A Renewed Emphasis on Therapeutic Angiogenesis Research for CAD

Up to now, unblocking clogged arteries for so-called no-option patients with coronary artery disease or congestive heart failure hasn't been possible. Angiogenesis research, despite previous setbacks, may be the answer for this group of patients.

By Jack Jacobs, PhD

Any experienced traffic cop knows that an overturned truck blocking a major artery into a city requires a rapid, two-pronged response. The ultimate goal is to right and remove the truck, thus relieving the backup. In the meantime, diverting traffic away from the clogged artery and identifying a free-flowing detour into the city can alleviate the crisis by keeping traffic moving, allowing commuters to get to their jobs to provide the life force needed to keep the city functioning at peak capacity.

A physician treating a patient with blocked coronary arteries due to atherosclerosis has much in common with a traffic cop. Ultimately the physician would like to clear the blocked arteries and reinstate maximal blood flow to the heart muscle that is being starved of oxygen and nutrients.

Several options are available to achieve this goal: pharmaceutical approaches to combat atherosclerotic plaque build-up; surgical bypass procedures to graft functioning arteries from other parts of the body onto the heart; balloon-based and laser techniques to clear the blockages; and the use of stents, plain or medicated, to keep the treated passages open (See "The Scope of CVD and the Economics of Angiogenesis Therapy," page 51). These options vary in effectiveness, risk, cost, and outcomes. No one solution is the answer for every patient, but for a significant share (5 to 21 percent) of patients with coronary artery disease (CAD) or congestive heart failure (CHF), there are no options (Lee 2006).


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So-called "no-option" heart patients, who have refractory symptoms despite maximal medical therapy, may have microvascular disease — more commonly seen in women — for which no main target for blockage-busting therapy exists. They also may suffer from comorbidities or physiologic complications that make them poor candidates for available treatments, or have disease that is too mild to warrant traditional bypass or artery-opening procedures (Mukherjee 2004).

FAILURE AND HOPE

No-option patients are ideal candidates for therapeutic angiogenesis — a proven and safe method of stimulating the natural process of new blood vessel formation (Simons 2005, Ruel 2004). The vessels formed are not different from those already present in the body. Early concerns that angiogenesis-inducing growth factors would promote tumor growth have not been supported by clinical study results. Although angiogenic growth factors may stimulate the growth of an existing malignancy, *de novo* tumor formation is not considered to be a significant risk (Simons 2005).

The challenge that therapeutic angiogenesis faces lies in demonstrating the ability to deliver angiogenic treatment to a target organ or tissue and to limit the effects of treatment to the target tissue, as well as provide evidence of new vessel growth and associated improve-

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ment in symptoms, clinical outcomes, and long-term patient management. The promise of therapeutic angiogenesis has been touted for many years but, until recently, performance has not lived up to the promise (Ruel 2004).

Numerous studies in animals with induced cardiac ischemia have demonstrated the potential value of angiogenic therapy, whether the angiogenic factors that promote the growth of new blood vessels have been delivered via a protein, gene, or cell. Yet, the wealth of positive animal data has translated into only limited success in human trials.

Why has this been the case? More importantly, what has changed to offer renewed hope that therapeutic angiogenesis can provide an effective and cost-efficient means of healing diseased hearts and improving the lives of patients with limited cardiac function?

ANGIOGENESIS ADVANTAGES

Treatments to promote angiogenesis mimic a natural process in which the body responds to a diminished blood supply to vital tissues and organs, whether due to disease, trauma, or physiologic abnormalities, by producing new collateral vessels to resupply the ischemic region. The road map of new vessels created provides multiple alternative paths for blood to reach the damaged tissue.

Angiogenesis is an attractive therapeutic target for three reasons:

- It is a natural bodily process.
- Its physiology and mechanisms are well understood and have been intensively studied.
- A variety of potent growth factors that drive and regulate this process in the body have been identified. These proteins can be manufactured in quantities required for therapeutic dosing, and the growth factors (or compounds that stimulate their production) can be administered to patients via various delivery methods and routes.

In adult organisms, in the absence of disease, the endothelial cells that line the blood vessels typically exist in a quiescent, non-proliferative state. Trauma or disease and the associated inflammation and reduced oxygen supply that may stimulate the natural angiogenic process and choreograph complex processes that lead to the formation of new vessels. These signaling molecules induce a cascade of events mediated by growth factors and other molecules that transmit signals within and between cells.

Human growth factors represent an excellent and well-studied family of proteins for pharmaceutical development.

Angiogenesis begins with the breakdown of the basement membrane and extracellular matrix underlying the endothelial cell lining of blood vessels, the migration, recruitment, and proliferation of endothelial cells that adhere to the site of new vessel formation, and the construction of a new three-dimensional hollow vessel capable of supporting blood circulation. The tubular structure lengthens as new matrix and cellular components are deposited at the growing end of the nascent vessel.

HELPING THE HEART TO HEAL

The three approaches to therapeutic angiogenesis currently in development are gene therapy, cell-based therapy, and the delivery of protein growth factors, either systemically or directly to the site of the diseased tissue. All have had mixed success in animal and human studies. Each offers advantages and disadvantages in terms of delivery, safety, efficacy, cost of commercialization and administration, and regulatory considerations. And each relies on a variety of growth factors, chemokines, and transcription factors that induce neovascularization (Losordo 2004).

Gene therapy. In general, gene therapy-based angiogenesis — using adenovirus or retrovirus vectors or nonviral plasmid DNA vectors to deliver genes that produce angiogenic factors in the heart's muscle cells — has not demonstrated sufficient efficacy or, like gene therapy in general, has been plagued by safety concerns. Most angiogenesis trials in CVD to date involve nonviral vectors and an intracoronary or intramyocardial route of delivery (Ruel 2004, Losordo 2004, Freedman 2002).

Atlanta-based Corautus Genetics initiated the Genetic Angiogenic Stimulation Investigational Study (GENASIS) trial in August 2004, using plasmid DNA to deliver the vascular endothelial growth factor (VEGF)-2 gene directly to the heart

The scope of CVD and the economics of angiogenesis therapy

Cardiovascular disease (CVD) comprises coronary heart disease (CHD), congenital vascular defects, heart failure, stroke, high blood pressure, and peripheral arterial disease (PAD). It affects 71.3 million Americans, and is the underlying cause of more than a third of all U.S. deaths each year. CVD is responsible for nearly half of all deaths in Europe, and CHD alone is the leading cause of death among both men and women. Every day, nearly 2,500 Americans die from CVD.

CVD exacts an enormous toll in terms of healthcare costs and quality of life. In 2006, combined direct and indirect costs of care for Americans with CVD exceeded $400 billion. As the population ages, the number of individuals with some form of CVD also is likely to grow.

Coronary artery disease (CAD) is characterized by atherosclerotic plaque buildup in the arteries that supply the heart muscle. Over time, accumulation of plaque and the scar tissue that may form as the body's inflammatory response tissue damage sites can cause blockage of the arteries. Plaque instability may result in a portion of the plaque breaking away and traveling through the circulation, with the potential to cause complete blockage of a smaller vessel, resulting in a myocardial infarction, ischemia, and death of the affected area of heart muscle. As CAD increases in severity, it can cause angina (pain on stress or exertion) and limited heart function.

The therapeutic market for CAD exceeds $25 billion in the United States. Diagnosis of early to intermediate-stage CAD with or without angina typically leads to medical management, with a range of options that may include coronary artery bypass graft (CABG), percutaneous transluminal coronary angioplasty (PTCA), or the use of stents to open a blocked artery. When these treatments fail or patients are not candidates for available pharmaceutical or surgical therapies, they are classified as "no-option" patients. Of the 100 million or more Americans with CAD risk factors, about 13 million will develop CAD; 6 to 7 million, angina; 1.5 to 2 million will be at high risk of an acute event. About 25 percent of this latter group — 250,000 to 500,000 patients — will ultimately fall into the no-option category.

The no-option population is an ideal target for new treatment strategies such as therapeutic angiogenesis. In the future, the definition of no-option patients could expand to include individuals with the following conditions:

Mild CAD (less than 50 percent occlusion) — not yet candidates for CABG or PTCA, but drug therapy is insufficient
Silent ischemia — increased risk of acute events despite absence of angina
Chronic total occlusion — high failure rate with CABG and PTCA
Comorbidities (e.g., diabetes, elevated cholesterol) — no therapeutic options beyond pharmaceutical management
Multiple (five or more) sites of vessel occlusion — may not be able to target or successfully treat all with CABG/PTCA
Occlusions affecting the smaller vessels (diffuse microvascular disease) or dysfunction of the microvasculature — no good treatment options beyond routine drug therapy

Economic models demonstrating the potential for substantial cost savings in the no-option population predict that angiogenic therapy can reduce by about half the costs associated with treating refractory angina, and can eliminate the need for "mercy" CABG/PTCA procedures, with no related complications and a five-year duration of effect. The projected cost of fibroblast growth factor (FGF)-1 therapy, for example, is $20,000 per treatment. If all no-option patients were treated with FGF-1, the model forecasts a savings of as much as 20 percent of current standard of care costs over a 5-year period. FGF-1 use could cut in half the number of MIs over 5 years, allowing more than 160 of each 1,000 no-option patients who likely would have had one or more MIs to go unafflicted. The economic model projects that overall cumulative cost savings over 5 years for treating 1,000 no-option patients with FGF-1 could reach $48 million. Indirect savings recouped from restored work time and increased productivity as a result of raising work capacity from 80 percent to 90 percent for these patients has been estimated at $7 million per year.

SOURCES: AMERICAN HEART ASSOCIATION; CARDIOVASCULAR BIOTHERAPEUTICS 2007 ANNUAL REPORT

muscle in patients with Class III and IV angina who are not candidates for conventional revascularization techniques. The U.S. Food and Drug Administration put the phase 2b trial on hold in March 2006, and eventually it was stopped due to safety concerns and a lack of demonstrated efficacy of the treatment.

Cardium Therapeutics is sponsoring the AWARE trial, a phase 3 study of its Ad5FGF-4 (Generx), a one-time coronary infusion of adenoviral vector-based fibroblast growth factor (FGF)-4 gene therapy in women with angina pectoris who are not candidates for revascularization. The primary endpoint is the time to onset of myocardial ischemia on exercise treadmill testing and improved myocardial blood flow on single photon emission computer tomography (SPECT) imaging. A phase 2b trial in a more diverse patient population has been halted due to lack of efficacy.

A planned clinical trial led by Douglas Losordo, MD, at Tufts University School of Medicine, will evaluate the intramyocardial gene transfer of $VEGF_{165}$ delivered via a catheter inserted through the groin in patients with ischemic heart failure. The large Euroinject One phase 2 trial, involving direct percutaneous intramyocardial transfer of plasmid VEGF-A_{165} in patients with severe CAD who were not candidates for conventional revascularization therapy, did not show significant improvement in stress-induced myocardial perfusion compared with placebo (Kastrup 2005).

Genzyme is pursuing a phase 2 trial of locally delivered hypoxia inducible factor (HIF)-1 alpha adenoviral vector-based gene in patients with peripheral arterial disease (PAD) to study its safety and effectiveness in promoting new blood vessel growth in limbs affected by intermittent claudication. AnGes MG, based in Japan, recently completed enrollment in its phase 3 clinical trial of hepatocyte growth factor (HGF) plasmid gene therapy in patients with critical leg ischemia. The plasmid is delivered via intramuscular injection.

Overall, the promise of gene therapy to deliver growth and transcription factors is currently limited by the inability to regulate gene therapy vectors and to control the level and duration of gene expression, and by continuing safety concerns (Simons 2000, Post 2001).

The potential of angiogenic therapy lies in the delivery of potent and safe angiogenic growth factors directly to the damaged heart.

Cell-based therapy. In cell-based treatments, the therapeutic agent is usually a stem cell or progenitor cell isolated from the patient, enriched and expanded in numbers outside the body, and then reintroduced at the site of tissue damage. Successful cell-based angiogenesis studies in animals have relied on transplanted progenitor or precursor endothelial cells directly into the heart, where they proliferate and are incorporated into new blood vessels in a process called neovascularization. The potential for translating these successful efforts into an acceptable human therapy has been hampered by the lack of well-controlled trials to demonstrate that the risk of stimulating new tumor formation (or of promoting the growth of undetected, existing lesions) is minimal or nonexistent.

BioHeart is conducting a phase 1 study of its MyoCell therapy in 15 no-option patients using cells isolated from thigh tissue and injected into the heart via a catheter. An expanded phase 2a trial is underway in Europe. BioHeart's MyoCath-SR-II cell therapy, using local endocardial delivery and direct injection of myoblasts to deliver its product, is also in two phase 1/2 safety studies in Europe. TheraVitae has not yet begun recruiting patients for its planned phase 1/2 trials of Vescell — intracoronary injection of autologous angiogenic cell precursors — in patients with severe angina. The Texas Heart Institute's Stem Cell Center is recruiting patients for a trial involving intramyocardial injection of autologous aldehyde dehydrogenase-bright stem cells for therapeutic angiogenesis.

Protein therapy. The history of protein therapy dates back to the early years of recombinant protein production and the emergence of recombinant human insulin and human growth hormone in the early 1980s. Most human diseases result from the over- or underexpression, missing or misplaced activity, or malfunction of one or more proteins. Some highly successful forms of protein therapy involve blocking or at least limiting protein production by administering small-molecule drugs capable of inhibiting key cellular receptors and interfering with intricate sig-

naling cascades, or of preventing activation of transcription factors in the nucleus. Other small-molecule agonists bind to cellular or nuclear receptors to stimulate production of a specific protein. A growing number of monoclonal antibody-based therapeutics bind directly to errant proteins, targeting them for destruction or removal.

Direct administration of therapeutic proteins is challenging. Typically, these are large, structurally complex molecules, the correct three-dimensional conformation of which is critical for their function. Oral administration of these molecules is complicated by their propensity to be broken down in the intestinal tract and cleared from the circulation. Thus, routes of delivery other than by mouth often include intramuscular, intravenous, or intra-arterial injection, along with various techniques for direct delivery to the target site of action.

Another challenging feature of protein therapy stems from the existence of large multidimensional protein families, the individual members of which may differ only minimally in composition or structure but may exhibit significant variation in their targets, activity, pharmacodynamics, and potential for toxicity or unanticipated adverse effects. When identifying and developing a protein drug, it is crucial to have a full understanding of the scope and diversity of a protein family and the potential for cross-reactivity and unintended effects of its members.

Human growth factors represent an excellent and well-studied family of proteins for pharmaceutical development. They are capable of stimulating cellular proliferation, maturation, and differentiation, including the various types of cells that comprise mature blood vessels. At least two families of growth factors have proven angiogenic potency: FGFs and VEGFs (Losordo 2004). Numerous clinical studies have explored the potential for using these and other proteins known to play a role in tissue repair and angiogenesis — including colony granulocyte stimulating factor (CGSF), HGF, and platelet-derived growth factor (PDGF) — to simulate angiogenesis in an ischemic tissue or organ. These early trials typically employed intravenous delivery of the therapeutic protein and, although they showed this treatment approach to be safe, they did not consistently demonstrate statistically significant efficacy. As a result, the hope of using protein-based angiogenic therapy to treat ischemic CAD was abandoned in recent years.

STILL THE WAY TO GO?

The unsuccessful efforts with gene or cell-based angiogenic therapy has rekindled interest in developing protein drugs capable of stimulating the growth of new blood vessels. Newer approaches to treating CAD involve the injection of angiogenic proteins directly into the heart. Whereas localized perivascular delivery requires open-chest surgery or, perhaps, thoracoscopy, intrapericardial instillation of growth factors can be achieved by way of catheter delivery (Simons 2000). Even with intracoronary administration of angiogenic protein factors, the potential risks associated with systemic recirculation still exist (Simons 2000). Overall, protein therapy offers controlled dosing, the ability for combined delivery of multiple proteins, and a well-accepted safety profile (Post 2001).

VEGF, FGF-2, and FGF-1 represent the most extensively studied recombinant proteins for myocardial angiogenesis (Freedman 2002). Early studies have shown evidence of improvement in blood perfusion, anginal symptoms, exercise capacity, quality of life, and reduced use of anti-anginal drugs compared with placebo or baseline findings (Freedman 2002, Syed 2004). These studies, however, often involved patients undergoing concomitant bypass surgery, making it difficult to differentiate the effects of the two procedures. Further, the results of VEGF and FGF-2 studies suggest that readministration of the proteins is not associated with anti-growth factor antibody production (Simons 2000).

The FGF family of growth factors consists of 22 known proteins, which tend to have a high affinity for heparin-based compounds and can induce a range of cellular functions when they bind to cell surface receptors in the presence of heparin proteoglycans. They are expressed on endothelial cells, smooth muscle cells, and myoblasts. FGF-1, -2, and -4 are highly angiogenic and may function synergistically with VEGF (Losordo 2004). FGFs trigger a signaling cascade that results in the activation of genes required for growth and replication of endothelial cells, fibroblasts, and smooth muscle cells. FGF-1 is the broadest acting known member of the FGF family and is capable of stimulating the development of new blood vessels ranging in size and maturation from small capillaries to larger arterioles and fully functional arteries.

Clinical studies using localized administration of human FGF-1

have yielded promising results in no-option heart patients. Stegmann (2000) followed 33 patients with three-vessel CAD treated with intramyocardial injection of FGF-1 as an adjunct to bypass surgery for 3 years. No side effects or signs of tumor induction were reported and, compared with a control group that received heat-denatured FGF-1, the patients had a three-fold increase in vascular density (Schumacher 1998). Evidence of neoangeogenesis and improved blood flow and function of the heart muscle persisted for the three-year study period. A second trial of no-option CHD patients treated with FGF-1, completed in 2000, also demonstrated improved myocardial perfusion, with patients both at rest and under stress, as well as improved maximum working capacity in 90 percent of treated patients (Stegmann 2000). Findings from these two European studies were subsequently confirmed in a U.S. phase 1 clinical trial (Simons 2000).

In general, these three trials used, in various combinations, angiograms to localize the ischemic region of the heart, SPECT imaging to assess blood flow, and direct injection of FGF-1 into the heart via surgical mini-thoracotomy. None showed serious adverse events. Pharmacokinetic data revealed that once FGF-1 leaves the heart, the body clears it from circulation within three hours, minimizing risk of the protein stimulating unwanted angiogenesis in other organs or tissues, including the kidneys and the retina.

ON THE HORIZON

Various gene, cell-based, and recombinant protein therapies continue to progress through early-stage clinical testing. CardioVascular BioTherapeutics will initiate a phase 2 clinical trial of its FGF-1 drug later this year in patients with severe CHD, with a 1-year follow-up to assess adverse events. The trial will involve administration of the protein via a catheter introduced into an artery in the leg and snaked up to the heart, similar to the technique used to perform angioplasty and stent placement. This delivery route will allow the protein to be administered by an interventional cardiologist in an outpatient setting, which could translate into cost benefits and, perhaps, make this treatment option attractive to patients unwilling to undergo surgery.

Gene and protein therapies that deliver angiogenic growth factors are being tested as treatments for ischemia associated with peripheral artery disease and for wound healing of dermal ulcers. Various strategies for protein and gene-based delivery of VEGF, FGF, and HGF have advanced to phase 2/3 studies to treat limb ischemia. Combined with ongoing clinical studies in myocardial angiogenesis, these trials demonstrate the heightened interest and activity in protein therapy designed to stimulate the body's natural ability to repair tissue damage and to establish new vascular routes through which the nourishing and revitalizing blood supply can reach ischemic organs.

The potential medical and economic benefits of angiogenic protein therapy are well documented, while its promise for restoring function and improving quality of life are incalculable.

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DISCLOSURE

Jack Jacobs, PhD, is an officer and on the board of directors of CardioVascular BioTherapeutics.