Therapeutic Angiogenesis: A Treatment for the New Millennium or Passing Fad?

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Angiogenesis is defined as the growth and proliferation of blood vessels from existing vascular structures. Currently, in cardiovascular medicine, our interests are focused on “therapeutic angiogenesis,” a process that seeks to treat disorders of inadequate tissue perfusion by promoting the growth and proliferation of blood vessels or the modulation of endothelial function. The presentation of therapeutic angiogenesis in this issue of Cardiology Rounds is divided into 5 parts:

Part 1: An overview

Part 2: Clinical trials of therapeutic angiogenesis in coronary artery disease

Part 3: Clinical trials of therapeutic angiogenesis in peripheral arterial disease with an emphasis on the TRAFFIC study

Part 4: Therapeutic angiogenesis “outside the box”

Part 5: Conclusions

No discussion on therapeutic angiogenesis would be appropriate without honoring the person who was clearly the leader in this field, Dr. Jeffery M. Isner, who passed away on October 31, 2001 at the age of 53. Jeff was the consummate Clinician/Scientist and without question, the pioneer of this area. On a personal note, Jeff was my first cardiology attending physician when I was an intern only 4 weeks out of medical school. Over the years, Jeff served as my advisor, and increasingly, as my colleague and friend. The pain of Jeff’s absence is still palpable. Jeff would have wanted us to carry on and continue to advance the field that he forever changed with his insights and passion.

Part 1: An overview

Over the past two decades, a number of cytokine growth factors and the corresponding receptors that appear to play a role in angiogenesis have been identified and methods to modulate angiogenesis have become an area of great interest and excitement. Perhaps the most extensively studied angiogenic cytokines are vascular endothelial growth factor (VEGF) and basic and acidic fibroblast growth factor (bFGF and aFGF). Prior to reviewing the human studies that have been conducted using angiogenic growth factors to promote therapeutic angiogenesis, two facts are worth noting.

First, in some fields of medicine, angiogenesis is noted more for its association with pathologic disease processes (Table 1) as opposed to its potential therapeutic benefits. An exciting and active area of investigation in cancer employs antiangiogenic factors to reduce tumor mass. Moreover, there are reports that link angiogenesis to atherosclerosis. Therefore, in some respects, we are attempting to employ a process that may well be involved in advancing a pathologic disease that has end-stage symptoms we are attempting to treat.

Secondly, an understanding of the complexity inherent in the angiogenesis system is also worth considering. VEGF and its receptor(s) will be used as an example. VEGF is a family of at
least 5 genetically distinct, but structurally related, proteins plus placental growth factor. VEGF (also known as VEGF-A) is a prototypic, angiogenic agent that causes endothelial proliferation in vitro and angiogenesis in vivo. VEGF-A exists in at least four isoforms (121, 165, 189, or 206 amino acids) that are produced by alternative splicing of a single mRNA transcript. VEGF exists as dimers that include both homodimers (ie, VEGF121-VEGF121 or VEGF165-VEGF165) and heterodimers (ie, VEGF121-VEGF165). All of these forms of VEGF are biologically capable of mediating receptor-specific interactions. The smallest isoform lacks a heparin-binding domain and is freely soluble. Despite the known differences in heparin affinity, relatively little is known about any biologic differences amongst the VEGF isoforms. In addition, VEGF-B and VEGF-C are expressed (predominantly) in cardiac and peripheral skeletal muscle. VEGF-C is also expressed in lymphatic tissue. VEGF-B is also referred to as VEGF-3 and VEGF-C is also referred to as VEGF-2. VEGF-B can interact with VEGF-A and VEGF-B with VEGF-C. VEGF-D is expressed in the lung and in muscle and VEGF-E has no known mammalian homologue.

These numerous permutations and combinations of VEGF must then interact with a family of tyrosine kinase receptors that are found almost exclusively on vascular endothelium. There are three known VEGF receptors:

- VEGFR-1 (often referred to as flt-1)
- VEGFR-2 (often referred to as flk-1 or kdr)
- VEGFR-3 (also referred to as flt-4).

Expression of flt-4 is found in lymphatic endothelium. As shown in Figure 1, the VEGF ligands have variable affinity for the VEGF receptors, (eg, VEGF-A will bind to both VEGFR-1 and VEGFR-2; VEGF-B binds to VEGFR-1, but not to VEGFR-2; VEGF-C will bind to both VEGFR-2 and VEGFR-3, but not to VEGFR-1.) To further complicate the picture, soluble receptors (receptors that bind ligands, but lack the intracellular part of the protein that is needed for intracellular signaling) can play a role in modulating angiogenesis. As well, integrins located on the cell membrane are capable of amplifying or dampening the signaling that results from ligand receptor binding. Although some data are available regarding the changes that occur in a few of the VEGF ligands and receptors in controlled, pre-clinical models of hindlimb and myocardial ischemia, far less is known about the changes that occur in humans in response to ischemia. Moreover, the VEGF system is relatively simple compared to the FGF system that contains far more ligands and receptors. Given the complexities that exist in the system, it could be considered remarkable if a single agent given at single time would be able to modulate the angiogenic response in humans.

**Part 2: Clinical trials of therapeutic angiogenesis in coronary artery disease**

Ischemic heart disease is the leading cause of morbidity and mortality in the Western World. Each year, in the United States alone, over a million people undergo coronary artery bypass surgery or percutaneous revascularization. Despite improvements in surgical methods, catheter technologies, and pharmacologic therapies, many patients continue to suffer from symptoms related to atherosclerotic vascular disease. Many patients are not optimal candidates for surgical or catheter-based revascularization because of co-morbid diseases, unsuitable anatomy, or the risks of the procedure. This pool of patients is likely to increase as the population ages and more patients with prior bypass surgery present with symptoms of ischemic heart disease. A number of agents, including VEGF and bFGF, have been able to improve perfusion and reduce ischemia in pre-clinical models of myocardial ischemia.

Although several phase I angiogenesis studies have been conducted in patients with advanced ischemic heart disease with encouraging results, this discussion will be limited to the two completed phase II, randomized, double-blind, placebo-controlled trials.

**Table 1: Some pathologic conditions where angiogenesis plays a role**

- Diabetic retinopathy and age-related macular degeneration
- Non-osteoarthritis
- Oncology
- Atherosclerosis

**Figure 1: Diagram of the majority of ligands and receptors that are involved in the VEGF system. NRP = neuropilin receptor, sVEGFR-1 = soluble VEGF receptor, PLGF = placental growth factor**
The VIVA study

The VIVA study (VEGF in Ischemia for Vascular Angiogenesis) was completed in 1999. VIVA was a double-blind, placebo-controlled trial designed to evaluate the safety and efficacy of combined intracoronary and intravenous infusions of rhVEGF for therapeutic angiogenesis. In total, 178 patients with stable exertional angina who were considered unsuitable for standard revascularization were randomized to receive placebo, low dose rhVEGF (17 ng/kg/min), or high dose rhVEGF (50 ng/kg/min) by intracoronary infusion for 20 minutes on Day 0, followed by 4-hour intravenous infusions on Days 3, 6, and 9. Exercise treadmill tests, angina class, and quality of life (QOL) assessments were performed at baseline, Day 60, and Day 120, and myocardial perfusion imaging was performed at baseline and Day 60. The primary endpoint, change in exercise time from baseline to Day 60, was no different between the three groups. Indeed, exercise time, angina class, and QOL measures were improved in the placebo, as well as in the treatment groups, without a significant change in myocardial perfusion or function. Interestingly, 3 patients developed a malignancy despite the extensive cancer screening that was performed prior to randomization, and all 3 of these patients were in the placebo group. This demonstrates the need for placebo-controlled trials. By Day 120, there was a small, but not statistically significant, difference in the change in exercise time between the placebo and VEGF-treated patients, and the high-dose rhVEGF treated patients had an improvement in angina class (P=0.05) compared with placebo. The VIVA Study demonstrated the safety of the VEGF regimen that was used and the need for placebo controls.

The FIRST Study

The other phase II angiogenesis trial that was completed in patients with ischemic heart disease was FIRST (The FGF-2 Initiating Revascularization Support Trial). FIRST was a multicenter, randomized, double-blind, placebo-controlled trial of a single intracoronary infusion of bFGF at 0, 0.3, 3, or 30 µg/kg. In total, 337 patients were randomized into these 4 groups. Efficacy was evaluated at 90- and 180-days by exercise tolerance test and myocardial nuclear perfusion imaging. The Seattle Angina Questionnaire (SAQ) and Short-Form 36 (SF-36) were used to assess changes in quality of life. Similar to VIVA, exercise tolerance was increased to a similar degree at 90 days in all groups and was not significantly different between the placebo- and FGF-treated groups. However, at 90 days, the bFGF-treated patients did have reduced angina symptoms as measured by the angina frequency score (AFS) of the SAQ (overall p = 0.035) and the Physical Component Summary Scale (PCSS) of the SF-36 (pair-wise p = 0.033, all FGF groups vs. placebo).

These differences were more pronounced in highly symptomatic patients and were no longer apparent at 180 days due to continued improvement in the placebo group. Significant adverse events were similar across all groups. This study demonstrated that bFGF, given as a single intra-coronary infusion was well tolerated, and although it did not improve exercise tolerance or myocardial perfusion, there were symptomatic improvements at 90 (but not at 180) days.

Part 3: Clinical trials of therapeutic angiogenesis in peripheral arterial disease

Peripheral artery disease (PAD) affects up to 15% of adults >55 years. One-third of patients with PAD are estimated to have typical intermittent claudication (IC), exertional muscular leg pain that is relieved promptly by rest. Patients with IC are frequently symptomatic despite optimal conservative therapy. These patients are quite symptomatic. Approximately one-third of patients with IC cannot walk one city block and an additional one-third experience leg pain during routine activities at home. Revascularization for patients with IC may be appropriate for focal or aorto-iliac obstruction but, in general, revascularization is less desirable or unfeasible in the presence of diffuse, infra-inguinal obstructions.

TRAFFIC

TRAFFIC (Therapeutic Angiogenesis with Recombinant FGF-2 in Intermittent Claudication) was a Phase II, randomized, multicenter, double-blind, placebo-controlled, regimen-finding study of 1 or 2 intra-arterial infusions of rFGF-2 or placebo. Robert J. Lederman and I were the principle investigators. The vast majority of the investigators were from the Peripheral Atherosclerosis Research Consortium (PARC, www.arterial.org). The Duke Clinical Research Institute was the Coordinating Center. The sponsor of the TRAFFIC study was Chiron Corporation (Emeryville, CA).

The selected agent was bFGF or rFGF-2; the dose (30 µg/kg) was the maximum tolerated dose, limited by acute hypotension, in phase I studies of intracoronary infusion and the dose that was tolerated after peripheral artery infusion. The target population was patients with moderate to severe IC due to infra-inguinal atherosclerotic disease. The major inclusion and exclusion criteria for entry into the TRAFFIC study can be found in Table 2 and additional details on the study design can be found in the references for Part 3. TRAFFIC employed both clinical and anatomic criteria in an attempt to maximize patient homogeneity. In total, 190 patients were randomized to:

- PLACEBO (bilateral intra-arterial placebo on Day 1 and Day 30)
- SINGLE (bilateral intra-arterial bFGF on Day 1 and placebo on Day 30), or
• DOUBLE (bilateral intra-arterial bFGF on Day 1 and Day 30).

The primary endpoint of the study was change in peak walking time (PWT) from baseline to Day 90. Compared with baseline, patients in the PLACEBO group increased PWT by 0.60 minutes, SINGLE by 1.77 minutes, and DOUBLE by 1.54 minutes. By analysis of variance (ANOVA), the differences of log-transformed data for the 174 patients with paired baseline and day-90 PWT assessment did not achieve statistical significance (p=0.075), but the intention-to-treat demonstrated a significant difference between the 3 groups (p=0.034). In addition, pair-wise analysis demonstrates a significant increase in PWT in the SINGLE versus the PLACEBO group (p=0.026). DOUBLE was not superior to SINGLE. There was also a small improvement in the ankle-brachial systolic blood pressure index (ABI) in both rFGF-2 groups at 90 days compared with baseline (ΔABI –0.01 for placebo, 0.06 for SINGLE, 0.06 for DOUBLE; pair-wise p=0.037 for SINGLE versus placebo, and p=0.047 for DOUBLE versus placebo). Full details on the results of TRAFFIC will be published in the near future. When taken together, the increase in PWT and ABI provides the most compelling evidence to date, in a sizeable, randomized, double-blind, placebo-controlled trial, to suggest therapeutic angiogenesis in humans.

At present, a number of therapeutic angiogenesis agents are in Phase I and Phase II clinical trials in patients with peripheral arterial disease. Many of these studies are examining plasmid DNA and adenoviral-mediated, gene delivery. TRAFFIC demonstrated that protein therapy still has the potential to be effective. Over the next year or two, additional details on the designs of these trials and preliminary data will become available in this exciting area.

Table 2: The major inclusion and exclusion criteria used in TRAFFIC

<table>
<thead>
<tr>
<th>INCLUSION</th>
<th>EXCLUSION</th>
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<tbody>
<tr>
<td>• Age &gt; 40 years</td>
<td>• Suspcion of malignancy (screening per ACS guidelines)</td>
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<tr>
<td>• Exercise limited by claudication</td>
<td>• Creatinine &gt; 2.0 mg/dL</td>
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<tr>
<td>• Paired baseline PWTs within 20% of each other</td>
<td>• Urine protein ? 2+ or &gt; 300 mg/day</td>
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<tr>
<td>• Index ABI &lt; 0.8 at rest</td>
<td>• Proliferative retinopathy</td>
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<tr>
<td>• Patent femoral inflow</td>
<td>• Other conditions impacting safety or compliance</td>
</tr>
<tr>
<td>• Medically stable for &gt; 4 mos</td>
<td>• Informed consent</td>
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Part 4: “Unconventional” approaches for therapeutic angiogenesis

Ultimately, some of the most frequent uses of therapeutic angiogenesis agents may not reside solely in their ability to make blood vessels grow. Endothelial cells in the vascular endothelium play a central role in the regulation of vascular tone and vasomotor reactivity. Endothelial cells in the adult vascular bed produce nitric oxide and thereby interact with other layers of vascular cells. An array of pathophysiologic stimuli including, but not limited to, hypertension and hypercholesterolemia, cause endothelial injury that disturbs the interactions between the endothelium and other layers of the blood vessels. This results in endothelial dysfunction. Conversely, a deficiency in normal endothelial function, including the maintenance of normal vascular tone, contributes to the pathogenesis of a number of diseases including diabetes mellitus and atherosclerosis. Both in vitro and in vivo angiogenic growth factors have the ability to protect endothelial cells from injury. When extended to cells other than endothelial cells, angiogenic growth factors have the ability to improve the survival of other cells types. These could include, for example, cardiomyocytes and skeletal muscle.

Endothelial cell-mediated venous-occlusion, by vascular smooth muscle in the corpus cavernosum, is the principle vasomotor event that is required for normal erectile function and nitric oxide is a mediator of this endothelial-smooth muscle cell interaction. Endothelial dysfunction is now well-recognized as the major cause of erectile dysfunction. Endothelial dysfunction and atherosclerotic vascular disease share common epidemiologic risk factors such as hypertension, diabetes mellitus, tobacco use, and hypercholesterolemia. In men, the magnitude of cholesterol elevation is directly correlated with the risk of erectile dysfunction. Hypercholesterolemia causes endothelial injury and a loss of integrity, even in the absence of gross structural changes. In hypercholesterolemic animal models, there is dose- and time-dependent endothelial injury (Figure 2). This is accompanied by a quantifiable abnormality in endothelial-dependent vasomotor reactivity since the ability of acetylcholine (ACH) to produce smooth muscle relaxation is dependent on the presence of an intact endothelium.

In collaboration with Dr. Craig Donatucci from the Division of Urology, and Dr. Per-Otto Hagen from the Division of Experimental Surgery at Duke, we have begun to explore the role of angiogenic growth factors in erectile function. Pre-clinical models of ath-
Angiogenesis is a fundamental biologic process and further understanding and exploration of endothelial cell growth, proliferation, and function will offer the opportunity to markedly advance and perhaps revolutionize therapies for patients with cardiovascular disease. Therapeutic angiogenesis offers the promise of new treatment strategies to treat hundreds of thousands of patients with ischemic heart and peripheral disease, while the TRAFFIC study provides the most promising evidence to date for therapeutic angiogenesis in humans. With further research and refinements, I believe that angiogenic therapy will become a major part of our therapeutic armamentarium in cardiology.

Editor’s note: Dr. Annex appropriately acknowledges his mentor, Dr. Jeffrey M. Isner, for his pioneering leadership in both the animal and early clinical studies of therapeutic angiogenesis in cardiovascular disease. Dr. Isner was a highly productive and innovative researcher and deeply respected for his zeal, compassion, and integrity. The most fitting tributes to Dr. Isner’s scientific contributions will be realized in the years ahead when his trainees, colleagues, and others around the world determine the full clinical benefits of therapeutic angiogenesis.
Selected references – Part 1

Selected references – Part 2

Selected references – Part 3

Selected references – Part 4

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