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Angiogenesis: An emerging technology for the treatment of coronary artery disease

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Ischemic heart disease is the major cause of death in adults in most developed and many developing countries and is now the most common cause of death worldwide. Effective treatments of coronary artery disease involve the percutaneous revascularization techniques of balloon angioplasty and stenting or coronary artery bypass grafting (CABG). The long-term success of these approaches is limited by the development over time of native vessel restenosis and graft occlusions. In addition, despite continued advances in the prevention and treatment of coronary artery disease, there is still a large number of patients who are not candidates for conventional treatments.

Vasculogenesis, angiogenesis, and arteriogenesis are processes that are 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 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.

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 tumour 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 resembling capillaries (diameter of 5 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;
- remodeling of the neovascular network, with maturation and stabilization of the blood vessels.

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-1β (or the aryl hydrocarbon-receptor nuclear translocator, ARNT), HIF-1α, and HIF-2α. They mediate the response to hypoxia by binding to specific DNA sequences – the hypoxia-response promoter elements – that regulate the transcription of an array of genes critical to the cellular response to hypoxia, including several genes that regulate angiogenesis.[2]

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 (α and β) that mediate attachment of cells to their foundation, but are

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also involved in intracellular signal transduction.[3-5] Endothelial cells express a number of different integrins, and of these, $\alpha_v\beta_3$ and $\alpha_v\beta_5$ have been shown to be particularly important during angiogenesis.[6] Alpha$_v\beta_3$ 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 factor. Although the $\alpha_v\beta_3$ receptor is not widely expressed, it is prominent on cytokine-activated endothelial cells or smooth muscle cells, suggesting its relevance in angiogenesis.[7] A number of angiogenic cytokines have been shown to increase the expression of the α_v and β_3 subunits on endothelial cells,[8-11] and it has been demonstrated that $\alpha_v\beta_3$ antagonists (antibodies and cyclic RGD peptides) inhibit angiogenesis.[12-15] Newer data suggest that endothelial cell survival and proliferation in response to vascular endothelial growth factor (VEGF) may require the association of one of its receptors with $\alpha_v\beta_3$.

Basement membrane degradation, extracellular matrix invasion, and capillary lumen formation are also essential components of the angiogenic process; all 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, or 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.[16-18]

Subsequent steps in angiogenesis – including endothelial cell migration, proliferation, new vessel formation and maturation – result in a functional vascular conduit.[4,19-21] 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.[21-23] In the setting of coronary ischemia, NO is required for vascular endothelial growth factor (VEGF) to function,[24] which may in turn, be mediated by endothelin release.[25] 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-ß) are thought to spur the differentiation and maturation of pericytes and smooth muscle cells.[21] The glycoprotein angiopoietin-1 (Ang-1) and its tyrosine receptor kinase Tie-2, stabilize the immature endothelial cell network, attract pericytes, and maintain biochemical interactions and vessel integrity[21] (Figure 1).

Vasculogenesis

The process of vasculogenesis is distinct from 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 hematopoeitic precursors and the outer cell population gives rise to the primitive endothelial cells that generate a functioning vascular network.[26-28] The primitive vascular plexus subsequently develops into a complex, interconnecting network of mature blood vessels.

Arteriogenesis

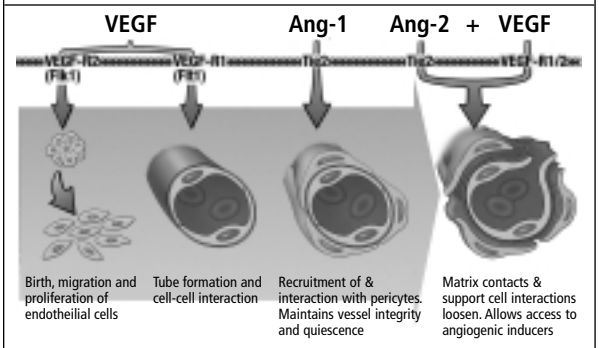
The importance of the collateral coronary circulation has long been known and the mechanisms governing the recruitment, growth, and proliferation of collateral vessels differs from those regulating angiogenesis and vasculogenesis.[19,29-34] Acute occlusion of a large- or medium-sized artery often results in the recruitment of pre-existing arteriolar connections 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 that 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, leading to marked inter- and intra-species variability.[35,36]

Increased shear stress is an important stimulator of arteriogenesis that leads to changes within the newly recruited artery. The most important change is activation of the endothelium. This results in an increased expression of a number of genes, partially via a protein that binds to the shear stress responsive element (SSRE) that is 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 that are 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. Indeed, 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.[22,37,38] 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, and the presence of Ang-1 may be required for cell differentiation, maturation, and the establishment of a mature vessel (Figure 1). The interdependence 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.[23] Finally, such a paradigm would suggest that therapeutic angiogenesis would require the provision of several factors at appropriate points in the

Figure 1: Vessel maturation



(Adapted from Hanahan)[90]

process to allow for desired product.[39] Many angiogenic factors have now been identified (Table 1).[89]

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.[40-42] 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.[43-47]

Both VEGF and FGF, administered by either an 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.

Models of ischemic porcine myocardium, induced by placement of an ameroid constriction of a coronary artery, have also demonstrated augmentation of myocardial vascularization after both protein and gene treatment administered via intracoronary or perivascular injection.[48-52] These pre-clinical studies supported the proof of principle that vascular growth factors can promote angiogenesis to improve blood flow to ischemic muscle.

Clinical trials

Until recently, human angiogenic experiments have been predominantly limited to small series in which VEGF or FGF, protein or gene, have been administered.[53-70] Delivery strategies have included intracoronary, epicardial, or direct myocardial injection of either VEGF or bFGF protein, or genetic material. The latter can be delivered as naked plasma DNA or in a viral vector.

Schumacher and colleagues were the first to report on therapeutic angiogenesis in human myocardium. In this phase I, randomized, blinded study, 40 patients undergoing CABG with a left internal mammary artery (LIMA) graft and a left anterior descending (LAD) artery stenosis distal to the anastomosis site were enrolled. Patients were randomly assigned to direct intramyocardial injection of aFGF or denatured protein control near the distal non-grafted segment.[53] At 3 months, they observed increased "coronary blush" (a surrogate measure [unvalidated] of collateral formation) among FGF injected patients compared to placebo. This effect persisted to 3 years, and was associated with improved echocardiographic ejection fraction and functional class.[54] Similar positive results were seen in small trials reported by Sellke et al,[55] Laham et al,[56,58,59] and Unger et al[57] (Table 2).

Table 1: List of angiogenic proteins[89]

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-α (TGF-α)	No
Transforming growth factor-ß (TGF-ß)	No
Tumour necrosis factor-α (TNF-α)	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

Table 2: Clinical trials of FGF delivery						
Parameter	Schumacher et al (1998)[53]	Laham et al (1999)[56]	Unger et al (2000)[57]	Laham et al (2000)[59]	Sellke et al (1998)[55]	FIRST
N	40	24	25	66	8	337
Design	RDB	RDB	RDB	observational	observational	RDB
Placebo-controlled	Y	N	Y	N	N	Y
Thoracotomy	Y	Y	N	N	Y	N
Agent	aFGF protein	bFGF protein	bFGF protein	bFGF protein	bFGF protein	bFGF protein
Vector	none	heparin/alginate microcapsules	none	none	heparin/alginate microcapsules	none
Dose	70 mg	10/100 µg	3-100 µg/kg	0.33-48 µg/kg	10/100 µg	200 µg
Delivery	intramyocardial	epicardial fat implantation	intracoronary	IC/IV	epicardial fat implantation	intracoronary
Endpoint	DSA	clinical/MPI	GXT	MPI	MPI	GXT/MPI/QOL
Result	positive	positive	safe	positive	safe	negative

GXT = graded exercise stress test; RDB = randomized, double blind; MPI = myocardial perfusion imaging; IC = intracoronary; IV = intravascular, DSA = digital subtraction angiography; QOL = quality-of-life

Six small studies have evaluated VEGF delivery to ischemic myocardium (Table 3). Protein therapy, performed with varying doses of intracoronary recombinant human VEGF, was studied by Henry et al[60] and Hendel et al.[61] DNA coding for VEGF has been delivered either as naked plasmid DNA (Losordo et al,[62] Vale et al,[63,66] Hendel et al,[64] and Symes et al[67]), or using an adenoviral vector (Rosengart et al[68,69]). The results of these small trials were promising, and suggested that both VEGF protein and DNA were effective for the production of new blood vessels.

Collectively, these phase 1 and 2 studies described the experience of 298 patients without blinded outcome assessment. Although data from these studies cannot be used to make conclusions concerning efficacy, they firmly establish the feasibility and safety of different methods of gene transfer and have set the stage for larger randomized trials.

Only two relatively large, randomized, double-blind, placebo-controlled studies have been performed in humans.

The FIRST Study (FGF-2 Initiating Revascularization Support Trial) recruited 337 patients with angina who were considered sub-optimal for traditional revascularization. In a double-blind, placebo-controlled manner, participants were randomized to 3 doses of intracoronary recombinant bFGF protein (0.3, 3.0 and 30 µg/kg). At 90 days, there was no difference between groups in the primary endpoint of exercise treadmill times, or in the secondary endpoints of nuclear perfusion parameters (p= 0.64), and quality-of-life indices (Seattle Angina Questionnaire [SAQ] or short-form 36 [SF-36]). On post-hoc analysis, a benefit was shown in older patients (>63 yrs), which was statistically significant (P=0.025) compared with younger patients.

The VIVA Trial (VEGF in Ischemia for Vascular Angiogenesis) involved a patient cohort similar to that of the FIRST Trial with evidence of a reversible perfusion defect on nuclear scans. Patients (n=178) were assigned randomly to two doses of VEGF (17 or 50 ng/kg) or placebo. VEGF protein was administered as a 20-minute intracoronary infusion during coronary angiography, followed by three 4-hour intravenous infusions on days 3, 6, and 9. Although no improvement was seen in the primary endpoint of treadmill score at 60 days, mean CCS (Canadian Cardiovascular Society) anginal class was significantly lower for the high dose group compared to placebo at 120 days (1.6 ± 0.1 vs 2.1 ± 0.1, $P = 0.04$).

No safety concerns were raised in either of these landmark trials. Although both trials were unable to demonstrate efficacy by their primary endpoint, several factors may account for the lack of effect. In the two randomized human trials, growth factor delivery was accomplished via an intracoronary or intravenous route. It is unclear if this method provides adequate tissue levels to stimulate and maintain angiogenesis. This is particularly true for bFGF, given the poor specificity for target endothelium. In fact, dose-ranging studies for both FGF and VEGF suggest a graded effect at higher doses.[59,61] It is possible that injection into myocardium or pericardial fat is necessary for clinically relevant dose delivery.[56]

Issues of study design

In planning controlled trials to assess the effectiveness of gene therapies, investigators must consider a number of factors. These include:

- selection of the appropriate means of delivery of therapeutic material
- determination of appropriate endpoints to be studied
- quantification and resultant objectification of the results
- assurance of adequate controls
- selection of patients to be included
- determination of the mechanisms of any observed clinical effects
- assessment of complications – potential, actual, local, systemic, immediate and long-term.

Delivery modalities and strategies

Delivery of growth factors has been accomplished using two means: either through the use of single or multiple doses

Table 3: Clinical trials of VEGF delivery								
Parameter	Hendel et al (2000)[64]	Henry et al (1998)[60]	Vale et al (2001)[63]	Symes et al (1999)[67]	Hendel et al (2000)[61]	Rosengart et al (1999)[68,69]	Losordo et al (1998)[62]	VIVA
N	30	15	30	20	14	21	5	178
Design	observational	Observational	observational	observational	observational	observational	observational	RDB
Placebo controlled	N	N	N	N	N	N	N	Y
Thoracotomy	N	N	Y	Y	N	Y	Y	N
Agent	VEGF-C DNA	rhVEGF protein	$VEGF_{165}$ DNA	$VEGF_{165}$ DNA	rhVEGF protein	$VEGF_{121}$ DNA	$VEGF_{165}$ DNA	rhVEGF protein
Vector	plasmid	none	plasmid	plasmid	none	adenovirus	plasmid	none
Dose	0.2/0.8/2.0 mg	0.005/0.017/ 0.05/0.167 µg/kg	125/250/500 µg	125 µg	0.005/0.017/ 0.05/0.167 µg/kg	1000 µg	125 µg	17/50 ng/kg/min
Delivery	intramyocardial	intracoronary	intramyocardial	intramyocardial	intracoronary	intramyocardial	intramyocardial	IC/IV
Endpoint	clinical/GXT/ MPI/NOGA	MPI	clinical/GXT/ MPI	clinical/MPI/ angiography	MPI	clinical/MPI/ angiography GXT	clinical/MPI/ angiography	clinical/GXT angiography
Result	positive	positive	positive	positive	positive	positive	safety	negative

GXT = graded exercise stress test; RDB = randomized, double blind; MPI = myocardial perfusion imaging; NOGA = NOGA electromechanical mapping, IC = intracoronary; IV = intravascular

of recombinant protein, or by a gene transfer approach. Each strategy has its limitations. Potential advantages for the use of proteins include the ability to adjust the dose and thus define a therapeutic window between efficacy and toxicity. This would allow withdrawal of treatment if and when necessary. Factors against the use of protein for therapeutic angiogenesis are the considerable cost involved in producing significant quantities of pyrogen-free materials; the appearance of secondary effects (prolonged administration of bFGF is associated with a decrease in arterial pressure, moderate thrombocytopenia, and moderate anemia); and the requirement for repeated or prolonged administration of protein. Local perivascular delivery via myocardial injection, pericardial fat implantation of coated microspheres, or pericardial instillation has been attempted in order to address the latter limitation.[56,70-72]

Delivery strategies for protein have recently been studied more thoroughly in an experimental model. In a pig model, tissue and myocardial distribution of labeled bFGF was determined at 1 hour and 24 hours after intracoronary or intravenous delivery by measuring ^{125}I-bFGF-specific activity.[73,74] At 1 hour, total cardiac activity was 0.88 with intracoronary delivery which dropped significantly to 0.05 at 24 hours. Cardiac-specific activity with intravenous administration was lower at 1 hour (0.26), but also decreased significantly to 0.04 by 24 hours. Intrapericardial delivery resulted in a cardiac-specific activity of 1.45 after 1 hour which increased to 2.98 by 24 hours.[74] The 1-hour cardiac-specific activity was highest with intramyocardial delivery at 4.31, which decreased to 2.30 by 24 hours. The study showed that intrapericardial and intramyocardial delivery results in a more favorable myocardial distribution of growth factor than intracoronary or intravenous delivery. Additional data from the study indicated that intrapericardial delivery was limited to the epicardial layers and required a normal pericardium.

In contrast to protein delivery, gene therapy results in the prolonged secretion of growth product by host cells, offering sustained protein levels with a single administration. However, the potential for extra-lesional uptake of the gene or vector and distant, unwanted effects in non-target tissues, related either to the vector or the gene product that it encodes, is of concern.

There are many means to deliver genes coding for angiogenic products. The simplest is through the delivery of naked plasmid DNA. Injection of naked DNA into myocardium has been shown to result in growth factor expression for a considerable period of time, without incorporation into host DNA.[69,75] Many facilitated means of delivery have also been studied. Liposomal encapsulation has been tested; however current techniques are associated with low transfection efficiencies. Retrovirus encapsulation and delivery allows for effective and long-term gene expression through DNA incorporation into the genome; however the potential for activation of retroviral genes in the host DNA is of concern. The use of adenoviral vectors is an effective means of delivery; however it is associated with an immune response that can lead to destruction of the vector or a significant systemic inflammatory response.[76]

Efficacy endpoints

The choice of efficacy endpoints for clinical trials remains an area of controversy. The ideal endpoint for angiogenesis trials should have the following characteristics:

- It should address the primary hypothesis and represent a direct marker of efficacy.
- It should be clinically meaningful.
- It should be easily measured and not be prohibitively costly to perform or analyze.
- It should provide insight into mechanisms.
- It should lend itself to statistical analysis.

The endpoints for trials of angiogenesis can be considered either clinical (angina status, functional capacity, or quality-of-life) or physiologic (improved myocardial perfusion,

improvement in vessel collateralization, improvement in global or regional wall motion).

Objective endpoints such as death, myocardial infarction, and repeat revascularization can provide solid data. However, results from several trials in patients with no therapeutic option indicate a 5% mortality rate over 2 years of follow-up. A prohibitively large study population would therefore be required to show a reduction in mortality. Exercise stress testing is one surrogate clinical assessment that has been considered as an endpoint for angiogenesis trials. The advantages are that exercise stress testing is often used in phase 1 and 2 studies, the results are quantitative, semi-objective (rate pressure product, time to ST depression), and the findings are fairly reproducible. The disadvantages are that comorbidities (peripheral vascular disease, COPD, arthritis) may limit exercise performance, day-to-day variability exists, and the reasons for test termination may still be subjective.

Changes in the Canadian Cardiovascular Society (CCS) score or response to the Seattle Angina Questionnaire (SAQ) have also been used as clinical endpoints in angiogenesis trials. Advantages are that they are highly relevant to patients and easy to interpret (ie, CCS), are sensitive to change, are fairly reproducible (esp. SAQ), and are familiar to most clinicians. Disadvantages are that they are more subjective than exercise stress testing (double-blinding necessary), the CCS score requires observer input (SAQ doesn't), changes in SAQ are not easily interpreted (lack of familiarity by clinicians), and the placebo effects are substantial (~40% in the DIRECT DMR study).

The advantages of using the Medical Outcome Study (Short Form, 36 items) (MOS SF-36) or Health Utility Index (HUI) are that they are both broadly applicable, sensitive to change, and normal values have been established for various disease states. Disadvantages of these types of analyses are that they are considered to be softer endpoints, are more subjective, and changes are not easily interpreted (lack of familiarity by clinicians).

One of the problems common to all clinical endpoints is that they are prone to placebo effects. One solution is to look for objective endpoints that can explain subjective outcomes such as reduction in CCS class. In this regard, "angiogenesis specific" quality-of-life or symptom assessment tools may be necessary. An additional problem with clinical endpoints is that small changes may be undetected, but may still be clinically meaningful (basement effect).

Although clinical endpoints are employed in trials of myocardial angiogenesis, physiologic assessments are preferred as primary endpoints. Several have been considered, including SPECT myocardial perfusion imaging, MRI, and PET. The advantages of nuclear scintigraphy are that it is sensitive to changes after revascularization, it is reproducible, and wall motion can be assessed. There are concerns, however, over the adequacy of the spatial resolution obtainable with nuclear imaging. MRI has enormous potential. It is able to provide excellent spatial resolution and information on structure, function, and flow, however, although it is gaining greater acceptance with time, prohibitive cost and restrictive availability limits its use. PET scanning is more sensitive than SPECT in measuring coronary flow reserve. It remains the only way to measure absolute blood flow. The limitations of PET imaging are the poor spatial resolution, the lack of widespread availability, and its cost.

Potential complications

Angiogenic agents are thought to have the potential to induce unintended neovascularization in nontargeted tissues. Mitigating this possibility are data that suggest that angiogenesis will occur in response to a cytokine only under appropriate conditions. Both FGF and VEGF receptors are upregulated when tissues become ischemic,[77-80] therefore, it would be expected that ischemic tissue would respond more sensitively to the biological effects of FGF and VEGF than would normal tissues. This concept was supported by a study in which normal and ischemic canine myocardium was exposed to high local levels of aFGF protein administered with an epicardial sponge over a prolonged time.[81] In this study, only ischemic myocardium responded with an angiogenic response. Although the high threshold for neovascularization in normal tissue is reassuring, there is still concern about patients who have co-existent conditions in which cytokine receptors are abnormally upregulated, such as malignant tumours and diabetic retinopathy.

There is a potential that angiogenesis therapy may trigger the growth of existent, but unrecognized tumours. FGF is a mitogen that stimulates a wide variety of cell types and therefore, may stimulate tumour growth. Although VEGF acts primarily on vascular endothelium, a number of non-endothelial tumor cells have been found to possess low levels of functional VEGF receptors.[82] In addition to direct effects of the angiogenic agents on tumour cell proliferation, there is evidence to suggest that solid tumours require an angiogenic stimulus to supply nutrients required for growth beyond a critical size. Induction of angiogenesis may therefore indirectly contribute to the growth of dormant tumours. Angiogenic factors may also contribute to *de novo* tumour development. In addition, the mechanisms by which FGF and VEGF have the potential to stimulate neoplastic growth are relevant to their reported proatherogenic effects.

The potent vascular permeability activity of VEGF can also have unwanted consequences. Transient peripheral edema was frequently observed in studies of VEGF administered to patients with lower extremity ischemia.

There may be several reasons for the disparate results of the reported clinical trials of angiogenesis and the results from animal models. In addition to the issues of dose, mechanisms of delivery, endpoints, and the choice of patient cohort studied may have confounded the clinical trials, making positive results unattainable. Unlike animal populations, the patient population of interest has demonstrated an inability to form or recruit adequate collateral vessels prior to inclusion in the trials. In addition, the response to simple growth factor delivery may differ in the presence of diffuse atherosclerosis and endothelial dysfunction, compared with the response in experimental ischemic models.[83] Also, various cardiac medications (ie, aspirin, captopril, lovastatin, and furosemide) and health states (ie, hypercholesterolemia, smoking, diabetes, and age), negatively impact the angiogenic response.[33,71,84-89] The recognition that patients enrolled in clinical trials of angiogenesis are highly selected on the basis of anatomy, symptoms, LV function, concurrent disease, and motivation, also has an impact on the generalization of the results of clinical trials.

Future research

Current animal studies are focusing on the mechanisms of angiogenesis, examining particularly the roles of different compounds and the local and host factors that govern their effectiveness. The action of angiogenic factors in the milieu of coronary artery disease is also an area of active research. The results of animal studies and early results of clinical trials suggest that delivery of a cocktail of angiogenic factors might be more effective than delivery of a single agent, and may more closely mimic the physiologic angiogenic response. Finally, stem or progenitor cell transplantation may allow for the development of all components required for new myocardium and functioning vascular network, and may provide a feasible therapy in the future.

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References

1. Folkman J. Anti-angiogenesis: new concept for therapy of solid tumors. *Ann Surg* 1972;175:409-416.
2. Wang GL, Semonza GI. Purification and characterization of hypoxia inducible factor 1. *J Biol Chem* 1995;270:1230-1237.
3. Brooks PC, Montgomery AM, Rosenfeld M, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. *Cell* 1994;79:1157-1164.
4. Tomanek RJ, Schatteman GC. Angiogenesis: new insights and therapeutic potential. *Anat Rec* 2000;261:126-135.
5. Hynes RO, Bader BL, Hodivala-Dilke K. Integrins in vascular development. *Braz J Med Biol Res* 1999;32(5):501-510.
6. Eliceiri BP, Cheresh DA. The role of alpha$_v$ integrins during angiogenesis: insights into potential mechanisms of action and clinical development. *J Clin Invest* 1999;103:1227-1230.
7. Varner JA, Brooks PC, Cheresh DA. The integrin $\alpha_v\beta_3$: angiogenesis and apoptosis. *Cell Adhes Commun* 1995;3:367-374.
8. Basson CT, Kocher O, Basson MD, Asis A, Madri JA. Differential modulation of vascular cell integrin and extracellular matrix expression in vitro by TGF-beta 1 correlates with reciprocal effects on cell migration. *J Cell Physiol* 1992;153:118-128.
9. Swerlick RA, Brown EJ, Xu Y, Lee KH, Manos S, Lawly TJ. Expression and modulation of the vitronectin receptor on human dermal microvascular endothelial cells. *J Invest Dermatol* 1992;99:715-722.
10. Sepp NT, Li L-J, Lee KH, et al. Basic fibroblast growth factor increases expression of the $\alpha_v\beta_3$ integrin complex on human microvascular endothelial cells. *J Invest Dermatol* 1994;103:295-299.
11. Senger DR, Ledbetter SR, Claffey KP, Papadoupoulos-Sergiou A, Perruzzi CA, Detmar M. Stimulation of endothelial cell migration by vascular permeability factor/vascular endothelial growth factor through cooperative mechanisms involving the $\alpha_v\beta_3$ integrin, osteopontin, and thrombin. *Am J Pathol* 1996; 149:293-305.
12. Brooks PC, Clark RAF, Cheresh DA. Requirement of vascular integrin $\alpha_v\beta_3$ for angiogenesis. *Science* 1994;264:569-571.
13. Brooks PC, Strömblad S, Klemke R, Sarkar FH, Cheresh DA. Antiintegrin $\alpha_v\beta_3$ blocks human breast cancer growth and angiogenesis in human skin. *J Clin Invest* 1995;96:1815-1822.
14. Drake CJ, Cheresh DA, Little CD. An antagonist of integrin $\alpha_v\beta_3$ prevents maturation of blood vessels during embryonic neovascularization. *J Cell Sci* 1995; 108:2655-2661.
15. Hammes H-P, Brownlee M, Jonczyk A, Sutter A, Preissner KT. Subcutaneous injection of a cyclic peptide antagonist of vitronectin receptor-type integrins inhibits retinal neovascularization. *Nat Med* 1996;2:529-533.
16. Dvorak HF. Tumours: wounds that do not heal. Similarities between tumour stroma generation and wound healing. *N Engl J Med* 1986; 315:1650-1659.
17. Haas TL, Madri JA. Extracellular matrix-driven matrix metalloproteinase production in endothelial cells: implications for angiogenesis. *Trends Cardiovasc Med* 1999;9:70-77.
18. Mignatti P, Rifkin DB. Plasminogen activators and matrix metalloproteinases in angiogenesis. *Enzyme Protein* 1996;49:117-137.
19. Buschmann I, Schaper W. The pathophysiology of the collateral circulation. *J Pathol* 2000;190:338-342.
20. Henry TD. Therapeutic angiogenesis. *BMJ* 1999;318:1536-1539.
21. Griffioen A, Molema G. Angiogenesis: potentials for pharmacologic intervention in the treatment of cancer, cardiovascular diseases, and chronic inflammation. *Pharm Rev* 2000;52:237-268.
22. Jang J, Ho HV, Kwan H, Fakardo L, Cooke JP. Angiogenesis is impaired by hypercholesterolemia: role of assymmetric dimethylarginine. *Circulation* 2000;102:1414-1419.
23. Babaei S, Teichert-Kuliszewska K, Monge JC, Mohamed F, Bendeck MP, Stewart DJ. Role of nitric oxide in the angiogenic response in vitro to basic fibroblast growth factor. *Circ Res* 1998;82:1007-1015.
24. Matsunaga T, Warltier DC, Weihrauch DW, Moniz M, Tessmer J, Chilian WM. Ischemia-induced coronary collateral growth is dependent on vascular endothelial growth factor and nitric oxide. *Circulation* 2000;102:3098-3103.
25. Goligorsky MS, Budzikowski AS, Tsukahara H, Noiri E. Co-operation between endothelin and nitric oxide in promoting endothelial cell migration and angiogenesis. *Clin Experiment Pharm Physiology* 1999; 26:269-271.
26. Flamme I, Frolich T, Risau W. Molecular mechanisms of vasculogenesis and embryonic angiogenesis. *J Cell Physiol* 1997;173:206-210.
27. Risau W, Sariola H, Zerwes HG, et al. Vasculogenesis and angiogenesis in embryonic-stem-cell-derived embryoid bodies. *Development* 1988;102:471-478.
28. Nicosia RF, Villaschi S. Autoregulation of angiogenesis by cells of the vessel wall. *Int Rev Cytol* 1999;185:1-43.
29. Fleisch M, Billinger M, Eberli FR, Garachemani AR, Meier B, Seiler C. Physiologically assessed coronary collateral flow and intracoronary growth factor concentrations in patients with 1- to 3- vessel coronary artery disease. *Circulation* 1999;100:1945-1950.
30. Sasayama S, Fujita M. Recent insights into coronary collateral circulation. *Circulation* 1992;85:1197-1204.
31. Charney R, Cohen M. The role of the coronary collateral circulation in limiting myocardial ischemia and infarct size. *Circulation* 1993;126:937-945.
32. Ito W, Arras M, Scholz D. Angiogenesis but not collateral growth is associated with ischemia after femoral artery occlusion. *Am J Physiol* 1997; 273:H2155-H2165.
33. Jones MK, Wang H, Peskar BM. Inhibition of angiogenesis by non-steroidal anti-inflammatory drugs; insight into mechanisms and implications for cancer growth and ulcer healing. *Nat Med* 1999;5:1418-1423.
34. Cohen M, Rentrop KP. Limitations of myocardial ischemia by collateral circulation during sudden controlled coronary artery occlusion in human subjects: a prospective study. *Circulation* 1986;74:469.
35. Marcus ML, Chilian WM, Kanatsuka H, Dellsperger KC, Eastham CL, Lamping KG. Understanding the coronary circulation through studies at the microvascular level. *Circulation* 1990;82:1-7.
36. Schaper W. Control of coronary angiogenesis. *Eur Heart J* 1995;16 (Suppl C):66-68.
37. Nugent MA, Iozzo RV. Fibroblast growth factor-2. *Int J Biochem Cell Biol* 2000;32:115-120.
38. Nakagawa K, Chen YX, Ishibashi H, et al. Angiogenesis and its regulation: roles of vascular endothelial cell growth factor. *Semin Thromb Hemost* 2000;26:61-66.
39. Asahara T, Bauthers C, Zheng LP. Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. *Circulation* 1995;92:635-371.
40. Broadley KN, Aquino AM, Hicks B, et al. Growth factors bFGF and TGB beta accelerate the rate of wound repair in normal and in diabetic rats. *Int J Tissue React* 1988;10:345-353.
41. Greenhalgh DG, Sprugel KH, Murray MJ, Ross R. PDGF and FGF stimulate wound healing in the genetically diabetic mouse. *Am J Path* 1990;136:1235-1246.
42. Thompson DW, Li WW, Maragoudakis M. The clinical manipulation of angiogenesis: pathology, side-effects, surprises, and opportunities with novel therapies. *J Path* 2000;190:330-337.
43. Baffour R, Berman J, Garb JL, Rhee SW, Kaufman J, Friedmann P. Enhanced angiogenesis and growth of collaterals by in vivo administration of recombinant basic fibroblast growth factor in a rabbit model of acute lower limb ischemia: dose-response effect of basic fibroblast growth factor. *J Vasc Surg* 1992;16(2):181-191.
44. Isner JM, Kaufman J, Rosenfield K, et al. Combined physiologic and anatomic assessment of percutaneous revascularization using a Doppler guidewire and ultrasound catheter. *Am J Cardiol* 1993;14:70D-86D.
45. Pu LQ, Sniderman AD, Brassard R, et al. Enhanced revascularization of the ischemic limb by angiogenic therapy. *Circulation* 1993;88:208-215.
46. Takeshita S, Pu LQ, Stein LA, et al. Intramuscular administration of vascular endothelial growth factor induces dose-dependent collateral artery augmentation in a rabbit model of chronic limb ischemia. *Circulation* 1994;90:II228-II234.
47. Takeshita S, Zheng LP, Brogi E, et al. Therapeutic angiogenesis. A single intraarterial bolus of vascular endothelial growth factor augments revascularization in a rabbit ischemic hind limb model. *J Clin Invest* 1994;93:662-670.
48. Harada K, Friedman M, Lopez JJ, et al. Vascular endothelial growth factor administration in chronic myocardial ischemia. *Am J Physiol* 1996; 270:H1791-H1802.
49. Lazarous DF, Shou M, Stiber JA, et al. Adenoviral-mediated gene transfer induces sustained pericardial VEGF expression in dogs: effect on myocardial angiogenesis. *Cardiovasc Res* 1999;44:294-302.
50. Lopez JJ, Edelman ER, Stamler A, et al. Basic fibroblast growth factor in a porcine model of chronic myocardial ischemia: a comparison of angiographic, echocardiographic and coronary flow parameters. *J Pharmacol Exp Ther* 1997;282:385-390.
51. Lopez JJ, Edelman ER, Stamler A, et al. Angiogenic potential of perivascularly delivered aFGF in a porcine model of chronic myocardial ischemia. *Am J Physiol* 1998;274:H930-H936.

52. Shou M, Thirumurti V, Rajanayagam S, et al. Effect of basic fibroblast growth factor on myocardial angiogenesis in dogs with mature collateral vessels. *J Am Coll Cardiol* 1997;29:1102-1106.
53. Schumacher B, Pecher P, von Specht BU, Stegmann T. Induction of neoangiogenesis in ischemic myocardium by human growth factors. First clinical results of a new treatment for coronary heart disease. *Circulation* 1998;97:645-650.
54. Pecher P, Schumacher BA. Angiogenesis in ischemic human myocardium: clinical results after 3 years. *Ann Thorac Surg* 2000; 69:1414-1419.
55. Sellke FW, Laham RJ, Edelman ER, Pearlman JD, Simons M. Therapeutic angiogenesis with basic fibroblast growth factor: technique and early results. *Ann Thorac Surg* 1998;65:1540-1544.
56. Laham RJ, Sellke FW, Edelman ER, et al. Local perivascular delivery of basic fibroblast growth factor in patients undergoing coronary bypass surgery: results of a phase I randomized, double-blind, placebo-controlled trial. *Circulation* 1999;100:1865-1871.
57. Unger EF, Goncalves L, Epstein SE, et al. Effects of a single intracoronary injection of basic fibroblast growth factor in stable angina pectoris. *Am J Cardiol* 2000;85:1414-1419.
58. Udelson JE, Dilsizian V, Laham RJ, et al. Therapeutic angiogenesis with recombinant fibroblast growth factor-2 improves stress and rest myocardial perfusion abnormalities in patients with severe symptomatic chronic coronary artery disease. *Circulation* 2000;102:1605-1610.
59. Laham RJ, Chronos NA, Leimbach M, et al. Results of a phase I open label dose escalation study of intracoronary and intravenous basic fibroblast growth factor (rFGF-2) in patients (pts) with severe ischemic heart disease: 6 months follow-up [abstract]. *J Am Coll Cardiol* 2000;35(suppl A):73.
60. Henry TD, Rocha-Singh K, Isner JM, et al. Results of intracoronary recombinant human vascular endothelial growth factor (rhVEGF) administration trial (abstract). *J Am Coll Cardiol* 1998;31(Suppl A):65A.
61. Hendel RC, Henry TD, Rocha-Singh K, et al. Effect of intracoronary recombinant human vascular endothelial growth factor on myocardial perfusion: evidence for a dose-dependent effect. *Circulation* 2000; 101:118-121.
62. Losordo DW, Vale PR, Symes JF, et al. Gene therapy for myocardial angiogenesis: initial clinical results with direct myocardial injection of phVEGF165 as sole therapy for myocardial ischemia. *Circulation* 1998; 98:2800-2804.
63. Vale PR, Symes JF, Esakof DD, et al. Direct myocardial gene transfer of VEGF165 in patients with end-stage coronary artery disease: 12-month results of a phase I/II clinical trial (abstract). *J Am Coll Cardiol* 2001;37(Suppl A):285A.
64. Hendel RC, Vale PR, Losordo DW, et al. The effects of VEGF-2 gene therapy on rest and stress myocardial perfusion: results of serial SPECT imaging (abstract). *Circulation* 2000;102 (Suppl):II-769.
65. Fortuin FD Jr., Vale P, Losordo DW, et al. Direct myocardial gene transfer of vascular endothelial growth factor-2 (VEGF-2) naked DNA via thoracotomy relieves angina pectoris and increases exercise time: one-year follow-up of a completed dose-escalating phase 1 study (abstract). *J Am Coll Cardiol* 2001;37(Suppl A):285A-286A.
66. Vale PR, Milliken CE, Fortuin D, et al. Correlation of NOGATM left ventricular electromechanical mapping and radionuclide perfusion imaging demonstrating augmented perfusion of ischemic myocardium in patients undergoing direct myocardial VEGF-2 gene transfer (abstract). *J Am Coll Cardiol* 2001,37(Suppl A):370A.
67. Symes JF, Losordo DW, Vale PR, et al. Gene therapy with vascular endothelial growth factor for inoperable coronary artery disease. *Ann Thorac Surg* 1999;68:830-836.
68. Rosengart TK, Lee LY, Patel SR, et al. Crystal RG. Six-month assessment of a phase I trial of angiogenic gene therapy for the treatment of coronary artery disease using direct intramyocardial administration of an adenovirus vector expressing the VEGF121 cDNA. *Ann Surg* 1999;230:466-470.
69. Rosengart TK, Lee LY, Patel SR, Sanborn TA, Parikh M, Bergman GW, Hachamovitch R, Szulc M, Kligfield PD, et al. Angiogenesis gene therapy: phase I assessment of direct intramyocardial administration of an adenovirus vector expressing VEGF121 cDNA to individuals with clinically significant severe coronary artery disease. *Circulation* 1999;100:468-474.
70. Rosengart TK, Patel SR, Crystal RG. Therapeutic angiogenesis: protein and gene therapy delivery strategies. *J Cardiovasc Risk* 1999;6:29-40.
71. Simons M, Bonow RO, Chronos NA, et al. Clinical trials in coronary angiogenesis: issues, problems, consensus: An expert panel summary. *Circulation* 2000;102:E73-E86.
72. Laham RJ, Garcia L, Baim DS, Post M, Simons M. Therapeutic angiogenesis using basic fibroblast growth factor and vascular endothelial growth factor using various delivery strategies. *Curr Interv Cardiol Rep* 1999;1:228-233.
73. Laham RJ, Rezaee M, Post M, et al. Intracoronary and intravenous administration of basic fibroblast growth factor: myocardial and tissue distribution. *Drug Metab Dispos* 1999;27:821-6.
74. Laham RJ, Rezaee M, Garcia L, et al. Tissue and myocardial distribution of intracoronary, intravenous, intrapericardial and intramyocardial 125-I-labeled basic fibroblast growth factor (bFGF) favor intramyocardial delivery (abstract). *J Am Coll Cardiol* 2000;35(Suppl A):10A.
75. Anderson ED, Mourich DV, Leong JA. Gene expression in rainbow trout (Oncorhynchus mykiss) following intramuscular injection of DNA. *Mol Mar Biol Biotechnol* 1996;5:105-113.
76. McElvaney NG. Is gene therapy in cystic fibrosis a realistic expectation? *Curr Opin Pulmonary Med* 1996; 2:466-471.
77. Brown LF, Detmar M, Claffey K, et al. Vascular permeability factor/vascular endothelial growth factor: a multifunctional angiogenic cytokine. *EXS* 1997;79:233-269.
78. Masumura M, Murayama N, Inoue T, Ohno T. Selective induction of fibroblast growth factor receptor-1 mRNA after transient focal ischemia in the cerebral cortex of rats. *Neurosci Lett* 1996;213:119-122.
79. Detmar M, Brown LF, Berse B, et al. Hypoxia regulates the expression of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) and its receptors in human skin. *J Invest Dermatol* 1997; 108:263-268.
80. Takami K, Kiyota Y, Iwane M, et al. Upregulation of fibroblast growth factor-receptor messenger RNA expression in rat brain following transient forebrain ischemia. *Exp Brain Res* 1993;97:185-194.
81. Banai S, Jaklitsch MT, Casscells W, et al. Effects of acidic fibroblast growth factor on normal and ischemic myocardium. *Circ Res* 1991; 69:76-85.
82. Herold-Mende C, Steiner HH, Andl T, et al. Expression and functional significance of vascular endothelial growth factor receptors in human tumor cells. *Lab Invest* 1999;79:1573-1582.
83. Schultz A, Lavie L, Hochberg I, et al. Interindividual heterogeneity in the hypoxic regulation of VEGF: significance for the development of the coronary artery collateral circulation. *Circulation* 1999;100:547-552.
84. Volpert OV, Ward WF, Lingen MW. Captopril inhibits angiogenesis and slows the growth of exprimental tumours in rats. *J Clin Invest* 1996;98:671-679.
85. Felesko W, Balkowiec E.Z., Sieberth E. Lovastatin and tumour necrosis factor-alpha exhibit potentiated antitumour effects against Ha-ras-transformed murine tumour via inhibition of tumour-induced angiogenesis. *Int J Cancer* 1999;81:560-567.
86. Panet R, Markus M, Atlan H. Bumetanide and furosemide inhibited vascular endothelial cell proliferation. *J Cell Physiol* 1994;158:121-127.
87. Van Belle E, Bauters C, Bertrand ME. From the angiogenic response to ischemia to the validation of the concept of "therapeutic angiogenesis". *Arch Mal Coeur Vaiss* 1998;91:1159-1170.
88. Rivard A, Silver M, Chen D, et al. Rescue of diabetes-related impairment of angiogenesis by intramuscular gene therapy with adeno-VEGF. *Am J Pathol* 1999;154:355-363.
89. Hamawy AH, Lee LY, Crystal RG, Rosengart TK. Cardiac angiogenesis and gene therapy: a strategy for myocardial revascularization. *Curr Opin Cardiol* 1999;14:515-522.
90. Hanahan D. Signaling vascular morphogenesis and maintenance. *Science* 1997;277:48-50.

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