● Review ●

# Therapeutic Angiogenesis for the Treatment of Coronary Artery Disease: Can We Improve the Results?

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**Abstract:** The goal of therapeutic angiogenesis is to restore blood flow to ischemic myocardium using growth factors or gene therapy, without intervening on the epicardial coronary arteries. Although angiogenesis has recently received considerable scientific attention, it has not yet been shown to provide significant clinical benefit. Thus, it is still reserved for patients who have failed conventional therapies, and even then only in an experimental setting. Nevertheless, angiogenesis is a very potent physiologic process involved in the growth and development of both animals and humans. Thus, once its underlying mechanistic basis is better understood and effects of endogenous inhibitors can be overcome, it is likely that its use for therapeutic purposes may one day become a clinically significant modality for the treatment of patients with severe coronary artery disease. (J Jpn Coll Angiol, 2005, **45**: 221–232)

**Key words:** angiogenesis, growth factor, collateral vessel, fibroblast growth factor, hypercholesterolemia

## Introduction

Cardiovascular disease is a major cause of morbidity and death, not only in the Western world, but also in Asia and in other areas previously spared from severe atherosclerotic vascular disease, since poor dietary habits and other life style changes predisposing to atherosclerosis are becoming standard in these regions. Despite better management of cardiovascular risk factors, and improved non-surgical and surgical treatment options, coronary artery disease (CAD) may involve the epicardial vasculature of patients in such a diffuse and severe manner that repeated attempts at catheter-based interventions and coronary artery bypass grafting (CABG) may be unsuccessful at restoring normal myocardial blood flow. Patients with myocardial ischemia who are not eligible for CABG on the basis of poor graftability have an increased incidence of myocardial infarction and death.[1] It has been estimated that these patients constitute approximately 5% of patients who undergo coronary angiography at large referral centers. Other patients may have one or more myocardial territories that are ungraftable at the time of CABG,[2, 3] a situation associated with decreased survival and diminished freedom from angina after bypass surgery.[4]

Therapeutic angiogenesis aims to restore perfusion to chronically ischemic myocardium using growth factors, gene therapy or more recently, cell-based therapy, without intervening on the epicardial coronary arteries. However, despite initial enthusiasm, angiogenesis has not yet provided significant clinical benefit and is still reserved as an experimental treatment for patients who have failed conventional therapies.[5] Angiogenesis is a very potent physiologic process involved in the growth and development of all animals and humans, and it is plausible that its routine use for therapeutic purposes will one day become a practical reality, once the process is better understood and its endogenous inhibitors

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**Table 1** Substances involved in signal transduction of angiogenesis

| | Role in angiogenesis |
|---|---|
| Nitric Oxide | Vasodilation; co-factor for VEGFs, FGFs and other angiogens |
| Cyclooxygenase-2 | Vasodilatation; stimulation of angiogenesis |
| Vascular endothelial growth factors | Vasodilation; increased permeability; sequestration of VE-cadherin and PECAM-1; endothelial cell proliferation; formation of cords and lumens |
| Angiopoietin-1 | Prevention of excessive vascular permeability; endothelial cell chemotaxis; formation of cords and lumens; vessel stabilization via smooth muscle to endothelial cell interactions |
| Angiopoietin-2 | Vessel destabilization; detachment of smooth muscle cells; degradation of extracellular matrix (in conjunction with matrix metalloproteinases) |
| Fibroblast growth factors | Endothelial cell proliferation; formation of cords and lumens; recruitment of inflammatory cells, pericytes and smooth muscle cells; vessel maturation and enlargement |

FGF = fibroblast growth factor; PECAM = platelet endothelial cell adhesion molecule; VE = vascular endothelial; VEGF = vascular endothelial growth factor

can be modified.

## Basic mechanisms of angiogenesis

Anigogenesis is actually three different processes that results in the growth of new blood vessels in animals and humans: vasculogenesis, arteriogenesis, and true angiogenesis.[6, 7] *Vasculogenesis* is a process that occurs mainly during fetal development, and consists of the differentiation of endothelial cells from angioblasts and endothelial progenitor cells followed by their proliferation, coalescence, and recruitment of other cell types to complete the process of vascular formation *in situ*.[8] Initially considered to play little or no role in the response to chronic ischemia of adult tissues, vasculogenesis has now been shown to play a potentially important impact postnatally, and is the subject of an increasing body of literature.[9, 10]

*Arteriogenesis* refers either to the process by which a postnatal vascular network remodels by maturation of preexisting collaterals in response to supply-demand imbalances or, secondly, to the de novo formation by sprouting of mature blood vessels that contain pericytes and smooth muscle cells, which actually constitutes the goal of therapeutic "angiogenesis" modalities.

*True angiogenesis* refers to the sprouting into surrounding tissues of newly formed capillaries derived from preexisting vessels. This process is spontaneously seen, for example, in the border zone of a myocardial infarct or in granulation tissue during wound healing. However, these newly formed capillaries lack a fully developed medial layer, have abnormal permeability, and show poor vasomotor regulation. It is uncertain and controvertial what importance true angiogenesis plays in the creation of physiologically important sources of new blood flow.

## Endogenous angiogenesis in adult tissues

Both arteriogenesis and true angiogenesis spontaneously occur under a variety of stresses such as wound healing and inflammation,[11] peripheral vascular disease, chronic coronary insufficiency,[12-14] and acute myocardial ischemia.[15-21] Endogenous angiogenesis is also enhanced by a number of commonly used substances. Nicotine, for instance, is proangiogenic and may worsen atherosclerotic plaques by promoting intimal proliferation.[22] Moderate ethanol concentrations and low-dose statins also have proangiogenic properties, and the use of statins has been associated with increased tissue perfusion in a hind limb ischemia model.[23, 24]

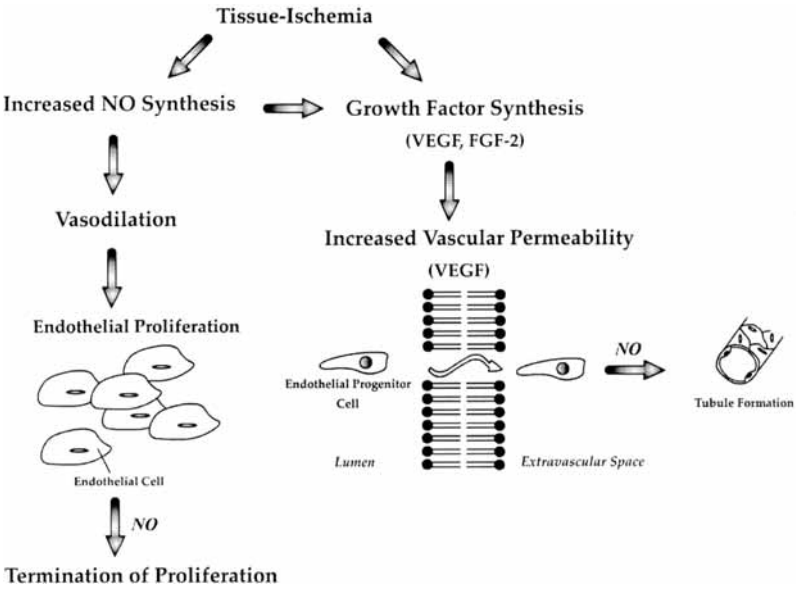


**Figure 1** Angiogenesis and nitric oxide (NO). NO synthesis following tissue ischemia results in vasodilation leading to endothelial proliferation. NO also induces growth factor synthesis including VEGF, which increases vascular permeability. Endothelial progenitor cells enter the extravascular space along with plasma proteins. At a later stage in the angiogenic process, as a likely result of NO signalling interactions with calveolin-1, the endothelial proliferative response is terminated to allow for the organization of newly formed endothelial cells into vascular tubules. Together these changes result in new vessel formation.

Adenosine and heparin also appear to independently stimulate angiogenesis.[25-27] Thus, some of the agents known to predispose to plaque rupture may actually enhance the development of new blood vessels.

Conversely, some commonly used medications inhibit angiogenesis. These include captopril,[28] isosorbide dinitrate,[29] furosemide,[30] spironolactone,[31] as well as ASA and other anti-inflammatory drugs, whose antiangiogenic effects are related to the inhibition of the inducible isoform of cyclooxygenase (COX-2).[32, 33] Endogenous angiogenic capacity decreases with age in animals, and correlates with a decrease in myocyte platelet-derived growth factor production.[34]

## Growth factors and delivery strategies

Although the development of new vascular networks is complex, therapeutic angiogenesis regimens have to date focused mainly on the administration of a single growth factor, either as a protein- or gene-based preparation. Protein- or gene-based approaches using select isoforms of VEGF-A ($VEGF_{121}$, $VEGF_{165}$) and FGF (FGF-1, FGF-2, FGF-4) have been most extensively studied. Delivery strategies may involve the actual angiogenic protein, the gene encoding for it by using naked plasmid DNA or a viral vector, or a native or transgenic cell preparation believed to stimulate or participate in the angiogenic cascade. Several routes of administration have been developed to deliver angiogenic substances to the heart in a single or repeated fashion; these include intravenous, intracoronary, left atrial, surgical perivascular/intramyocardial, intrapericardial via a catheter placed under echo guidance, and catheter-based intramyocardial approaches.

## Genetic modification

Gene therapy approaches require vectors to incorporate the angiogenic gene into a target host cell and induce production of the encoded protein. Naked plasmid DNA can be used for this purpose, but its efficiency is generally limited by the amount of plasmid DNA that actually enters the cell nucleus

and the transfection efficacy.[35, 36] Adenoviral vectors are associated with higher transfection efficiency, and can be readily produced as replication-deficient mutants for gene transfer applications.[37] However, circulating antibodies to adenoviruses are common in humans and may elicit an inflammatory response compromising the incorporation and expression of the gene.[38] Alternatives such as adeno-associated viruses, which are unique in their ability to transduce nondividing cells, allowing for more prolonged transgene expression, and retroviral vectors have been developed.[35] Retroviruses differ from plasmid and other viral vectors in that their RNA is reverse transcribed to DNA and integrated into the genome of the host cell (i.e. non-epichromosomal). While this induces long-lasting expression of the incorporated gene, it also raises safety concerns related to its potential overexpression.[7] In order to optimize the efficacy and safety of gene delivery vectors, viral vectors allowing for up- or downregulation of the gene of interest are currently being investigated.

## Preclinical studies of angiogenesis

In order to evaluate the potential of growth factors or other agents to produce a physiologically significant angiogenic effect, animal models of chronic myocardial ischemia must be used. Because of species specific heterogeneity of angiogenesis and its mechanisms, a large animal model of chronic myocardial ischemia is useful for the preclinical, functional evaluation of therapeutic angiogenesis modalities. Laboratory animals rarely or never spontaneously develop CAD, coronary insufficiency must be experimentally created. Vessel embolization, surgical ligation, and thrombogenic copper coil implantation mainly result in acute coronary occlusion and myocardial infarction; although a chronic ischemic area exists at the border of this infarct, this does not optimally model the ischemic myocardial territory of patients with severe angina.

There are several larege animal models of chronic myocardial ischemia. One of the most consistent and frequently used is created by surgical implantation and intermittent inflation of an external pneumatic coronary occluder,[39, 40] or by inserting an ameroid constrictor around a major coronary artery, usually the proximal left circumflex.[41] The ameroid method results in progressive stenosis and occlusion of the encircled vessel over a period of 2 to 4 weeks. The constrictor consists of hygroscopic casein compressed into a cylindrical shape and enclosed within a stainless steel collar; when in contact with fluid, the casein expands in an inward direction due to the fixed metal ring and occludes the artery. Experimental protocols typically involve administration of growth factors or cells 3–4 weeks after implantation of the ameroid, by which time it has closed and the myocardium has been made ischemic.[42]

The preclinical experience with VEGF in animal models of ischemia has mainly involved its $VEGF_{165}$ and $VEGF_{121}$ isoforms, derived from splicing of the VEGF-A gene. Perivascular or intramyocardial administration of VEGF has been led to impressive neovascularization in animals,[43, 44] but the use of intracoronary, intrapericardial or intravenous routes has not always been as successful. For instance, a 28-day course of intracoronary VEGF injections in dogs was effective in increasing flow to the collateral-dependent territory,[45] but a 7-day course was not and actually exacerbated neointimal accumulation following endothelial injury.[46] In another study, an adenoviral vector encoding for $VEGF_{165}$ injected through an indwelling pericardial catheter resulted in pericardial transgene expression, but no increase in perfusion of the collateral-dependent territory.[47] Intravenous injection of $VEGF_{165}$ was also found by Sato et al. to be relatively ineffective in a swine model.[48] Finally, there is concern that the type of vessels induced with VEGF isoforms are too small to provide sufficient increases in blood flow to be physiologically relevant.

Numerous biologically active isoforms of another growth factor, fibroblast growth factor (FGF), have been examined. Of these isoforms, FGF-1, FGF-2 and FGF-4 have been most studied. FGFs are believed to induce angiogenesis as well as arteriogenesis by stimulating growth of a variety of cell types, including vascular smooth muscle cells and pericytes.[49] Most experience in animals has been with FGF-2, using either protein or gene transfer approaches.[50] Unger et al. gave daily intracoronary bolus injections of 110 $\mu$g of FGF-2 in the distal circumflex artery of dogs for 28 consecutive days.[51] The transmural collateral flow in FGF-2-treated dogs exceeded that of controls by the second week of treatment and was

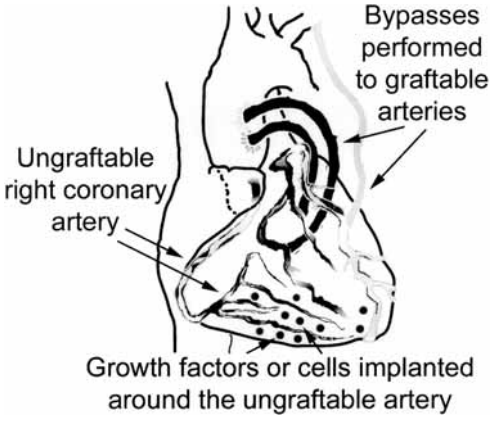


**Figure 2** Perivascular/intramyocardial angiogenesis in combination with coronary artery bypass grafting. In this example, sustained-release, protein-based angiogenesis was safely and successfully performed in the myocardial distribution of an ungraftable right coronary artery.[59]

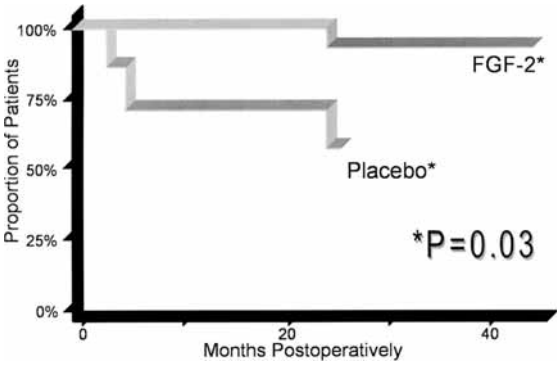


**Figure 3** Freedom from angina in patients who underwent surgical implantation of sustained-release FGF-2 protein versus placebo in a major, non-graftable myocardial territory at the time of coronary artery bypass grafting.[60]

associated with an increase in vessel density. Local perivascular administration of FGF-2 was also studied in swine and led to increased perfusion of the collateral-dependent territory and a dose-dependent improvement in left ventricular ejection fraction both at rest and during pacing.[52-54] The protocol used 10 sustained-release heparin-alginate capsules, each containing 1 or 10 $\mu$g of FGF-2, implanted 3 weeks after ameroid placement around the occluded vessel and in the transition zone between the normal and collateral-dependent territories. In other studies, single-dose intrapericardial and intracoronary delivery of FGF-2 led to perfusion and contractility improvements; however, single-dose intravenous infusion was not shown to be effective.[55, 56]

## Clinical trials of protein-based angiogenesis

### Surgery-based epicardial growth factor delivery

The first clinical demonstration of therapeutic angiogenesis in the heart was reported by Schumacher et al. Intramyocardial FGF-1 was used in a series of 20 patients, in which 0.01 mg/kg of FGF-1 protein was injected directly into the myocardium along a diffusely diseased left anterior descending coronary artery to which the left internal thoracic artery was also grafted.[57, 58] Patients were followed up 3 months and 3 years later with digital subtraction angiography, which revealed a local increase in collateral blush along the LAD. However, angina class status or nuclear imaging assessments of ischemia were not reported.

The safety and efficacy of perivascular FGF-2 administration was evaluated in a phase I, double-blind, randomized controlled trial of 24 patients who concomitantly underwent CABG (**Fig. 2**).[59] In this study, patients in whom high-dose FGF-2 sustained-release capsules were implanted in the ungraftable territory had complete relief from angina and significant improvements in stress perfusion defect size at 3-month follow-up. In addition, patients treated with either dose of FGF-2 experienced significantly more freedom from angina recurrence than controls when followed up at a mean of 32 months postoperatively (**Fig. 3**).[60] All but one patient in the control group had either persistence of a reversible perfusion defect or evidence of a new fixed defect in the ungraftable myocardial territory, while this was observed in only 1 of 9 patients treated with FGF-2. FGF-treated patients also showed better late global left ventricular perfusion scores during pharmacologic stress.

### Catheter-based growth factor delivery

A phase I trial of single-bolus intracoronary administration of FGF-2 was conducted by Laham et al. in 52 patients, and showed a trend in improvement in symptoms and myocardial perfusion.[61] A multicenter, randomized, double-

blind, placebo-controlled trial followed, in which 337 patients were allocated to receive single intracoronary infusions of FGF-2 at 0, 0.3, 3, or 30 μg/kg.[62] Exercise tolerance increased at 90 days in all groups but was not significantly different between placebo and FGF-treated groups. FGF-2 did reduce angina symptoms initially, with these differences being more pronounced in highly symptomatic patients with baseline CCS angina class scores of III or IV. However, despite the initial suggestion of a positive result, this benefit did not persist at 180 days because of continued improvement in the placebo group. Adverse events were similar across all groups, except for transient hypotension, which occurred more frequently in the 30 μg/kg FGF-2 group.

Intravenous and intracoronary administration of VEGF for myocardial ischemia was studied in a randomized, double-blind, phase II study: the Vascular Endothelial Growth Factor in Vascular Angina (VIVA) trial. This trial too was negative with respect to symptom improvement, exercise time, and nuclear imaging end-points.[63]

## Clinical trials of gene-based angiogenesis

### Surgical delivery of genetic material

There have been several gene-therapy based trials of myocardial angiogenesis. Rosengart et al. examined the effects of direct administration of an adenoviral vector encoding for $VEGF_{121}$ as an adjunct to conventional CABG in 15 patients and as sole therapy in 6 patients.[64] There was no control group and no systemic or cardiac-related adverse events related to vector administration were observed. All patients reported improvements in angina class, and postoperative nuclear imaging suggested increased contractility in the area of vector administration, but no increase in myocardial perfusion.

In another study, Losordo et al. examined the safety and bioactivity of direct myocardial transfer of the $VEGF_{165}$ gene in a small phase 1 trial using naked plasmid DNA in 5 patients with inoperable CAD.[65] The vector was administered by four 2.0-ml needle injections into the anterolateral wall of the left ventricle through a small left anterolateral thoracotomy. All patients had a significant reduction in angina with reduced nitroglycerin use, improved collateral scores on angiography, and reduced size of the ischemic defect on dobutamine nuclear imaging. These data were confirmed in a second open-label, uncontrolled phase 1 study from the same group in which 20 patients received either 125 or 250 μg of naked plasmid $VEGF_{121}$, again injected directly into the myocardium via thoracotomy.[66] Like in the previous study, patients reported decreased angina and reduced nitroglycerine use, and improvement was seen on radionuclide perfusion imaging.

Huwer et al. used a protocol invoving CABG and injection of plasmid DNA encoding for $VEGF_{165}$ and $VEGF_{167}$ at a dosage of 1,000 μg each, directly into an area of myocardium not amenable to surgical revascularization. Overall results were however marginal and an increase in nuclear perfusion in the region of gene application was observed in only 3 of 24 patients.[67]

In contrast, Stewart et al. recently reported the results of a phase 2, randomized, multicenter, 26-week study to assess the efficacy and safety of adenoviral $VEGF_{121}$ delivery via minithoracotomy versus maximum medical treatment in patients with severe angina and no other option for revascularization. Patients were randomized to receive $VEGF_{121}$ in 30 direct intramyocardial injections throughout the free wall of the left ventricle via minithoracotomy, or continuation of optimal medical management. Exercise treadmill time to 1-mm ST depression, time to angina, and total exercise duration were significantly improved in the VEGF group compared to the control group at 26 weeks. There were also significant improvements in CCS angina class and Seattle Angina Questionnaire scores at 12 and 26 weeks. Although the protocol was not blinded and improvements could have been due to a placebo-effect or inflammation resulting from thoracotomy and/or vector administration, the study, in addition to suggesting efficacy, demonstrated no significant differences in adverse events between groups, no positive adenoviral cultures, and no significant changes in systemic VEGF levels.[37]

### Catheter-based gene therapy

Recently, the results of a pilot study of catheter-based myocardial gene transfer was reported in which 6 patients with chronic myocardial ischemia were randomized to receive 200 μg of naked plasmid VEGF-2 or placebo.[68] A

**Table 2** Randomized, placebo controlled clinical trials of therapeutic angiogenesis

| Authors | Intervention | Outcome |
|---|---|---|
| Henry et al, 2003 | Intracoronary VEGF-1 Protein | No difference in nuclear perfusion or angiography |
| Grines et al , 2002 | Intracoronary FGF-4 Gene | No improvement in excercise treadmill testing |
| Simons et al, 2002 | Intracoronary FGF-2 Protein | No significant difference in excercise time or nuclear perfusion |
| Ruel et al, 2002<br>Laham et al, 1999 | Intramyocardial FGF-2 Protein | Perfusion scanning, ventricular stress perfusion defects, left ventricular ejection fraction improved in FGF-2 treated groups |

All trials except one have failed to provide objective evidence of efficacy in humans.

steerable, deflectable 8F catheter incorporating a 27-gauge needle was advanced percutaneously and guided into the left ventricular myocardium by left ventricular electromechanical mapping. Despite the small number of patients, end-points of angina frequency, nitroglycerin consumption, and stress myocardial perfusion on nuclear imaging revealed a trend in favor of the group transfected with VEGF-2 versus controls. These investigators subsequently proceeded to a trial involving 19 patients randomized to receive 6 injections of placebo or naked plasmid VEGF-2 in doses of 200, 800, or 2,000 $\mu$g guided by left ventricular electromechanical mapping. A significant improvement in Canadian Cardiovascular Society (CCS) angina class was noted at 12 weeks in VEGF-treated versus placebo-treated patients.[69]

## Safety and efficacy issues

### Safety profile of protein- and gene-based approaches

In murine models, the overexpression of VEGF has been associated with the formation of angiomas and vascular tumors,[70, 71] but the occurrence of these adverse events or proliferative retinopathy has not been reported in large animal studies of growth factor therapy. VEGF and FGF-2 are known to be associated with systemic hypotension in a dose-dependent fashion, with the doses of FGF-2 leading to hypotension being higher than that of VEGF.[72, 73] FGF-2 has also been associated with proliferative membranous nephropathy leading to proteinuria in mice, but this complication has not been observed in preclinical or clinical studies.[74, 75] VEGF, on the other hand, has been linked with hypercoagulability, and a relationship between serum VEGF and tissue factor levels has been demonstrated in patients with atrial fibrillation and CAD.[76]

### Delivery routes and duration of effect

Based on evidence currently available,[59, 60, 69] perivascular or intramyocardial administration of angiogenic factors or cells likely constitutes the route of choice for therapeutic angiogenesis, since it presents a more specific tissue distribution profile than intravascular techniques, does not result in rapid washout, is not limited by the endothelial barrier, and does not carry the potential of exacerbating intimal plaques.[22] The myocardial and tissue distribution of $I^{125}$ labeled FGF-2 after intracoronary and intravenous administration in swine has been studied with organ autoradiography.[77] The liver accounted for the majority of $I^{125}$ labeled FGF-2 activity at 1 h after injection with either route; total cardiac specific activity at 1 h was 0.88% for intracoronary and 0.26% for intravenous administration, and further decreased to 0.05% and 0.04% at 24 h, respectively. In another study, the amount of FGF-2 deposited in arteries adjacent to sustained-release devices was 40 times that deposited in animals who received a single intravenous bolus of FGF-2.[78]

### Adjuvant therapy to improve angiogenesis

There is interaction between the local availability of NO and the regulation of blood vessel growth mediated by the actions of VEGF and FGF-2.[79-82] Diminished NO availability, due to inactivation by interaction with superoxide anion and decreased prodiction, has been implicated in the inhibition of endothelial cell migration and capillary-like tube formation *in vitro*,[83] of basal and exogenous angiogenic

responses in hypercholesterolemic rodents,[84, 85] of basal angiogenesis in dogs,[86] and of the angiogenic response to exogenous FGF-2 in a hypercholesterolemic swine model of endothelial dysfunction.[87] Given these data and the fact that therapeutic angiogenesis is not nearly as effective in patients with inoperable CAD as it has been in laboratory animals, the failure of effect observed in clinical trials may relate to a deficiency in the stimulated release of NO, whose production as well as that of other endothelium-derived substances is altered in end-stage CAD.[88, 89] The current clinical indications for angiogenic therapy may therefore paradoxically target patients for whom the modality therapy is least likely to work, and it is possible that the clinical efficacy of therapeutic angiogenesis may therefore benefit from concomitant modulation of the coronary microvascular endothelium in patients with end-stage CAD. Such research is ongoing and could bridge the missing link between successful animal models and disappointing clinical trials of angiogenic therapy. We have recently reported that the concomitant administration of the nitric oxide substrate L-arginine may improve the efficacy of VEGF in producing an angiogenic response in a hypercholesterolemic porcine model of myocardial ischemia (x). Other approached that are being considered are the concomitant administration of oral antio-oxidant vitamins or other anti-oxidant agents, or the administration of HMG-co-reductase inhibitors (statins) that have been associated with decreased inflammation and a reduction in myocardial and vascular oxidative stress. However, the angiogenic response to certain growth factors such as VEGF are known to be in part dependent on the presence of oxygen-derived free radicals. Thus, drugs which markedly alter oxygen free radical concentration of tissues may negatively impact on the angiogenic potential of these growth factors. Experiment in the laboratory and in clinical trails will be required to determine the answer.

### Multi-agent therapy and master-switch genes

The physiologic events that result in angiogenesis are complex and incompletely understood. Therapeutic angiogenic approaches so far have concentrated on the administration of a single growth factor or cell type to induce the development of new large blood vessels that have the potential to provide incremental blood flow to the ischemic myocardium. Practicality and safety considerations as well as by the limited knowledge of potential interactions between growth factors led to this approach. However, it is debatable whether this approach will ever result in clinically reproducible formation of long-lasting, functional vessels.[90] The safety and efficacy of therapeutic approaches could be increased by stimulating endogenous angiogenesis in response to ischemia or with combinations of exogenous growth factors. Experimental stimulation of endogenous angiogenesis without administration of exogenous growth factors was previously achieved by modulating the proangiogenic properties of the gastric submucosa in a swine model of chronic myocardial ischemia.[87]

The use of master-switch agents, which induce the basal cascades of angiogenesis-related genes are upregulated in presence of ischemia, and mediate the endogenous angiogenic responses of animals and humans.[20, 90, 91] It has been suggested that the delivery of such agents may be an alterative approach to the optimal formation of new blood vessels. Hypoxia induced factor (HIF)-1-alpha is a prototype gene expressed in ischemic tissues and which initiates the cascade of VEGF-dependent angiogenesis.[90] PR39, relaxin and sonic hedgehog are other master-switch agents with the propensity to induce the VEGF, FGF and angiopoietin systems, respectively.[90, 92-95] It is unknown, however, whether the therapeutic use of these master-switch genes could lead to excessive, uncontrolled angiogenesis; their use should therefore be restricted until they are better understood and their tissue distribution reliably confined to a target tissue.

## Conclusion

Therapeutic angiogenesis is a promising modality for the treatment of patients with otherwise untreatable coronary artery disease. It is still experimental, reserved as an experimental treatment for selected patients with inoperable diffuse distal coronary disease, and likely to be more effective using a perivascular/intramyocardial approach rather than non-specific intravascular administration. Whether a sustained-release protein- or gene-based approaches will prove safer and more effective than the other remains to be deter-

mined. Continued research efforts need to be directed at overcoming the numerous limitations of current angiogenic regimens, including the effects of oxidative stress and endogenous inhibitors of angiogenesis. With these efforts, it is hoped that stimulation of angiogenesis for therapeutic purposes will one day effectively and safely recreate the natural process of vascularization that humans undergo during growth and development, and become a major modality for the treatment of coronary artery disease.

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