

Cardiac Angiogenesis: Part I

An Emerging Technology for the Treatment of CAD

Part I of this article defines angiogenesis, vasculogenesis and arteriogenesis and looks at pre-clinical studies which support the fact that vascular growth factors can promote angiogenesis to improve blood flow to ischemic muscle.



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

Introduction

Vasculogenesis, angiogenesis and arteriogenesis are processes responsible for the development and maintenance of the circulatory system. The growth

of new vasculature that occurs in the post-embryological phase has been termed “angiogenesis.” Angiogenesis is of critical importance, not only during normal growth, but also in pathological situations. Some conditions, like neoplastic diseases, are enhanced

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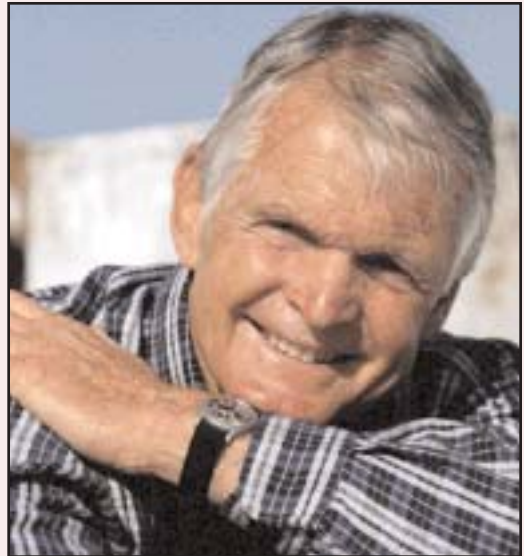


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Case

J.F., a 68-year-old male, presented with a strong family history of coronary artery disease (CAD) and a significant past cardiac history.

In 1973, at the age of 40, J.F. suffered a myocardial infarction (MI). He was doing well and remained pain-free for several years after the MI. In 1983, because of recurrent symptoms of angina, J.F. underwent four-vessel, coronary artery bypass grafting (CABG). He was well for several years post-bypass, but developed recurrent exertional angina in 1991. The anginal symptoms were progressive and, in 1992, he underwent a second CABG. Once again, he was symptom-free after the surgery, but the recurrence of angina led to coronary re-angiography



in 1997. The angiogram revealed an 80% stenosis at the ostium of the left anterior descending (LAD) artery. The LAD was totally occluded beyond the first diagonal. The intermediate artery had an 80% stenosis at its origin and the circumflex (CX) artery had a 90% stenosis after the first obtuse marginal branch. The first posterolateral branch of the CX was occluded and there was a 70% stenosis at the origin of the second posterolateral branch. The right coronary artery (RCA) was dominant, but totally occluded proximally, and filled with collaterals from the left system. There were patent saphenous vein grafts to the mid-portion of the LAD, and to the first diagonal branch. There was a vein graft to the second posterolateral branch, which had a flow-limiting stenosis at its insertion site. Based on this angiogram, J.F. was deemed inoperable.

Despite maximal medical therapy, J.F.'s symptoms progressed. By mid-1999, he was essentially housebound, suffering from nocturnal and rest angina. Normal activities of daily living provoked anginal symptoms, and he often experienced more than 20 episodes of angina per day. A repeat angiogram in 1999 was similar to that of 1997, but also revealed a new 70% stenosis in the saphenous vein graft to the LAD. Nuclear perfusion imaging revealed evidence of a previous infarction in the CX territory and significant ischemia in the LAD and RCA territories.

Question: What are other therapeutic options for this patient?

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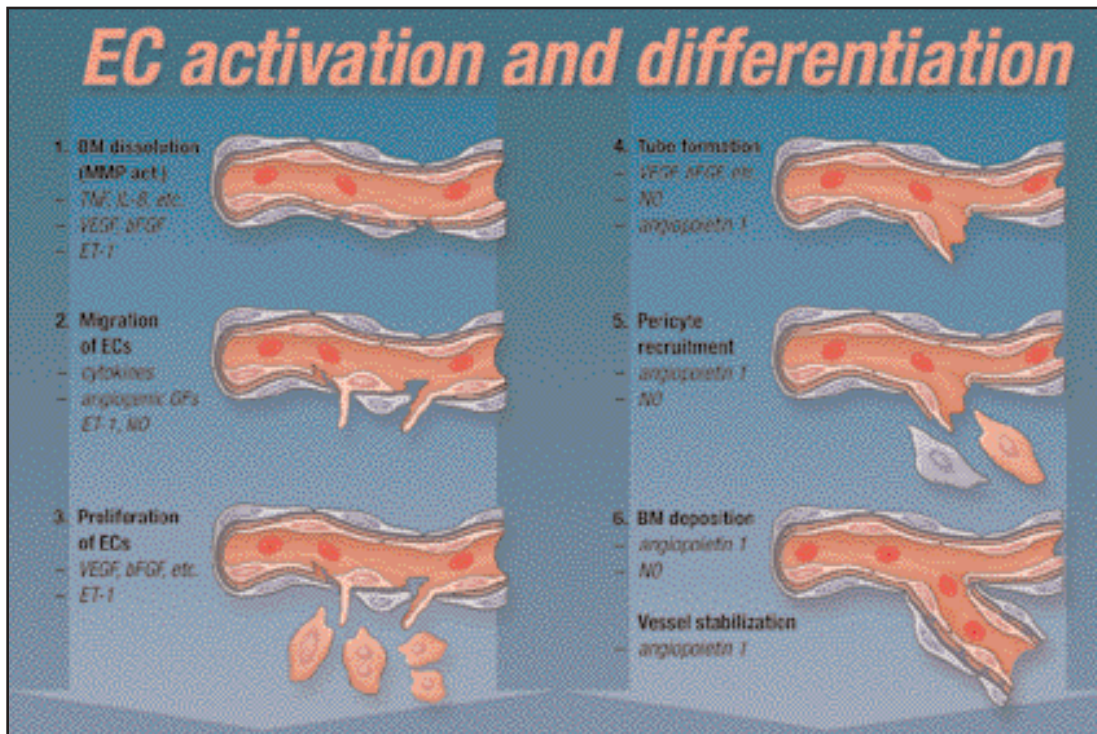


Figure 1

Sequential events in angiogenesis.

1) Basement membrane disintegration opens the way for 2) endothelial cell migration. 3) Cords of cells proliferate and 4) define a new vascular channel. 5) Cessation of cell migration and proliferation coincides with the recruitment of perivascular support cells 6) with the formation of a new basement membrane and vessel maturation and stabilization.

by excessive vascular growth, whereas in others, like ischemic heart disease, inadequate vascular growth contributes to morbidity and mortality. Therapeutic angiogenesis, through growth factor protein administration or gene therapy, has emerged as a promising new method of treatment for patients with coronary artery disease (CAD).

Angiogenesis

The term angiogenesis, first used by Hertig in 1935 to describe the growth of blood vessels in the placenta, was re-introduced by Folkman in 1972 to describe neovascularization accompanying solid tumor growth.¹

Angiogenesis is the process by which new capillaries sprout and differentiate from pre-existing microvascular networks. This process results in newly developed microvessels, most of which resemble capillaries (diameter of 5 μm to 8 μm). Although the exact mechanisms are not fully understood, angiogenesis is thought to involve a series of events, including:

- Activation of endothelial cells within a pre-existing vessel and vasodilation of the parent vessel;
- Degradation of the basement membrane and extracellular matrix;
- Migration of activated endothelial cells from the parent vessel directed by chemotactic factors liberated from fibroblasts,

monocytes, platelets, mast cells and neutrophils, towards the site where angiogenesis is required;

- Proliferation of endothelial cells in the newly forming vessels;
- Differentiation of these endothelial cells back to a quiescent phenotype with lumen formation;
- Recruitment of pericytes along the newly formed vascular structures;
- Formation of a new basement membrane by the newly organized endothelial cells and pericytes; and
- Remodeling of the neovascular network, with maturation and stabilization of the blood vessels (Figure 1).

Angiogenesis is rapidly initiated in response to hypoxia or ischemia and endothelial cell activation is the first process to take place in physiological or pathophysiological angiogenesis. Hypoxia induces increased levels of a family of hypoxia inducible transcription factors (HIFs), including HIF-1 β (or the aryl hydrocarbon-receptor nuclear translocator [ARNT]), HIF-1 α and HIF-2 α . The HIFs mediate the response to hypoxia by binding to specific deoxyribonucleic acid (DNA) sequences and the hypoxia-response promoter elements (which regulate the transcription of an array of genes critical to the cellular response to hypoxia, including several genes that regulate angiogenesis).

Leukocytes and platelets are potent producers of angiogenic growth factors, and several adhesion, chemoattractant and activator molecules govern their emigration from the blood stream. Integral membrane proteins, including integrins, play an important role in the process of angiogenesis. Integrins are heterodimeric cell surface receptors composed of two non-covalently associated transmembrane glycoproteins (a and b) that mediate attachment of cells to their foundation, but also are involved in intracellular signal trans-

duction. Endothelial cells express a number of different integrins, and $\alpha_n\beta_3$ and $\alpha_n\beta_5$ have been shown to be particularly important during angiogenesis. $\alpha_n\beta_3$ is a receptor for many proteins with an exposed Arg-Gly-Asp (RGD) tripeptide component, including vitronectin, fibronectin, fibrinogen, laminin, collagen, thrombospondin, osteopontin and von Willebrand's factor. Although the $\alpha_n\beta_3$ receptor is not widely expressed, it is prominent on cytokine-activated endothelial cells or smooth muscle cells, suggesting its relevance in angiogenesis. A number of angiogenic cytokines have been shown to increase the expression of the α_n and β_3 subunits on endothelial cells, and it has been demonstrated that $\alpha_n\beta_3$ antagonists (antibodies and cyclic RGD peptides) inhibit angiogenesis. Newer data suggest endothelial cell survival and proliferation in response to vascular endothelial growth factor (VEGF) may require the association of one of its receptors with $\alpha_n\beta_3$.

Basement membrane degradation, extracellular matrix invasion, and capillary lumen formation are also essential components of the angiogenic process, all of which are dependent on a cohort of proteases and protease inhibitors. Although a number of enzymatic systems have been implicated in extracellular proteolysis, many of the enzymes belong to one of two families, the serine proteases (in particular the plasminogen activator [PA]/plasmin system), and the matrix metalloproteases (MMPs). Plasminogen activators u-PA and t-PA convert the ubiquitous plasma protein plasminogen to plasmin. Plasmin activates certain MMPs, has a broad trypsin-like activity and degrades proteins such as fibronectin, laminin and the protein core of proteoglycans.

Subsequent steps in angiogenesis, including endothelial cell migration, proliferation, new vessel formation and maturation, result

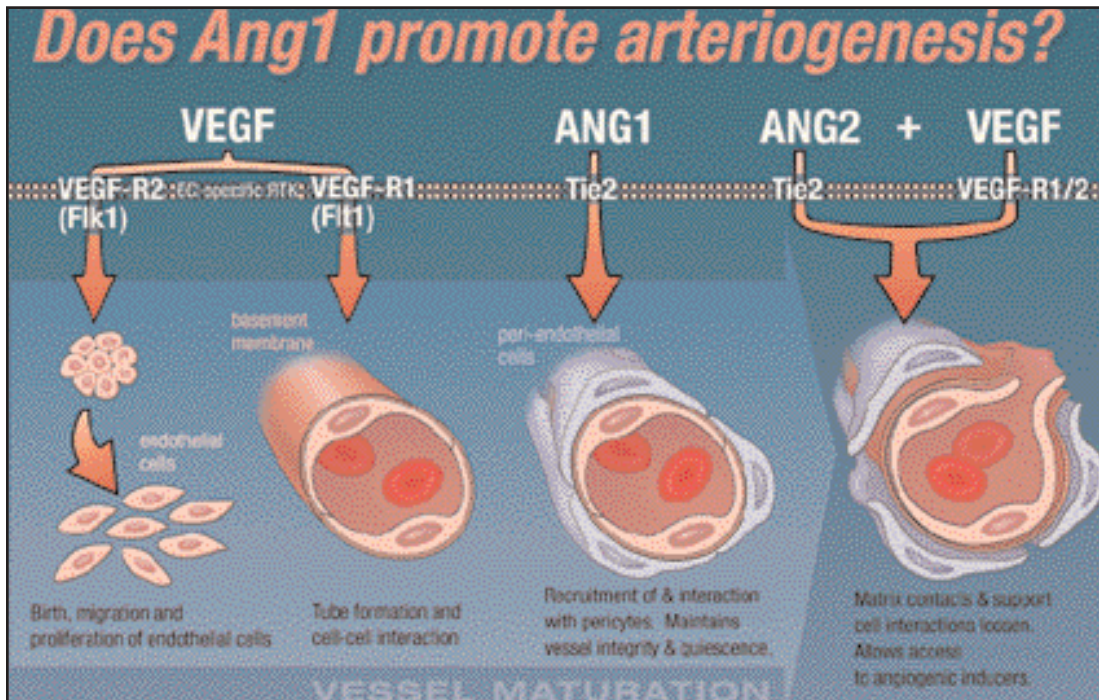


Figure 2

Co-ordinated and complimentary angiogenic activities of VEGF and the angiopoietins. VEGF, angiopoietin-1, and angiopoietin-2 bind to receptor tyrosine kinases (RTKs) that have similar cytoplasmic signaling domains. Binding of the ligands to their receptors elicits downstream signals with distinctive cellular responses. Only VEGF binding to the VEGF-R2 receptor sends a classical proliferative signal. VEGF binding to VEGF-R1 elicits endothelial support cells (pericytes, smooth muscle cells, myofibrocytes), thereby stabilizing a newly formed blood vessel. One property of ANG-2 is that it binds and blocks kinase activation in endothelial cells. The ANG-2 negative signal causes vessel structures to become loosened, reducing endothelial cell-cell contacts with matrix and dissociating peri-endothelial support cells. This loosening likely renders the endothelial cells more accessible and responsive towards the angiogenic inducers like VEGF.

Adapted from: Hanahan D: Signaling vascular morphogenesis and maintenance. Science 1997; 277:48-50

in a functional vascular conduit. Nitric oxide (NO) appears to play a crucial role in mediating various processes, including terminating the proliferative actions of growth factors, and promoting the formation of vascular tubes. In the setting of coronary ischemia, NO is required for VEGF to function. This, in turn, may be mediated by endothelin release. Secretion of platelet-derived growth factor (PDGF) helps attract other elements to the neovascular platform. Cell-to-cell contact, and the presence of transforming growth factor-beta (TGF- β) are thought to spur the differentiation and maturation of pericytes and

smooth muscle cells. The glycoprotein angiopoietin-1 (ANG-1) and its tyrosine receptor kinase TIE-2, function to stabilize the immature endothelial cell network, attract pericytes, and maintain biochemical interactions and vessel integrity (Figure 2).

Vasculogenesis

The process of vasculogenesis is distinct from that of angiogenesis. The term vasculogenesis is strictly reserved for the formation of new blood vessels during embryogenesis.



Initially, mesenchymal cells differentiate *in situ* into early hemangioblasts that form cellular aggregates (blood islands), in which the inner cell population differentiates into hematopoietic precursors. Meanwhile, the outer cell population gives rise to the primitive endothelial cells that generate a functioning vascular network. The primitive vascular plexus subsequently develops into a complex, inter-connecting network of mature blood vessels.

Arteriogenesis

The importance of the collateral coronary circulation has long been known and the mechanisms governing the recruitment, growth and proliferation of collateral vessels differs from those regulating angiogenesis and vasculogenesis. Acute occlusion of a large- or medium-sized artery often results in the recruitment of pre-existing arteriolar connec-

tions that can bypass the site of occlusion. Although this process does not require new vessel formation, the subsequent growth and proliferation of these collateral vessels occurs through a process called arteriogenesis. Collateral arteries are able to proliferate into large conductance arteries, which can efficiently restore blood flow to ischemic territories.

Adequate development of these collaterals may take days to weeks in order to compensate for critical stenoses of the nutrient branches of the coronary tree. Genetic factors are responsible for the variable number of pre-existing intracoronary connections and their capacity to grow, and lead to marked inter and intra-species vari-

ability. An important stimulator of arteriogenesis is increased shear stress that leads to changes within the newly recruited artery. The most important change is the activation of the endothelium. The result is an increased



Table 1

List of angiogenic proteins

Angiogen	Endothelial cell specific
Acidic fibroblast growth factor (aFGF)	No
Basic fibroblast growth factor (bFGF)	No
Fibroblast growth factor 3 (FGF-3)	No
Fibroblast growth factor 4 (FGF-4)	No
Fibroblast growth factor 5 (FGF-5)	No
Fibroblast growth factor 6 (FGF-6)	No
Fibroblast growth factor 7 (FGF-7)	No
Fibroblast growth factor 8 (FGF-8)	No
Fibroblast growth factor 9 (FGF-9)	No
Angiogenin 1	Yes
Angiogenin 2	Yes
Hepatocyte growth factor/scatter factor (HGF/SF)	No
Platelet-derived growth factor (PDE-CGF)	Yes
Transforming growth factor-a (TGF-a)	No
Transforming growth factor-b (TGF-b)	No
Tumor necrosis factor-a (TNF-a)	No
Vascular endothelial growth factor 121 (VEGF 121)	Yes
Vascular endothelial growth factor 145 (VEGF 145)	Yes
Vascular endothelial growth factor 165 (VEGF 165)	Yes
Vascular endothelial growth factor 189 (VEGF 189)	Yes
Vascular endothelial growth factor 206 (VEGF 206)	Yes
Vascular endothelial growth factor B (VEGF-B)	Yes
Vascular endothelial growth factor C (VEGF-C)	Yes
Vascular endothelial growth factor D (VEGF-D)	Yes
Vascular endothelial growth factor E (VEGF-E)	Yes
Vascular endothelial growth factor F (VEGF-F)	Yes
Placental growth factor	Yes
Angiopoietin-1	No
Angiopoietin-2	No
Thrombospondin (TSP)	No
Proliferin	Yes
Ephrin-A1 (B61)	Yes
E-selectin	Yes
Chicken chemotactic and angiogenic factor (cCAF)	No
Leptin	Yes
Heparin affin regulatory peptide (HARP)	No
Heparin	No
Granulocyte colony stimulating factor	No
Insulin-like growth factor	No
Interleukin 8	No
Thyroxine	No

Adapted from: Hamaway AH, Lee LY, Crystal RG, et al: Cardiac angiogenesis and gene therapy: A strategy for myocardial revascularization. *Curr Opin Cardiol* 1999; 14:515-22.

Case Discussion

In the year 2000, J.F. was enrolled in a clinical trial designed to evaluate the efficacy of direct intra-myocardial injection of plasmid DNA, incorporating the gene for vascular endothelial growth factor (VEGF 165) at the time of bypass surgery in patients undergoing incomplete revascularization.

In March 2000, J.F. underwent a third CABG operation. The LAD was bypassed using a left internal mammary graft, the intermediate artery was bypassed with a left radial arterial graft, and saphenous vein grafts were anastomosed to the first diagonal and the third obtuse marginal branches.

A total of 10 injections of 10 µg each of VEGF were administered along each side of the posterior descending artery, into the septum and the left posterior left ventricular wall.

At one-year follow-up after bypass surgery, J.F. remains virtually symptom-free and has experienced only one mild episode of angina during a strenuous walk against a cold wind.

expression of a number of genes, partially *via* a protein that binds to the shear stress responsive element (SSRE), present in the promoter of many of these genes, including nitric oxide synthase (NOS), platelet-derived growth factor (PDGF) and monocyte chemoattractant protein (MCP-1). Adhesion molecules are also upregulated, allowing for the adhesion and invasion of monocytes and platelets, which are also potent producers of growth factors. The process of arteriogenesis does not require hypoxia as a physical stimulus.

Neovascularization


Neovascularization depends on two distinct processes; cell proliferation and vessel differentiation. These processes must occur in harmony in order for functioning vessels to arise. It is likely that cell proliferation and differentiation occur in concert, and growth modulators may preferentially promote one process over the other in response to specific signaling mechanisms. Most angiogenically active factors are present in normal resting conditions and up- and down-

regulation of these substances is determined by physiological and pathophysiological moderators. Growth promoting factors are generated and active to varying degrees in response to the local environment and, depending on the local milieu, may be capable of promoting neovascularization. While VEGF and fibroblast growth factor (FGF) may regulate basement membrane disintegration, leukocyte and precursor cell recruitment, proliferation and adhesion, the presence of ANG-1 may be required for cell differentiation, maturation, and the establishment of a mature vessel (Figure 2). The inter-dependence of angiogenic factors is exemplified by FGF and NO. In the presence of NO, the action of FGF may switch from one that causes endothelial cell activation to one responsible for differentiation. Finally, such a paradigm would suggest therapeutic angiogenesis would require the provision of several factors at appropriate points in the process to allow for the establishment of a functional neovascular network. Many angiogenic factors have now been identified (Table 1).

Studies of Growth-Factor-Induced Myocardial Angiogenesis

Pre-Clinical Studies

Numerous animal experiments have demonstrated the link between growth factors and new vessel formation. Initial studies with FGF demonstrated accelerated wound healing in diabetic mice, leading to the first indicated use of topical growth factors for debrided diabetic ulcers.^{2,3} Animal studies of therapeutic angiogenesis have centered around two models: the rabbit hind limb model of peripheral ischemia, and the porcine model of myocardial ischemia.⁴⁻⁷ Both VEGF and FGF, administered by either intra-arterial or intra-muscular routes, can promote collateral blood vessel development after ligation of the rabbit femoral artery. In these studies, treated animals had more angiographically and histologically visible collateral vessels, greater hind limb blood flow, higher distal perfusion pressure, and enhanced muscle performance. Porcine and canine models of myocardial ischemia, induced by placement of an ameroid constrictor on a coronary artery, have also demonstrated augmentation of myocardial vascularization after both protein and gene administration *via* intra coronary or perivascular injection.⁸⁻¹² These pre-clinical studies supported the fact that vascular growth factors can promote angiogenesis to improve blood flow to ischemic muscle.

In next month's issue of *Perspectives in Cardiology*, the authors will look at the clinical applications of angiogenic therapy and issues of clinical trial design in Part II of this article. 

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