Angiogenesis

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Several clinical trials of therapeutic angiogenesis in patients with coronary artery disease have shown clinical improvement and provided some objective evidence of improved perfusion and left ventricular function. Larger-scale, placebo-controlled trials, as well as studies of combinations of growth factors and the use of endothelial progenitor-cell or stem-cell supplementation, are in progress. Revascularization of ischemic myocardium with angiogenic compounds and without the mechanical manipulation of atherosclerotic vessels has great potential in the treatment of coronary artery disease. If it is proven to be both safe and efficacious, the revascularization of tissue biologically via medical or gene therapy will be a major advance in the treatment of patients with a diffuse disease that is not amenable to conventional therapy and in the augmentation of revascularization in patients undergoing traditional surgical therapies. [Rev Cardiovasc Med. *2002;3(3):138–144*]

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Life is dependent upon the body's complex system of vasculature in order to deliver oxygen and nutrients and remove waste from organs and tissues. The blood vessels vary from large, strong conduits to tiny lace-like networks. The initial de novo stage of vasculature formation is termed vasculogenesis. Angiogenesis refers to the sprouting and growth of small vessels and the branching and extension of existing capillaries by the assembly of endothelial cells from preexisting vessels. Angiogenesis is necessary for development and embryogenesis, as well as for wound healing and reproductive function in the adult.[1] The development of blood vessels is held in balance by proangiogenic and antiangiogenic factors.

Table 1
Selected Angiogenic Growth Factors

Growth Factors	Endothelial Receptors	Growth Factor Tissue Distribution	Target Tissues
FGF-1 (acidic)	FGFR-1, 2, 3, 4	Brain, bone, kidney, retina, heart	Multiple
FGF-2 (basic)	FGFR-1, 2	Brain, retina, pituitary gland, kidney, placenta, testes, heart	Multiple
VEGF	Flt-1, flk-1	Pituitary cells, macrophages, smooth muscle, heart, lung, skeletal muscle, prostate	Monocytes, coagulation system, hematopoietic stem cells
TGF	TGFR	Macrophage, tumor cells	Multiple

FGF, fibroblast growth factor; VEGF, vascular endothelial growth factor; TGF, transforming growth factor.
Adapted from Ware JA. Cellular mechanisms of angiogenesis. In: *Angiogenesis and Cardiovascular Disease*. Ware JA, Simons M, eds. New York, NY: Oxford Press; 1999.

Angiogenesis occurs in a number of disease states, the most obvious of which are diabetic retinopathy and tumor growth. In tumor growth, angiogenesis allows the growth of small vessels to feed the tumor and also aids in the cancer's ability to metastasize. Antibodies, or naturally occurring angiogenesis inhibitors, are being investigated clinically to antagonize key angiogenic factors. A complementary strategy is likely to emerge for the treatment of cardiovascular disease. This article will review the status of angiogenesis as a treatment for cardiovascular disease.

Background

In the adult heart, angiogenesis can occur in a number of pathological conditions associated with myocardial ischemia and/or tissue hypoxia. Myocardial infarction resulting from coronary atherosclerosis is one of the leading causes of death in developed countries. Contemporary medical therapy consists of antianginal and antithrombotic medications and treatment of risk factors such as hypertension and hyperlipidemia. In patients who have failed medical therapy and who continue to have symptoms with evidence of ongoing ischemia, additional treatment options consist of percutaneous revascularization (eg, angioplasty) and/or bypass surgery.

Considerable advances in both surgical bypass and percutaneous revascularization techniques have occurred in the past two decades. These interventions are effective in most patients, but there are some patients who are not candidates because they have diffuse coronary artery disease or microcirculatory impairment. There are also patients with recurrent narrowing or occlusion of their bypass grafts after initial surgery; these patients are often symptomatic again and have no further surgical options. For patients with severe myocardial ischemia who are not candidates for percutaneous or surgical revascularization, the need for an alternative therapeutic strategy is compelling.

The strategy to promote the development of supplemental collateral blood vessels that can create a natural bypass conduit around a patient's occluded artery is termed "therapeutic angiogenesis." Therapeutic angiogenesis is the development of new vessels induced by the administration of an angiogenic stimulus in order to treat disease. For the many reasons discussed, alternative medical therapies as well as new therapies to achieve therapeutic angiogenesis have interested scientists and clinicians alike.

New Therapies

The identification of angiogeneic growth factors and cytokines that have angiogenic activity has created the opportunity for new therapies in the treatment of a variety of diseases. Several potential regulators of angiogenesis have been identified, including fibroblast growth factors (FGF) 1 and 2, which were previously called acidic and basic FGF, respectively; transforming growth factor (TGF); and vascular endothelial growth factor (VEGF) (see Table 1). The angiogens that have been best studied in animal models and clinical trials are VEGF and FGF-2.

VEGF is a protein that is an endothelial-cell mitogen with high-affinity binding sites that are limited to endothelial-cell-specific receptors known as flk-1 and flt-1.[2,3] This specificity of VEGF for endothelial cells is a potential advantage in therapeutic angiogenesis, because endothelial cells represent the most critical structures on a cellular level for the formation of new vessels.[4] The functions of the VEGF protein culminate in endothelial-cell migration, proliferation, and aggregation and, ultimately, in the formation of a network of arterial and venous systems.[5] However, VEGF is also called vascular permeability factor because it leads to capillary leakiness and tissue edema. FGF-1 and FGF-2 are mito-

genic for a wider variety of cell types than VEGF is, and they bind to receptors on fibroblasts and smooth-muscle and endothelial cells.[6]

Preclinical Research

Preclinical studies have indicated that angiogenic growth factors can stimulate the development of collateral arteries, resulting in therapeutic angiogenesis. In animal models, studies have found that exogenous administration of VEGF can stimulate development and growth of collateral vessels in both peripheral[7] and myocardial ischemia.[8] Basic fibroblast growth factor, or FGF-2, has also been found to improve collateral development in animal models of myocardial[9] and hind-limb[10] ischemia. In addition, in a rat model of acute myocardial infarction there was a rapid and profound increase in VEGF expression, suggesting that VEGF plays an important role in angiogenesis that occurs in response to myocardial infarction.[11]

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In general, therapeutic agents for angiogenesis can be given as a protein, or more recently, as a gene. Because of their short half-life, both VEGF and FGF have been found to be poor candidates for traditional protein delivery. Thus, investigations ensued to see if gene therapy and gene transfer might be used to successfully accomplish therapeutic angiogenesis. In one approach to gene therapy, DNA coding for VEGF is delivered to the myocardium, which then allows the myocardial cells to secrete VEGF.[12] Takeshita and colleagues[13] found that naked DNA encoding for VEGF could be used in a strategy of arterial gene therapy to stimulate collateral artery development. In that study, transfection of phVEGF resulted in the development of augmented collateral vessels, documented by serial angiography, and improvement in the calf blood-pressure ratio. Arterial gene transfer of naked DNA encoding for a secreted angiogenic cytokine represents a potential alternative to recombinant protein administration.

Using recombinant VEGF DNA for therapeutic angiogenesis in animal models has resulted in successful revascularization of ischemic myocardium, but there have been some limitations. In a rat model of myocardial infarction, the effects of direct intramyocardial injection of the plasmid-DNA-encoding VEGF in the border zone of myocardial infarction included macroscopic angioma-like structures at the VEGF injection sites.[14] The formation of angioma at the injection sites did not appear to contribute to regional myocardial blood flow. In this model and in this application of direct myocardial injection, the formation of angioma is an obvious limitation of gene therapy.

Arterial gene transfer of naked DNA encoding for a secreted angiogenic cytokine represents a potential alternative to recombinant protein administration.

FGF-2 has also been studied extensively in animals. Yanagisawa-Miwa and colleagues[15] found that the intracoronary administration of FGF-2 during acute myocardial infarction in dogs resulted in a reduction in the infarct size and an increase in the number of capillaries and arterioles in the treated territory. Harada and coworkers[16] showed that FGF-2 improved myocardial function and provided improved collateral flow when it was administered as a slow-release capsule formulation placed on chronically ischemic porcine hearts.

Clinical Trials

The use of recombinant genes and growth factors to augment collateral blood-vessel mass in humans represents a new approach to the treatment of cardiovascular disease. Human studies to evaluate the therapeutic potential of proangiogenic factors have now been done; they have attempted to achieve therapeutic angiogenesis either through direct administration of vessel-growth promoters, such as FGF-2 or VEGF, or through gene therapy by giving a patient either a genetically engineered virus or cell or pieces of naked DNA encoding an angiogenic factor. The targets of clinical trials of therapeutic angiogenesis have been patients with myocardial ischemia and peripheral vascular disease. Most strategies for gene delivery have used a transcatheter intracoronary route, which may have limitations because of its imprecise localization of genes or proteins and its possible delivery to noncardiac tissue via systemic blood flow. Direct intraoperative intramyocardial injection of angiogenic factors or plasmid vectors is being studied during open-heart surgery.[17]

For peripheral vascular disease, a phase 1 clinical study by Baumgartner

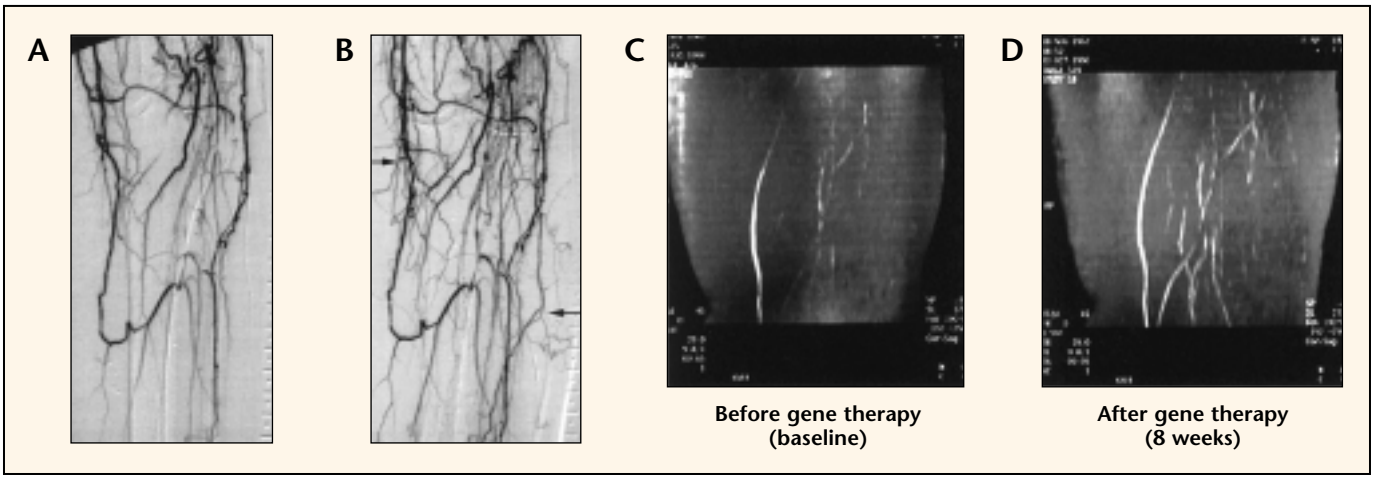

Figure 1. A and B: *Newly visible collateral vessels at calf level 8 weeks after phVEGF$_{165}$ gene transfer. The luminal diameter of newly visible vessels ranged from 200 µm to >800 µm (arrow); most were closer to 200 µm, and these frequently appeared as a blush of innumerable collaterals.* **C and D:** *Magnetic resonance angiography before and 8 weeks after gene therapy. After gene therapy, signal enhancement is clearly evident, consistent with improved flow in ischemic limb. Reproduced with permission from Baumgartner I, Pieczek A, Manor O, et al. Constitutive expression of phVEGF after intramuscular gene transfer promotes collateral vessel development in patients with critical limb ischemia.* Circulation. *1998;97:1114–1123.*

and colleagues[18] showed the safety and efficacy of intramuscular gene transfer using naked plasma DNA of VEGF that achieved constitutive overexpression of VEGF sufficient to induce therapeutic angiogenesis in patients with critical limb ischemia (see Figure 1). The ankle-brachial index improved significantly, and newly visible collateral blood vessels were seen by contrast angiography. Basic fibroblast growth factor has also been studied for peripheral vascular disease in humans. A phase 1 trial by Lazarous and associates[19] found that intra-arterial administration of FGF-2 was feasible and well tolerated in patients with peripheral vascular disease; calf blood flow increased significantly at 1 month and 6 months. Findings in the recently completed Therapeutic Angiogenesis with FGF-2 for Intermittent Claudication (TRAFFIC) trial showed that a single intra-arterial infusion of FGF-2 resulted in a significant increase in peak walking time within 90 days.[20] Trials are now in progress using hypoxia-inducible factor 1-α, FGF-2, or VEGF as either plasmid or adenovirus vectors in relation to angiogenesis and limb blood flow or ulcer healing.

Findings in the recently completed TRAFFIC trial showed that a single intra-arterial infusion of FGF-2 resulted in a significant increase in peak walking time within 90 days.

Several small studies of VEGF or VEGF-DNA treatment in coronary artery disease have been completed (see Figure 2). A phase 1, placebo-controlled trial of VEGF gene transfer in patients with myocardial ischemia by Vale and colleagues[21] found that myocardial gene transfer of naked plasmid-DNA-encoding phVEGF-2 by catheter-based injection in patients with chronic myocardial ischemia resulted in reduced angina and nitroglycerin consumption as well as reduced ischemia, shown by electromechanical mapping. In addition, catheter injections showed no sustained ventricular arrhythmias, no change in heart rate or blood pressure, and no evidence of infarction. This trial of phVEGF-2 myocardial gene transfer provided preliminary indications regarding its feasibility, safety, and potential efficacy. In a 2-year follow-up study there was persistent clinical improvement as well as no adverse effects, including no development of vascular tumors or cancer.[22] Another phase 1 assessment for angiogenesis gene therapy used direct intramyocardial administration of an adenovirus vector expressing VEGF DNA to individuals

It is possible that VEGF expression resulting from gene transfer could promote the development of a tumor that is too small to be recognized for months or even years.

with clinically significant severe coronary artery disease.[23] In this study there was no evidence of systemic or cardiac-related adverse events. There was evidence via coronary angiography and an assessment of a stress sestamibi scan of improvement in perfusion after therapy. In addition, all patients reported improvement in their angina class after therapy. There was, moreover, no evidence of excess or deranged angiogenesis, such as hemangioma formation, myocardial edema, or pericardial effusion. Another clinical study failed to show beneficial effects: patients received either VEGF or placebo via intracoronary infusion followed by intravenous infusion,[24] and exercise-time improved equivalently in both the treated and placebo groups. This lack of improvement may have occurred because of the selected route of administration (intravascular) or the use of protein rather than DNA. This study also underscores the general importance of having a placebo control group, as well as the continued unknowns in the optimal way to package and deliver growth factors to achieve therapeutic angiogenesis.

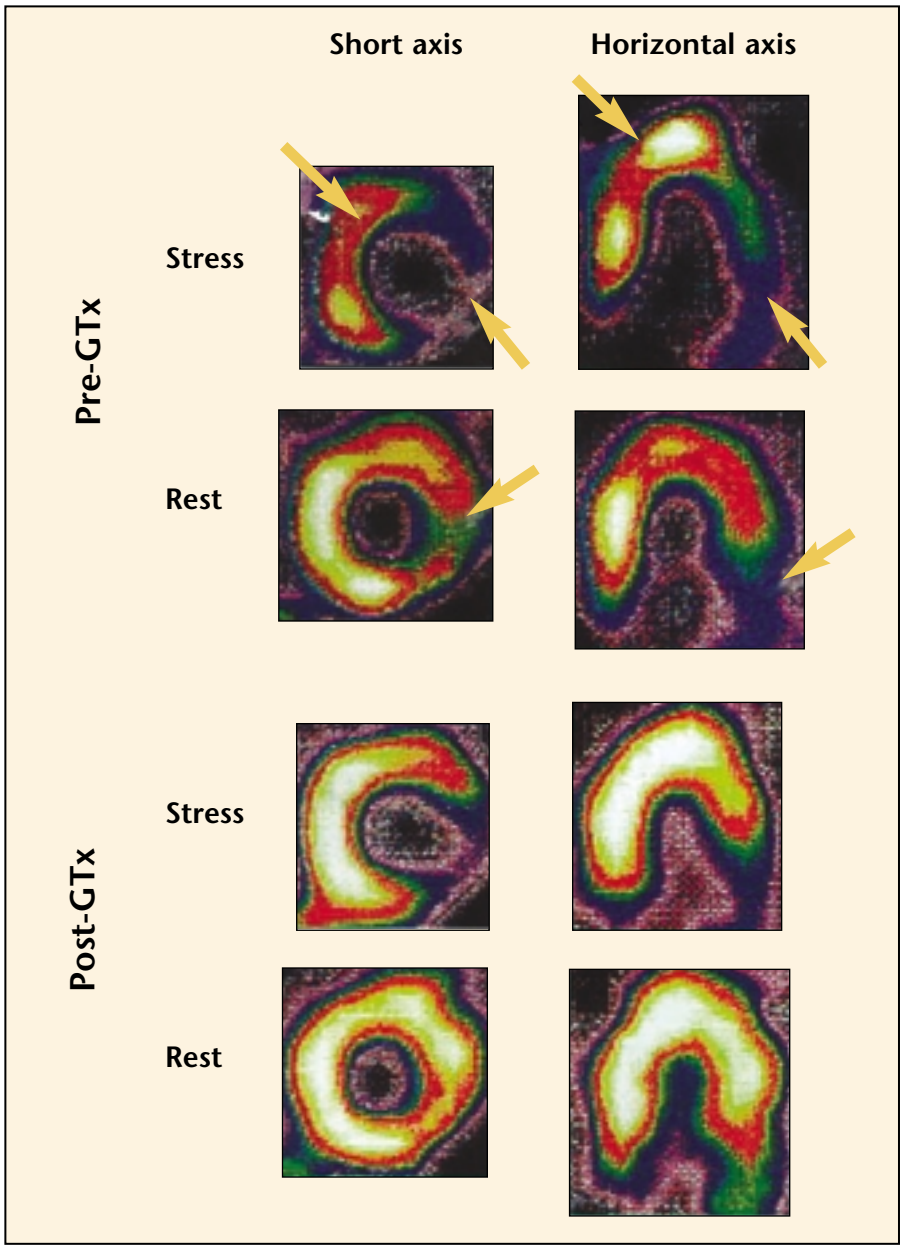

Figure 2. *Single photon emission computed tomography Persantine sestamibi imaging before VEGF gene transfer (pre-GTx) and after gene transfer (post-GTx) delivered intraoperatively by direct myocardial injection in a patient with chronic myocardial ischemia. White/yellow color indicates maximal radionuclide uptake, and red indicates impaired uptake. Arrows show ischemic areas before gene therapy, which are clearly improved following GTx. Reproduced with permission from Isner JM, Vale P, Symes J, et al. Angiogenesis and cardiovascular disease.* Dialogues Cardiovasc Med. *2001:6;145–170.*

The Future

Future clinical studies are needed in order to determine how to achieve optimal myocardial angiogenesis. Many aspects of gene transfer, including the appropriate vector dose, formulation, and administration route, are unknown (Table 2). Potential risks also accompany the promise of proangiogenic therapy. For example, therapeutic angiogenesis could increase a patient's future risk of cancer by creating an additional blood supply to small tumors in the body, thereby increasing their growth.[25] It is possible that VEGF expression resulting from gene transfer could promote the development of a tumor that is too small to be recognized for months or even years. It is also theoretically possible that VEGF might worsen deteriorating eyesight resulting from diabetic retinopathy, although there has been no evidence of this to date.[26] There is also concern that VEGF might increase the rate of atherosclerotic plaque development. A study in mice showed that VEGF significantly increased macrophage

Table 2
Unresolved Issues in Therapeutic Angiogenesis

■ Protein vs gene therapy
■ Single bolus vs repeated administration vs sustained delivery
■ Local vs systemic delivery
■ Route of local delivery: coronary artery lumen vs wall, coronary vein retrograde, intramyocardial, perivascular space
■ Dose of agent
■ Combinations of delivery techniques and substrates
■ Safety

levels in bone marrow and peripheral blood and increased the area of atherosclerotic plaque.[27]

To date, there has been no evidence of inflammatory or other complications, including death, directly attributable to cardiovascular gene therapy. A recent review of the risks associated with gene therapy found that the most common morbidity reported after cardiovascular gene transfer is lower extremity edema and that concerns regarding the potential for angiogenic cytokines to promote the progression of atherosclerosis have not been supported in angiographic follow-ups.[28] Similarly, there has been little evidence as yet from either preclinical or clinical studies to support the notion that the administration of angiogenic growth factors results in the growth of neoplasms.

Conclusions

Although some clinical trials of therapeutic angiogenesis in patients with coronary artery disease have shown clinical improvement and have provided some objective evidence of improved perfusion and left ventricular function, larger-scale, placebo-controlled trials are needed. Indeed, such trials, as well as studies of combinations of growth factors and the use of adjunctive endothelial progenitor-cell or stem-cell supplementation, are in progress. The development of strategies to revascularize ischemic myocardium with angiogenic compounds without the need of mechanical manipulation of atherosclerotic vessels has the potential to be of profound importance in the treatment of coronary artery disease. The ability to revascularize tissue biologically via medical therapy or gene therapy, if proven to be both safe and efficacious, will be a major advance in the treatment of patients with a diffuse disease that is not amenable to conventional therapy. In addition, there is the possibility and the

Main Points

- Angiogenesis is the growth of small vessels and the extension of existing capillaries by the assembly of endothelial cells from preexisting vessels. Angiogenesis is necessary for development and embryogenesis as well as for wound healing and reproductive function in the adult, but it also occurs in disease states, eg, diabetic retinopathy and tumor growth.
- Several potential regulators of angiogenesis have been identified, including fibroblast growth factors (FGF) 1 and 2, which were previously called acidic and basic FGF, respectively; transforming growth factor (TGF); and vascular endothelial growth factor (VEGF).
- Human studies have used either vessel-growth promoters, such as FGF-2 or VEGF, administered directly, or gene therapy given to a patient either as a genetically engineered virus or cell or as pieces of naked DNA encoding an angiogenic factor.
- Clinical trials of therapeutic angiogenesis have involved patients with myocardial ischemia and peripheral vascular disease. Most strategies for gene delivery have used a transcatheter intracoronary route. Direct intraoperative intramyocardial injection of angiogenic factors or plasmid vectors is being studied during open-heart surgery.
- Future clinical studies are needed to determine how to achieve optimal myocardial angiogenesis and minimize potential risk, such as the possibility of increasing tumor growth, diabetic retinopathy, or the rate of atherosclerotic plaque development. Many aspects of gene transfer, including the appropriate vector dose, formulation, and administration route, are unknown.
- To date, studies have found no evidence of inflammatory or other complications directly attributable to cardiovascular gene therapy and little evidence to support the notion that the administration of angiogenic growth factors results in the growth of neoplasms.

advantages of providing additional revascularization to patients undergoing traditional surgical therapies. Only time will tell whether these goals can be achieved. ■

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