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Angiogenesis in Cardiovascular Disease

Current Status and Therapeutic Potential

Frank W. Sellke and *Michael Simons*

Beth Israel Deaconess Medical Center, Division of Cardiothoracic Surgery and Cardiovascular Division, Harvard Medical School, Boston, Massachusetts, USA

Abstract

Therapeutic angiogenesis, in the form of growth factor protein administration or gene therapy, has emerged as a new method of treatment for patients with severe, inoperable coronary artery disease. Improved myocardial perfusion and function after the administration of angiogenic growth factors has been demonstrated in animal models of chronic myocardial ischaemia. Recently, preliminary clinical trials using growth factor proteins or genes encoding these angiogenic factors have demonstrated clinical and other objective evidence of relevant angiogenesis. Thus, therapeutic angiogenesis has the potential to extend treatment options to patients who are not optimal candidates for conventional methods of myocardial revascularisation.

Angiogenesis, the development and growth of blood vessels, has been familiar to scientists and clinicians for most of the present century. Since collateral vessels develop and grow in the interface between the ischaemic and normally perfused myocardium during gradual coronary occlusion, and certain mitogenic growth factors and cytokines can induce angiogenesis, it logically follows that the administration of angiogenic substances has a potential role in the management of patients with occlusive coronary artery disease (CAD). Recently, therapeutic angiogenesis, in the form of growth factor protein administration or gene therapy, has emerged as a method for the treatment of patients who are not suitable candidates for more conventional methods of revascularisation, such as percutaneous transluminal angioplasty (PTCA) or coronary artery bypass surgery (CABG).

Endothelial and vascular smooth muscle cells are mitotically inactive in normal adult coronary arteries.[1] However, during growth and development, and under conditions of ischaemia, hypoxia, inflammation or other stresses, these cells may begin to migrate and divide, especially in the microcirculation. This eventually results in the development of new intramuscular blood vessels. This process passes through several steps including the dissolution of the bond between the endothelium and the underlying basement membrane, migration, adhesion and reattachment of the endothelial cells, and proliferation and tube formation culminating in the development of a new capillary or rudimentary blood vessel. The cellular and molecular changes required for this process to occur are numerous and complex, and have only recently become understood. The development of larger, muscular epicardial arteries in the face of ischaemia, hypoxia or inflammation, or during vascular development is technically referred to neo-arteriogenesis, and the *de novo* embryological formation of

blood vessels from angioblasts is generally known as vasculogenesis.[1-3] While angiogenesis, vasculogenesis and arteriogenesis are distinct developmental events with distinct regulation, these processes all ultimately lead to the same end result; namely, increased blood flow to the underperfused myocardium. Therefore, a separate distinction will not be made in this brief review.

1. Mechanisms of Angiogenesis

Angiogenesis in the heart is most often associated with acute or chronic occlusion of a major coronary artery. If the occlusion occurs gradually, sufficient time may exist for the development of an extensive collateral network with relatively few clinical manifestations of ischaemia. All clinical cardiovascular specialists recognise that even extensive coronary occlusions can be evident in patients with normal left ventricular function. However, since even gradual occlusion of a major coronary artery in patients is usually associated with areas of infarction or at least reduced myocardial perfusion, angiogenesis is generally not sufficient to completely prevent signs and symptoms of coronary insufficiency. When abrupt coronary occlusion occurs, native collateral vessels are recruited but are seldom sufficient to completely prevent ischaemia or infarction in patients.

Hypoxia and ischaemia certainly play a significant role in the angiogenic process, but the relative contributions and interdependence of these factors with such influences as inflammation and shear forces are not known with certainty. The presence of an interface between normal and ischaemic or hypoxic tissues may contribute or be critical to the angiogenic process. Thus, other processes additional to tissue ischaemia or hypoxia, such as inflammation, must be involved in the angiogenic mechanism.

Almost universally, myocardial ischaemia and angiogenesis are associated with the production and release of growth factors. This suggests that these protein substances are mediators of angiogenesis and critical for the formation of new vascular networks. Indeed, elevated levels of fibroblast growth factor (FGF)-2 and vascular endothelial growth factor (VEGF) have been detected in the pericardial fluid of patients with unstable angina pectoris.[4] Not only is the presence of these growth factors critical in the initiation of angiogenesis, but their respective receptors must also be up-regulated and the actions of inhibitory factors must themselves be inhibited. Angiogenesis in the heart may be associated with increased protein and gene expression of FGF-1 and FGF-2, VEGF and platelet-derived growth factor, in addition to many other angiogenic factors. Increased expression of the FGF receptor FGFR1 and the VEGF receptors flt-1 and flk-1 occurs in both acute[5,6] and chronic[5,7] myocardial ischaemia, implicating the role of growth factors in the angiogenesis process. Some of these growth factors are constitutively expressed in the myocardium, whereas others[8,9] are induced mainly in response to a stimulus.

2. Therapeutic Angiogenesis

2.1 Studies in Animals

Improved myocardial perfusion and function after the administration of angiogenic growth factors has been demonstrated in animal models of myocardial ischaemia, including the ameroid occluder model. In this model, a hydrophilic plastic (ameroid) ring with a lumen of similar diameter to a coronary artery is surrounded by a circular casing made of either steel or hard plastic. The ameroid occluder is placed around the vessel and creates a chronic obstruction of the coronary artery, generally over 10 to 20 days, with minimal evidence of infarction. This is in contrast to acute ligation of the vessel, which generally causes a significant transmural infarction and is associated with a high rate of mortality.

Calcium alginate micro-capsules containing FGF-2 have been used to continuously deliver the growth factor over several weeks. This resulted in improved myocardial perfusion and contractility in the ischaemic zone[10] and normalisation of endothelium-dependent relaxation of the collateral-dependent microcirculation.[11] Attempts at the in-

travascular infusion of FGF-1 have met with minimal success,[12] but the chronic epicardial application of a longer half-life mutant form of FGF-1, in which one of the cysteines is replaced with a serine residue, resulted in improved coronary blood flow, myocardial function and normalised vasomotor regulation in the chronically ischaemic territory.[13,14] Similar beneficial results have been obtained with the daily injection of FGF-2 into an indwelling catheter in the left atrium,[15] or the intracoronary, perivascular, transvascular or intrapericardial administration of VEGF.[16-19]

Gene therapy has been used to deliver angiogenic factors to the ischaemic myocardium. The delivery of FGF by an adenoviral–mediated gene transfer has been reported to augment myocardial perfusion and increase capillary density.[20] The transfection of DNA encoding VEGF has been performed in animal models of chronic myocardial ischaemia. This can be accomplished with gene transfection using adenoviral vectors, adenoviral-associated vectors, liposomal vectors or with the injection of naked DNA.[21] Thus, the feasibility of growth factor delivery by both perivascular or intravascular delivery of the protein or by gene therapy has been demonstrated.

A novel method to improve vascularity of the heart, although not clinically tested, is the use of myocyte transplantation.[22] Not only has myocyte transplantation been found to improve contractility, but it may provide a method to augment local vascularity and improve myocardial perfusion. Recently, the co-administration of growth factor protein or gene therapy in conjunction with transmyocardial laser revascularisation (TMR) has gained popularity.[23] Indeed, angiogenesis may be the principle mechanism of increased perfusion to the ischaemic myocardium after TMR,[24,25] and increased expression of growth factors has been observed in the thermally injured tissue.

2.2 Clinical Experience

The successful augmentation of collateral vessel formation was reported by Isner et al.[26] in a patient with peripheral vascular disease 4 weeks after direct intra–arterial gene transfer of a plasmid encoding for VEGF. The plasmid was applied to a hydrogel polymer coating of an angioplasty balloon. With balloon inflation the plasmid was released into the vessel wall and, presumably, improved distal perfusion. In a preliminary report by Losordo et al.,[27] the use of gene therapy for myocardial angiogenesis was described. Five patients who had failed conventional therapy were treated with naked plasmid DNA encoding $VEGF_{165}$. The plasmid was injected into the myocardium through a small lateral thoracotomy. All patients experienced a significant reduction in angina symptoms, and postoperative ejection fraction was either unchanged or improved. Objective evidence of reduced ischaemia was demonstrated by dobutamine SPECT-sestamibi imaging in all patients and coronary angiography showed an improved Rentrop score in all patients.

Recently, Schumacher et al.[28] reported the results of a randomised trial in which patients undergoing CABG given an intramyocardial injection of FGF-1 had evidence of distal neovascularisation compared with those patients given a heat inactivated form of FGF-1 who showed no evidence. No adverse effects were evident. Henry and coworkers[29] have demonstrated that the intracoronary infusion of VEGF markedly improved perfusion in approximately one-half of patients by thallium scintigraphy.

In a phase I clinical trial recently completed at our institution, basic-FGF (FGF-2) protein was administered with the use of calcium alginate polymer slow release devices to a nonbypassable myocardial region during otherwise conventional CABG surgery (fig. 1). Patients were evaluated by nuclear magnetic resonance perfusion imaging and nuclear imaging before and 3 months after initiation of growth factor treatment. Preliminary results have been encouraging,[30,31] with greater recovery of myocardial function and perfusion in those hearts receiving FGF-2 100μg compared with those patients in whom placebo or a lower dose of FGF-2 (10μg) was administered. The intracoronary and intravenous administration of VEGF have

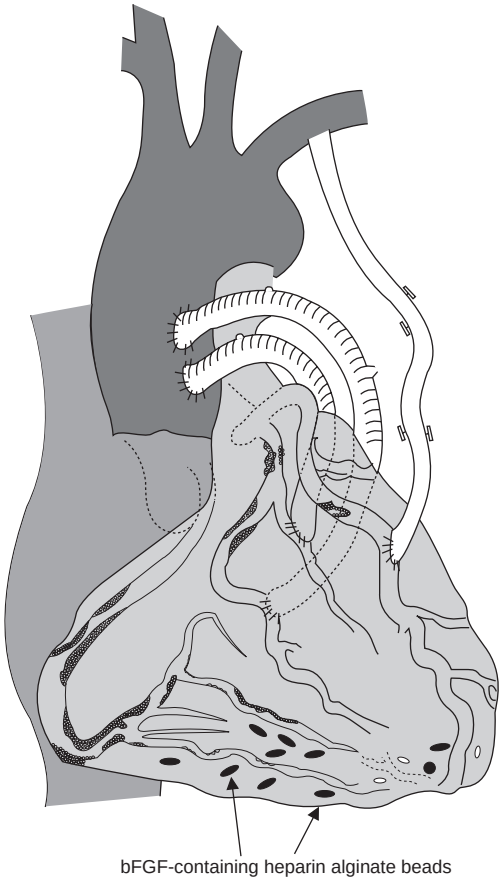

Fig. 1. Placement of fibroblast growth factor (b-FGF; FGF-2) containing heparin-alginate slow release devices in the ischaemic, and in the border zone between the ischaemic and non-ischaemic, myocardial territories (reproduced from Sellke et al.,[29] with permission).

the advantage of simplicity. Despite promising preliminary results, a phase II, randomised, placebo-controlled trial showed no improvement over placebo with either intravascular method of delivery.[32] This underscores the importance of rigorous, placebo-controlled trials prior to the initiation of the wide-spread application of angiogenic therapy.

2.3 Clinical Considerations

The optimal method or route of administration of growth factor protein or gene therapy is not currently known. The direct administration of the angiogenic protein has a simplicity in that it does not require the incorporation of a gene into the nucleus. However, gene therapy encoding the protein may be more preferable for several reasons. The cost involved in scaling up from research grade to human quality recombinant protein and the reimbursement for recombinant protein therapies in the future remains uncertain. The potential to maintain a desired concentration of VEGF or other growth factor over a period of time by direct application of the protein may be less than that by the prolonged endogenous local production of the growth factor resulting from gene transfection in the arterial wall. However, the ability of gene therapy to result in prolonged production of VEGF, FGF-2 or other growth factors is in doubt, as is the ability to 'turn off' production in some cases once it is initiated. In addition, the adverse effects of prolonged production of these factors are unknown.

Although improvement in myocardial perfusion has been documented in patients receiving either angiogenic proteins or genes encoding the proteins, the ability of angiogenic therapy to normalise perfusion and to maintain normal perfusion is in serious doubt, at least at present. In addition, it is unrealistic to expect that a single administration of an angiogenic substance would optimally increased myocardial blood flow. Therefore, repeated administrations during cardiac catheterisation, open invasive surgery or during thoracoscopy or other minimally invasive techniques, may be required.

As with all new forms of therapy, therapeutic angiogenesis is associated with potential problems and complications that need to considered before the treatment can be expanded in use. The exogenous administration of growth factors or genes encoding the growth factors may hasten the development of atherosclerosis in patients with preexisting disease and at risk for progression. In addition, there is a theoretical concern that the growth factors may augment the growth of small dormant tumours, or may interfere with the normal malignant cell surveillance. Patients with diabetes mel-

litus may have a further deterioration of vision or renal dysfunction as a result of the induction of neovascularity or by direct toxicity. Although few problems have been reported with gene transfer using viral vectors in clinical trials, events could occur within normal cells that take up foreign DNA that allow them to be transformed and become abnormal. This may lead to catastrophic results.

However, on the basis of all preclinical animal studies in non–atherosclerotic coronary vessels and preliminary phase I studies, patients with diffusely diseased or very small coronary vessels and who are not candidates for CABG or PTCA may benefit from therapeutic angiogenesis for the alleviation of their ischaemic symptoms. It is anticipated that ongoing large clinical trials will determine the practical feasibility, investigate the bioactivity the growth factors, establish safety parameters, and determine the optimum delivery method of this new promising therapy.

Which growth factor, combination of growth factors, or inducers of growth factor such as hypoxia inducing factor-1α, [33] leads to optimal, clinically relevant angiogenesis for the treatment of myocardial ischaemia needs to be established. Most animal studies have found little direct advantage for one growth factor over another when administered alone. However, a synergistic effect of FGF-2 and VEGF on angiogenesis *in vivo* has been observed when these proteins were given in combination.[34] As mentioned in the opening paragraph of this section, options for growth factor delivery are numerous. Growth factor proteins or genes may be applied to the myocardium in conjunction with CABG surgery as is being performed presently at our institution. Hybrid forms of treatment consisting of a minimally invasive CABG (MIDCAB) along with local injection of growth factor protein or DNA may become an acceptable standard treatment for patients with co-morbid conditions excluding them from traditional redo CABG or from TMR. Alternatively, growth factors may be injected directly in paste or liquid form through a sternotomy, by thoracoscopic methods, or percutaneously with a long needle. Several methods of catheter-based delivery are being investigated, such as bolus intracoronary infusion and transvascular injection using either a needle or balloon catheter with side perforations, and intrapericardial delivery.

3. Conclusion

The investigation of angiogenesis in the heart is an exciting area of research on a purely scientific basis, but more importantly, it has the potential for improving and reducing the cost of care of patients with ischaemic CAD. It is unlikely that therapeutic angiogenesis will significantly reduce the need for CABG or PTCA in the near future. However, therapeutic angiogenesis has the potential to augment these treatment modalities and may extend treatment options to patients who are not otherwise candidates for conventional methods of myocardial revascularisation.

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Correspondence and reprints: Dr *Frank W. Sellke,* Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, 330 Brookline Avenue, Boston, MA 02215, USA.
E-mail: fsellke@bidmc.harvard.edu