in nuclear perfusion or angiography.</td>
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<tr>
<td>AGENT</td>
<td>79</td>
<td>Chronic angina, ST shift on ETT; 1, 2, or 3-vessel CAD, 1 vessel&lt;70%</td>
<td>Intracoronary FGF4 gene, adenoviral vector</td>
<td>4 and 12 weeks</td>
<td>No significant difference in ETT. Subgroup analysis showed significant improvement in ETT in patients with prolonged baseline ETT.</td>
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<tr>
<td>REVASC</td>
<td>71</td>
<td>Chronic angina, ineligible for revascularization</td>
<td>Intramyocardial VEGF121 gene, adenoviral vector</td>
<td>12 and 26 weeks</td>
<td>Significant improvements in exercise treadmill time to additional 1-mm ST depression at 26 weeks and exercise time to angina, total exercise duration, and CCS class at 12 and 26 weeks.</td>
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FIRST indicates FGF-2 Initiating Revascularization Support Trial18; VIVA, VEGF in Ischemia for Vascular Angiogenesis19; AGENT, Angiogenic Gene Therapy20; REVASC, Randomized Evaluation of VEGF for Angiogenesis in Severe Coronary Disease; FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; ETT, exercise treadmill testing; CAD, coronary artery disease; and CCS, Canadian Cardiovascular Society.

≤10 minutes in the Ad5-FGF4 treated group.

The REVASC study (Randomized Evaluation of VEGF for Angiogenesis in Severe Coronary disease) is the first large randomized gene therapy trial to demonstrate sustained clinical benefit in patients with severe coronary disease.21 The study randomized 71 patients with advanced angina and no options for conventional revascularization to receive either replication-defective adenovirus containing the VEGF121 gene (AdVEGF121) in 30 direct intramuscular injections throughout the free wall of the left ventricle via mini-thoracotomy or continuation of optimal medical therapy. Because this trial could not be blinded, an objective measure of exercise-induced myocardial ischemia was used for the primary analysis: ETT to additional 1-mm ST depression. This was significantly improved in the AdVEGF121 group compared with the control group at 26 weeks, but not after 12 weeks. In addition, exercise time to angina, total exercise duration, and Canadian Cardiovascular Class (CCS) showed significant improvement at both 12 and 26 weeks. Similarly, significant improvements were seen in all 5 functional domains of the Seattle Angina Questionnaire at weeks 12 and 26. There were no significant differences in adverse events between groups. Serious cardiac events were attributed to the mini-thoracotomy/AdVEGF121 injection procedure in 4 patients. This trial demonstrated sustained and continuous clinical improvement from 3 to 6 months and serves as a proof-of-concept study for angiogenic myocardial gene delivery in patients with severe coronary disease. Given the risks associated with mini-thoracotomy, newer catheter-based strategies may provide a lower risk approach to myocardial gene delivery and allow more rigorous trial design.

#### Current Approaches to Therapeutic Coronary Angiogenesis

The clinical trials just described have raised several questions regarding the optimal dose and route of delivery of angiogenic growth factors. The delivery of growth factors as proteins via the intravenous or intracoronary route may be insufficient to provide adequate local tissue levels necessary to promote and sustain clinically relevant angiogenesis.8,14 These concerns have led to the use of gene therapy approaches to achieve longer-term tissue production of biologically active growth factors. Although there is less experience with gene transfer for therapeutic angiogenesis, it has the advantage of sustained production of growth factor by host cells.

A preliminary phase 1/2 placebo-controlled, double-blind study by Losordo and colleagues22 randomized 19 patients with CCS class III-IV angina who were not candidates for surgical revascularization or percutaneous coronary intervention to receive intramyocardial injections of either naked VEGF-2 plasmid DNA or placebo. They used a percutaneous catheter-based approach with left ventricular electromechanical (NOGA) mapping to guide endocardial injections into targeted regions of ischemic myocardium. Patients received a total of 6 injections at one time, and were followed-up for 12 weeks. Despite the small sample size, there was a significant improvement in the primary end point of CCS angina class in the VEGF-2 treated patients, with a strong trend toward improvement in exercise time and quality of life. The VEGF-2–treated patients also exhibited objective improvements in nuclear perfusion and electromechanical mapping parameters. Importantly, there were no reported complications in the study. Given this encouraging data, larger randomized trials have been undertaken.
Potential Complications of Therapeutic Angiogenesis

Although current efforts are focused on targeting angiogenesis to ischemic myocardium, there exists the theoretical risk of unwanted blood vessel growth in adjacent or distant tissue sites. In addition, VEGF is known to increase vascular permeability and tissue edema and to cause hypotension, whereas FGF therapy is associated with proteinuria. Broader safety concerns include the possibility of accelerating occult tumor growth, diabetic retinopathy, or atherosclerosis. Despite these concerns, no increased risk of aberrant neovascularization has been reported, although long-term clinical follow-up is needed.

Endothelial Progenitor Cells

EPCs represent an exciting avenue for therapeutic coronary angiogenesis. Asahara et al and Takahashi et al have demonstrated that bone marrow-derived EPCs present in peripheral blood home to and incorporate into sites of neovascularization, with an associated enhancement of capillary density and collateral vessel formation. Moreover, EPC transplantation has been shown to have beneficial effects in experimental models, and more recently, in humans. In 2 phase 1 trials, selective intracoronary transplantation of autologous mononuclear bone marrow cells (BMCS) or circulating blood-derived EPCs was used as an adjunct to standard therapy for acute transmural myocardial infarction. In both studies, patients underwent angioplasty and stenting of the infarct-related artery within 12 to 24 hours of symptom onset. Four to 9 days after MI, autologous BMCS or circulating EPCs were isolated and cultured and then selectively infused into the infarcted territory by using a balloon angioplasty technique. After 3 to 4 months of follow-up, there was a significant reduction in infarct size and a significant improvement in wall motion, stroke volume index, and myocardial perfusion in cell therapy patients compared with baseline as well as with the standard therapy group. Similar results were observed for both the BMC and circulating EPC groups. Importantly, there were no significant adverse events in any of the patients receiving cell therapy. Although preliminary, these novel results are compelling and need to be confirmed in large randomized controlled trials.

The potential applications for EPCs in therapeutic angiogenesis are considerable. As discussed earlier, vascular risk factors such as hypercholesterolemia, diabetes, and aging impair the normal angiogenic response, potentially diminishing the efficacy of angiogenic gene or protein therapy in patients with severe coronary disease. A combined approach using EPC transplantation and angiogenic gene or protein therapy might augment coronary neovascularization by providing an endothelial substrate that is better able to respond to vascular growth factors. Alternatively, a strategy of transplanting EPCs transfected with genes encoding for angiogenic factors may facilitate targeted angiogenesis of ischemic myocardium.

Conclusions

Novel and promising options are being explored for the treatment of patients with refractory myocardial ischemia who are not candidates for conventional revascularization by percutaneous coronary angioplasty or coronary artery bypass grafting. Although questions regarding safety, the optimal dose, route of delivery, and combination of angiogenic factors remain, these issues do not appear to be insurmountable. Moreover, recent fundamental insights into the underlying biology of vascular growth factors and endothelial progenitor cells may soon translate into improved clinical outcomes in ongoing trials of therapeutic coronary angiogenesis.


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