European Heart Journal (2001) **22,** 903–918
doi:10.1053/euhj.2000.2372, available online at http://www.idealibrary.com on IDEAL®

Review Article

Angiogenesis and the ischaemic heart

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Introduction

Historically, interest in the therapeutic implications of angiogenesis has centered on the inhibition of new vascular growth to retard the spread of cancer[1,2]. More recently, however, there has been equal enthusiasm for the therapeutic implications of inducing new vascular growth in ischaemic disorders of the coronary and peripheral vasculature. While medical, surgical and percutaneous catheter-based interventions provide symptomatic relief in a significant proportion of the ischaemic population, a substantial cohort of patients with advanced or refractory disease currently has no acceptable therapeutic alternative. In this context, the promotion of neovascularization within ischaemic vasculature is based upon a valid and intuitively rational scientific concept and presents an innovative approach to treatment of ischaemic diseases in the cardiovascular system. The purpose of this paper is to elucidate some of these scientific concepts and to review the recent advances in the field of angiogenesis.

Principles of angiogenesis

Angiogenesis can be defined as the budding of capillaries that leads to the formation of new microvessels from pre-existing vascular structures. This process can be differentiated from vasculogenesis, which describes the formation of blood vessels from committed mesenchymal stem cells, mainly during embryogenesis[3,4]. Enhancement of blood flow to ischaemic myocardium can result from either true angiogenesis, as defined above, or from the recruitment of pre-existing coronary collaterals. In fact, it is not entirely clear whether one or both of these mechanisms might be involved in the development of coronary collaterals in the ischaemic myocardium[5].

Key Words: Therapeutic angiogenesis, ischaemia, gene therapy.

Manuscript submitted 13 March 2000, and accepted 12 July 2000.

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Mechanisms of blood vessel formation

Formation of new blood vessels involves several steps[6]: dissolution of the matrix underlying the endothelium; migration, adhesion, and proliferation of endothelial cells; and, finally, formation and maturation of a new three-dimensional tubular structure to support the flow of blood[3,7]. Numerous elements stimulate angiogenesis and can influence each step of the process (Fig. 1). In theory, modification of the process at any of these stages may confer the ability to control angiogenesis and harness its effects to treat the ischaemic heart. These influences are briefly reviewed here.

Angiogenesis is a very complex process. Conceptually, the major triggers of this process can be simplified into three broad categories: mechanical, chemical, and molecular factors (Fig. 1). Clearly, these elements may work in concert and contribute to the regulation of the angiogenic process at various stages.

Mechanical influences

Haemodynamics

Haemodynamics may influence angiogenesis in several ways. Augmentation of blood flow during exercise[8], the hyperthyroid state, and the administration of certain drugs have all been shown to stimulate vascular sprouting[9–11]. Furthermore, it has been observed that, while large vessels with low flow tend to reduce or obliterate the endovascular diameter, the lumen of smaller vessels with chronically increased high flow tends to enlarge[12]. Augmentation of blood flow, therefore, may both stimulate vascular sprouting and maintain patency of the newly formed collateral vessels, thereby providing blood flow to the ischaemic myocardium.

Shear stress

Shear stress has an important influence on the development of collaterals in the ischaemic myocardium. Shear

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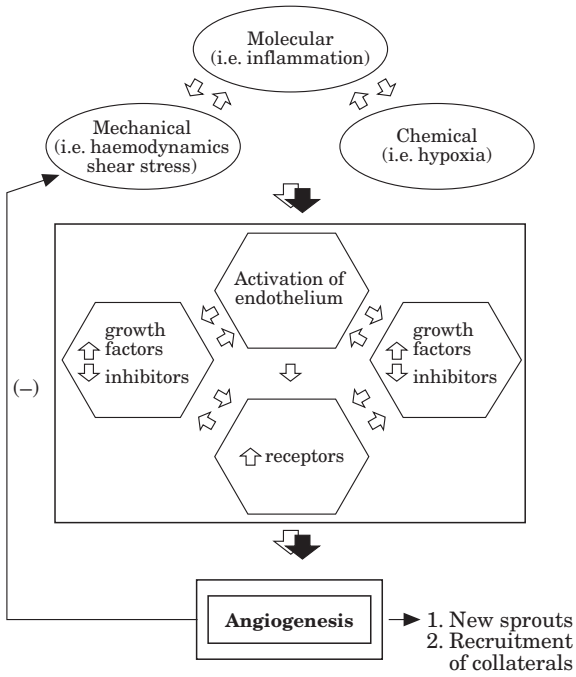

Figure 1 **Hypothetical scheme for number of factors that lead to angiogenesis and influence each step of the process. Conceptually, the major triggers of angiogenesis can be simplified into three broad categories: mechanical, chemical, and molecular factors. These elements may work in concert and contribute to the regulation of the angiogenic process at various stages. Formation of new blood vessels involves several steps: dissolution of the matrix underlying the endothelium; migration, adhesion, and proliferation of endothelial cells; and, finally, formation and maturation of a new three-dimensional tubular structure to support the flow of blood. In theory, modification of the process at any of these stages may confer the ability to control angiogenesis and harness its effects to treat the ischaemic heart.**

stress and stretch on the myocardium can lead to up-regulation of adhesion molecules in the endothelium[13], attraction of inflammatory cells[14,15], and stimulation of endothelial cells to produce growth factors[16,17]. Myocardial stretch, of the magnitude observed in clinically significant left ventricular dysfunction, has been shown to increase vascular endothelial growth factor expression in the heart[18,19]. Endothelial cells can be viewed as mechano-receptors that respond to physical forces and therefore play an important role in linking mechanical influences with the molecular signals of angiogenesis.

Chemical influences

Hypoxia and oxygen tension gradient

The molecular machinery that initiates angiogenesis may be driven by deprivation of oxygen and other important nutrients[20]. Hypoxia stimulates macrophages to release various factors including platelet-derived growth factor and fibroblast growth factor 1 and 2[21]. Hypoxia has also been shown to upregulate vascular endothelial growth factor[22,23], owing both to increases in the transcription mediated by hypoxia inducible factor 1 and an increase in the stability of vascular endothelial growth factor mRNA[24,25]. The augmentation of vascular growth induced by hypoxia might be mediated, at least in part, by nucleotides released from ischaemic tissues[26,27]. Hypoxia also increases the expression of vascular endothelial growth factor receptors (FLK and FLT)[28,29]. This renders the endothelium susceptible to either the systemic or the paracrine effects of pre-existing vascular endothelial growth factor[23,30]. Animal studies on localization of vascular endothelial growth factor expression during the vascularization process have emphasized the potential role for a hypoxic gradient in the regulation of angiogenesis[21,31]. The ability to induce vascular endothelial growth factor expression in response to hypoxia may play a crucial role in regulating angiogenesis in the ischaemic heart. A recent study has described inter-individual heterogeneity in the ability to induce vascular endothelial growth factor in response to hypoxia[32], although the mechanism has not yet been elucidated.

Molecular influences

Inflammation

Animal studies suggest that the presence of inflammatory cells, including macrophages and neutrophils, is sufficient to produce angiogenesis[33,34]. Following myocardial necrosis, the influx of inflammatory cells, including macrophages, monocytes, and platelets, produces the release of cytokines that are capable of stimulating fibroblast growth factor and vascular endothelial growth factor expression. Vascular endothelial growth factor, in turn, can stimulate and recruit other macrophages to augment the inflammatory response and further stimulate angiogenesis. The inflammatory response can induce the expression of receptors (e.g. selectins, ICAM-1, VCAM-1) for various extracellular matrix proteins, monocytes, macrophages, and also for the growth factors already mentioned[35]. Myocardial ischaemia, with an inflammatory response much inferior to infarction, may provide a very different mechanism for angiogenesis.

Angiogenic factors

The existence of angiogenic factors was first noted with the isolation of a tumour factor that was shown to be mitogenic for endothelial cells and later found to be a member of the fibroblast growth factor family[1]. Several other peptides have subsequently been recognized to play a role in angiogenesis (see discussion below). These include: acidic and basic fibroblast growth factor (1 and 2), vascular endothelial growth factor, platelet-derived growth factor, insulin-like growth factor-1, angiogenin, transforming growth factor (TGF_{α} and TGF_{β}), tumour necrosis factor (TNF_{α}), hepatocyte growth factors, granulocyte colony-stimulating factor, placental growth

factor, interleukin 8, and several others[36]. Of the large number of angiogenesis factors that have been described, the fibroblast growth factor and vascular endothelial growth factor families have been most intensively studied. Although several elements are likely to be involved in the process of angiogenesis, as previously discussed, in vivo studies have amply demonstrated that the simple administration of an angiogenic growth factor is sufficient to stimulate the cascade of events that lead to angiogenesis and to the augmentation of blood delivery.

Role of growth factor receptors

Several modulatory control mechanisms for the biological effects of growth factors have been elucidated. Administration of growth factors in the normal heart does not result in angiogenesis[12,37]. This phenomenon has been explained by the lack of expression of appropriate growth factor receptors within the normal tissue. Studies of tumour angiogenesis have documented that up-regulation of receptor density is an important modulatory mechanism in angiogenesis. It has been shown that the FLK and FLT (vascular endothelial growth factor) receptors are up-regulated by hypoxia[28,29] and, possibly, by the presence of other growth factors. Vascular endothelial growth factor and fibroblast growth factor, when administered simultaneously, potentiate one another's effect[38,39], perhaps reflecting reciprocal up-regulation of their respective receptors[12].

Down-regulation of angiogenic inhibitors

The presence of angiogenic growth factors and their respective receptors have been demonstrated in normal tissue[40]. Despite the coexistence of both angiogenic ligands and their receptors in normal tissues, persistent, active neovascularization is not typically observed. This implies the influence of an angiogenic inhibitor. Induction of the cascade of events that leads to angiogenesis would, therefore, require the appropriate down-regulation of the inhibitory pathway. One such putative inhibitor has been isolated from porcine heart[41] and other inhibitors have been described more extensively in the cancer literature[42].

Activation of endothelium

The role of the endothelium in angiogenesis cannot be over-emphasized[43]. Endothelial cells have a well-described, pivotal role in the endovascular regulation of cellular proliferation, migration, adhesion to extracellular matrix, and the formation of a three dimensional lumen for blood flow. In addition, once 'activated', these cells provide a substrate for the adherence of inflammatory cells, permit an increased receptor density, and propagate the release of crucial growth factors[7]. Activation of endothelial cells is one of the earliest events in the formation of new blood vessels. Endothelial cell activation, either by mechanical or chemical factors, increases the sensitivity to various growth factors. The endothelial cells respond by secreting growth factors and a variety of proteolytic enzymes that dissolve the extracellular matrix, releasing stored growth factors and providing substrate for endothelial cell proliferation.

Extracellular matrix

The extracellular matrix also plays an integral role in the process of angiogenesis. The dissolution of the matrix beneath the endothelium is the first step in the cascade that results in neovascularization. In addition, the extracellular matrix can serve as a reservoir for growth factors through the avid binding of these molecules to the heparin contained within the extracellular matrix. Furthermore, inflammation can elicit changes in the extracellular matrix that alter the binding of growth factors to the heparin molecules and their release into the adjacent cellular milieu.

Known angiogenic factors

Historical perspective

The potential relationship of angiogenesis to cancer therapy was first described in the early 1970s[1]. Although the applicability of therapeutic angiogenesis to myocardial ischaemia was initially conjectured in the same decade[44], intense experimental exploration of this concept has not occurred until quite recently. The existence of spontaneously occurring angiogenesis has been inferred from those individuals in whom vigorous endogenous collateralization of an underperfused region can prevent the symptomatic and functional consequences of chronic myocardial ischaemia. Moreover, the observation that infarcted human myocardium expresses angiogenic factors that resemble those produced by various tumours[45] has further stimulated interest in the ability to harness this phenomenon as a therapeutic tool. As individual growth factors have been identified throughout the years, their role in angiogenesis of the ischaemic heart has been systematically investigated[46].

Peptide angiogenic factors

Numerous peptide growth factors have been identified in relation to tumour angiogenesis[36,46]. Many of these same factors play a potential role in cardiac angiogenesis as well[47–52].

Fibroblast growth factor-2, also known as basic fibroblast growth factor, is an 18 kilo-Dalton (kD), single-chain peptide with extensive mitogenic capability. The fibroblast growth factors represent some of the most potent of the known angiogenic peptides. As a family of growth factors, fibroblast growth factors have almost ubiquitous distribution and a wide scope of biological activity. They act as regulatory proteins to induce the proliferation of a broad range of cell types, including cells with epithelial, mesenchymal, and neural origins[46], and mediate a broad spectrum of developmental and pathophysiological processes in vivo and in vitro[53]. They are produced by, and can act directly upon, vascular endothelial and smooth muscle cells, and function as angiogenic factors[36]. One of the characteristic properties of fibroblast growth factors is their ability to

bind to glycosaminoglycan heparin. This property allows them to be classified among the heparin-binding growth factors[54]. The failure of fibroblast growth factor to be secreted in tissue culture is another characteristic biological feature of these molecules. This property can be ascribed to the absence of a signal sequence to direct their secretion, and to their requisite association with extracellular matrix[55,56]. The biological responses to the fibroblast growth factors are induced through the activation of specific receptors and intracellular pathways in a tissue- and temporally-specific manner[57,58]. The fibroblast growth factor-receptor family consists of four membrane-spanning tyrosine kinases. Each of these receptors gives rise to multiple isoforms as a result of alternative splicing of their mRNAs[53,58]. Fibroblast growth factor-2 can be activated through physical stimuli, such as hypoxia, and through the activity of a variety of other growth factors[21,59]. Recent studies have suggested that vascular endothelial growth factor and fibroblast growth factor-2 may have synergistic effects, both in vitro[39] and in vivo[60]. Since fibroblast growth factor-2 is one of the most potent mitogens and chemotactic factors for the vascular endothelial cell, it has been considered the prime candidate for inducing angiogenesis in the ischaemic heart. Numerous studies, both in animal models and in humans, have begun to investigate the potential role of fibroblast growth factor-2 in the treatment of coronary artery disease.

Fibroblast growth factor-1, or acidic fibroblast growth factor, in its mature form, is a 16 kD polypeptide and, like other members of the fibroblast growth factor family, has a wide spectrum of activity. It has potent mitogenic and chemotactic effects on a variety of cell types, including fibroblasts, endothelial cells, and smooth muscle cells[46]. It has been implicated in the control of capillary proliferation during embryogenesis, tumour progression, and wound healing. Fibroblast growth factor-1 is upregulated during collateral formation[49] and in the regeneration of endothelial cells after injury[61]. Hypoxia has been shown to induce release of fibroblast growth factor-1 by macrophages[21]. Despite the apparent therapeutic potential of fibroblast growth factor-1, initial in vivo studies with this agent have proven disappointing[62].

Vascular endothelial growth factor, also known as vascular permeability factor, is a basic 45 kD heparin-binding glycoprotein. It was first described as a secreted mitogenic factor specific for endothelial cells in vitro and a pro-angiogenic molecule in vivo[63]. Numerous additional functions have since been attributed to the vascular endothelial growth factor molecule. These include: the induction of vascular permeability[64,65], the increased expression of serine proteases[66] and interstitial collagenases[67], that can promote a prodegradative environment during angiogenesis, and induction of vasodilatation[68]. Vascular endothelial growth factor exists in various forms and is expressed in various tissues in the body. Four isoforms of vascular endothelial growth factor ($VEGF_{121}$, $VEGF_{165}$, $VEGF_{189}$ and $VEGF_{206}$) arise from alternative splicing of the mRNA from a single gene[69]. All vascular endothelial growth factor isoforms are secreted glycoproteins that can homodimerize and bind to heparin, except for $VEGF_{121}$. $VEGF_{165}$ is the predominant form and is secreted by a variety of normal and transformed cells. The genetic and structural properties of vascular endothelial growth factor and its isoforms have been reviewed elsewhere[63]. Interestingly, regulation of vascular endothelial growth factor gene expression has been shown to be effected by hypoxia in both in vitro and in vivo models[22,23]. Other vascular endothelial growth factor-like proteins have also been cloned[70]. Among those, VEGF-B appears to be particularly highly expressed in cardiac tissue[71,72] whereas, VEGF-C may play a role in formation of lymphatics[73,74]. Nevertheless, some studies suggest that VEGF-C may also have some angiogenic properties in the vasculature of the blood capillary[75]. Vascular endothelial growth factor has a crucial role in embryogenesis, inasmuch as deletion of the vascular endothelial growth factor gene in early embryogenesis is a lethal mutation[76,77]. Vascular endothelial growth factor has also been implicated as a regulator of physiological angiogenesis[78]. The first evidence supporting this hypothesis was provided by in situ hybridization studies on the rat ovary. These investigations revealed that there is a temporal and spatial relationship between vascular endothelial growth factor expression and proliferation of microvessels in the ovary. In addition to its role in embryogenesis and physiological angiogenesis, vascular endothelial growth factor also has a role in the neovascularization response in pathological conditions like ischaemia[21,22,79]. Vascular endothelial growth factor is thought to function by interacting with two high-affinity receptors, flk-1 and flt-1[80,81]. It is believed that both receptors play an important role in the angiogenesis of the ischaemic heart, since their respective levels of expression have been observed to rise in response to hypoxia[27,82]. In vivo studies have demonstrated that acute myocardial infarction stimulates a rapid and prolonged increase in the expression of both vascular endothelial growth factor and its receptors, with characteristic spatial and temporal kinetics[28]. A role for vascular endothelial growth factor has also been conjectured in such diverse pathological entities as rheumatoid arthritis and proliferative retinopathies[42].

Other growth factors

Several other factors have been implicated in the angiogenesis process, and their role has been investigated to varying degrees and previously reviewed elsewhere[36,46]. Among these factors, TGF_{β} induces an increase in the level of vascular endothelial growth factor mRNA and protein expression[83] and, thus, its angiogenic effect maybe mediated by vascular endothelial growth factor. Although the expression of these growth factors has been studied extensively in vitro, their temporal and spatial expression in vivo models is not well-studied[84].

Non-peptide angiogenic factors

Several non-peptide molecules also have been reported to be angiogenic. These include: l-butyryl glycol[85,86],

the prostaglandins $PGE_{1\&2}$[87–90], nicotinamide[26,91], adenosine[89,92–94], certain degradation products of hyaluronic acid[95], and other uncharacterized low-molecular-weight factors[96]. Some of these factors are felt to have a secondary role, since they do not act through the direct stimulation of endothelial proliferation, migration, and protease production. The mechanism by which some of these agents induce angiogenesis is unclear. In any event, they do not appear to exert their effect through increasing the bioavailability of preformed heparin-binding growth factors sequestered in the extracellular matrix [97].

Among the low molecular-weight factors, adenosine may have a unique role in stimulating angiogenesis. Adenosine has been shown to increase vascular density in the chick chorioallantoic membrane model in a dose-dependent manner[93,98] and stimulate proliferation of human endothelial cells in culture[94]. Adenosine formation stems from the ischaemia-induced catabolism of high energy phosphates[99] and this has been shown to increase vascular endothelial growth factor mRNA stability via the A1 receptor[24,82]. Theoretically, adenosine might mediate the stimulating effect of hypoxia upon angiogenesis[100]. It is unclear whether adenosine or its metabolic by-products can induce angiogenesis in vivo[100].

A potential modulatory role for nitric oxide has also been entertained in both tumour angionesis[101,102] and other forms of neovascularization[103,104]. In fact, it is conjectured that a defective endothelial synthetic mechanism for nitric oxide may provide an additional therapeutic target in patients with advanced vascular obstruction[105].

From basic science to clinical practice

Many therapeutic advances in cardiology have contributed to the improved management of coronary artery disease. Clinical benefits have been accrued through multiple risk factor modification, enhancement of local oxygen delivery through direct percutaneous interventions and surgical revascularization, and through the pharmacological manipulations that maximize myocardial function after injury and prevent further ischaemic events. Nonetheless, there persists a large group of patients who cannot benefit from these interventions. It is for this subgroup of individuals with refractory myocardial ischaemia, in particular, that therapeutic angiogenesis may hold particular promise.

Since occlusion of a portion of the coronary vasculature may be tolerable in the presence of appropriate collateralization, the ability to pharmacologically induce the growth of coronary collaterals could, in principle, markedly change the natural history of ischaemic heart disease. Coronary collaterals are believed to develop either as a result of neovascularization or of the recruitment of neighbouring vessels. As discussed above, numerous biological factors stimulate these responses and can influence each step of the angiogenic process (Fig. 1). In theory, modification of any of these steps, individually or in concert, may confer the ability to control angiogenesis and change the natural history of ischaemic heart disease.

Insights derived from animal models of angiogenesis

Although it is likely that the concurrent effects of several factors produce a concerted response that results in new vessel formation, as previously discussed, it is entirely conceivable that therapeutic administration of a sole angiogenic growth factor might be sufficient to augment blood delivery to the ischaemic myocardium. Such phenomena have repeatedly been demonstrated through in vivo studies of experimental angiogenesis (some are reviewed below).

Fibroblast growth factor-2

The intra-coronary administration of fibroblast growth factor-2 has been studied in a canine acute infarct model. In this approach, when infusion of the growth factor immediately followed induction of acute myocardial infarction, reduction of the infarct size and increased neovascularization were both observed[106]. It is as yet unclear whether fibroblast growth factor-2 induces the observed neovascularization or whether it is responsible for the recruitment of preexisting collateral vessels, inasmuch as administration occurred in the acute setting. Some benefit of fibroblast growth factor-2 has also been shown in the chronic ischaemic setting, utilizing various modes of delivery[107–109]. Reduction of infarct size in the acute setting has repeatedly been demonstrated[106,110,111].

Fibroblast growth factor-1

Studies with fibroblast growth factor-1 have demonstrated conflicting evidence of benefit in the process of therapeutic angiogenesis[62,112–114]. In vivo studies have demonstrated that fibroblast growth factor-1 has minimal effect on non-injured quiescent endothelial cells, but exerts a marked mitogenic effect on injured smooth muscle cells[62]. In one series of experiments, administration of exogenous fibroblast growth factor-1 in myocardium has shown striking vascular smooth muscle hyperplasia exclusively within areas of myocardial infarction but without significant collateral formation[62]. Others have demonstrated radiographic and histological evidence of stimulated coronary collateral formation[113,114] and have shown that exogenous fibroblast growth factor-1 improves myocardial perfusion to the collateral-dependent ischaemic myocardium[115].

Vascular endothelial growth factor

While the $VEGF_{121}$ isoform has recently been receiving increasing attention, most of the earlier animal studies

have been performed with $VEGF_{165}$, the more abundant isoform in humans. Studies in various animal models have investigated the role of vascular endothelial growth factor administration in the setting of myocardial ischaemia. In a canine model of ameroid coronary occlusion, daily intracoronary vascular endothelial growth factor injection enhanced coronary collateral blood flow and increased myocardial distribution vessel density compared to control animals treated with saline[116]. In a similar study, although vascular endothelial growth factor administered via the left atrium did not increase collateral development, it significantly enhanced neointimal accumulation[117]. The difference in results between the two studies is not clear. Another study using intracoronary protein injection also demonstrated an increase in myocardial perfusion, although it was associated with systemic hypotension[118]. A different study in a porcine model showed that periadventitial and extraluminal mini-pump administration of vascular endothelial growth factor improved myocardial contractility and enhanced coronary blood flow and collateralization[119]. A more recent study using gene transfer methodology showed that direct myocardial injection of an adenoviral vector encoding vascular endothelial growth factor cDNA improved myocardial contractility and perfusion with enhanced collateralization[120]. While these studies have established the feasibility of therapeutic angiogenesis through local administration of angiogenic growth factors, site-specific stimulation of angiogenesis has also been demonstrated for systemic administration of vascular endothelial growth factor[121]. These studies demonstrated that vascular endothelial growth factor appears to be an effective angiogenic agent in animal models of the ischaemic heart and warranted further investigation for its applicability in clinical studies.

Considerable therapeutic efficacy has been demonstrated for vascular endothelial growth factor administration either as protein or gene-transfer in animal models of peripheral ischaemia. Earlier studies demonstrated a significant dose-dependent augmentation in perfusion accompanied by evidence of increased collateral formation after intramuscular administration of vascular endothelial growth factor in the ischaemic rabbit hindlimb[122]. Successful transfection of naked DNA encoding vascular endothelial growth factor in a rabbit model of hindlimb ischaemia showed augmented vascularity and perfusion to the ischaemic limb[123]. In a different study, site-specific transfection of plasmid encoding cDNA of $VEGF_{165}$ resulted in augmented development of collateral vessels and increased capillary density documented by serial angiography[124]. Vascular endothelial growth factor was shown to improve endothelium-dependent vasorelaxation in microvascular collaterals in a rat hindlimb model of peripheral ischaemia[125]. In a similar animal model, adenoviral vector encoding vascular endothelial growth factor stimulated an angiographic increase in the vascularity of the treated limb compared with each control group[126].

Therapeutic angiogenesis in human vascular disease

Although there are several mechanisms by which the angiogenic process is stimulated, early in vivo studies suggest that administration of individual growth factors may be sufficient to augment blood delivery to hypoxic tissues. These early studies have stimulated the current, extensive research effort to define the role of growth factors in the therapy of myocardial ischaemia. The high-affinity endothelial receptors for various angiogenic growth factors are now well-recognized to mediate proliferation, migration and differentiation of the cellular populations required for the augmented growth of new vascular structures in ischaemic tissues[46,127–129]. However, before therapeutic angiogenesis becomes a reality in human ischaemia, several critical issues will require resolution.

Which growth factors?

As already discussed, there are several known growth factors that might serve as suitable candidates for the stimulation of coronary angiogenesis. Among these, fibroblast growth factor-2 and vascular endothelial growth factor have been the most extensively studied, both in animal models and in humans. It is as yet unclear whether one or a combination of these growth factors can infer the maximal angiogenic response in human ischaemic heart disease. Moreover, no comparison studies have yet been undertaken. Furthermore, a synergistic effect of these two important factors has been demonstrated, at least in a rabbit model of hindlimb ischaemia[60]. The search for new angiogenic factors and their effects on angiogenesis of the ischaemic heart is ongoing and intense[130–132]. For example, investigation of the angiogenic potential of nucleotide metabolites has revealed a potential role for certain pyridines[26,92,96,97]. The study of vasculogenesis during embryonic development has traditionally led to the identification of important mediators of angiogenesis[4,131,133,134]. Other molecular modulators, that affect angiogenesis at the cellular receptor or the intracellular level, may also present a potential for novel pharmaceutical interventions for ischaemic heart disease.

Which patient population?

Currently, clinical studies of angiogenesis are focused upon the patient with severe, refractory angina. The question remains whether the benefits of therapeutic angiogenesis can be extended to other ischaemic disease states, like diabetes, ischaemic cardiomyopathy and congestive heart failure, and peri-infarction ischaemia. One study has shown that vascular endothelial growth factor–gene transfer restored the neovascularization response in a mouse model of diabetes[135,136]. In patients with acute myocardial infarction, where thrombus formation may pre-empt the adequate formation of collateral vascularization, angiogenic factors may accelerate the process of collateralization and thereby limit the size

Table 1 Various modes of drug delivery

Gene transfer	Native factor
Plasmid DNA	Systemic
	Single vs repeated administration
Liposomal DNA	
	Local
Viral vectors	Polymer-based devices (slow releasing polymers, hydrogel, microspheres, etc.)
Recombinant adenovirus	
Retrovirus	
Adeno-associated virus	Intravascular delivery (porous balloons, stents, iontophoresis, simple injection)
	Cardiac catheterization
	TMR/PMR
	'Mini' thoracotomy
	CABG surgery

of the infarct[45,137]. Animal studies with fibroblast growth factor-2 suggest that this kind of intervention is feasible[106,110,111]. A recent study has shown that mice deficient in vascular endothelial growth factor isoforms developed ischaemic cardiomyopathy and ultimately died of cardiac failure[138]. One implication of this study is that treament with angiogenic factors might promote revascularizaion and improve heart failure[139].

Strategies for drug delivery

In simplest terms, therapeutic angiogenesis can be effected by one of two mechanisms of drug delivery: through transfer of the relevant genetic material, or through therapeutic administration of the angiogenic factor itself (Tables 1 and 2). There has not yet been a systematic comparison to determine which mode of drug delivery is most efficacious. The native factor can be administered either via the systemic route or locally, as noted in Table 1. Alternatively, local sustained-release of the native factor or direct gene therapy may provide efficient delivery of angiogenic factors and facilitate more prolonged local exposure to the growth factors and minimize systemic side effects.

Native factors

While it might reflect the simplest mode of drug delivery, systemic administration of the native factors raises the spectre of several potential undesired effects of angiogenesis. These include: the acceleration of proliferative retinopathy and, perhaps, of atherosclerosis[140–143]; the stimulation of neoplastic growth; hypotension mediated through cytokine release[118], localized oedema and inflammation[144], telangiectasias[144], anaemia, thrombocytopenia, and membranous nephropathy[145]. In contrast, local delivery of growth factors obligates lower dosing requirements and therefore might reduce the likelihood of associated systemic side-effects. Local drug delivery, however, has its own limitations, such as the technical difficulty associated with delivery of the growth factors to the precise site of injury. Prolonged, yet time-limited exposure might realistically require multiple procedures for growth factor delivery. In addition, local injection of growth factors entails the potential risks associated with the required procedures, as well as the potential for local reactions to the drugs, including inflammation and arrhythmias. A number of slow-release matrices such as polymers (hydrogels and porous polymers), microspheres, stents, heparin beads, and porous balloon catheters have been studied to allow controlled local delivery of growth factors[146].

Gene transfer

Theoretically, the advantages of gene transfer include the reliability of gene expression at the target organ and the elimination of the technical difficulties that attend direct drug delivery[114]. Undesired effects of systemic drug administration are avoided[121]. Successful gene therapy of human disease poses distinct challenges[147]. The disappointing recent clinical trials of human gene therapy in such inherited diseases as cystic fibrosis and Duchenne's muscular dystrophy underscore the complexity of such endeavours[147]. Some of the noted disadvantages of gene therapy include the low transfection rates with currently available vectors, and the fact that the gene products were expressed for a short duration and only in limited amounts[147,148]. Local inflammatory responses to the introduction of viral vectors pose an additional potential side effect of gene transfer.

As previously discussed, the ideal mode of drug delivery for therapeutic angiogenesis would entail a simple mechanism for the delivery of growth factor to the precise site of injury. Exposure to the growth factor would be prolonged, yet time-limited. Fortunately, the same characteristics that pose difficulties in other disease processes, such as low transfection rates and transient, localized expression of gene product, suggest that the currently available vectors might be ideally suited for therapeutic angiogenesis[149]. Disappointing results in gene therapy have traditionally entailed the delivery of intracellular gene products; in contrast, vascular endothelial growth factor includes a leader sequence that allows its secretion from intact cells[150]. Therefore, despite low transfection rates, the paracrine effects of the secreted peptide may be sufficient to achieve a meaningful angiogenic effect[144,151–153]. Numerous animal and human studies have evaluated the safety and success of such gene therapy in the ischaemic heart[107,149,153–155].

Several important issues must be addressed to optimize the potential of angiogenic gene therapy. Chief among these is the identification of the ideal vector for gene transfer. Liposomes and plasmid DNA have been used in animal models of myocardial ischaemia[156–158] and the latter has also been evaluated in clinical trials for peripheral vascular disease[144,154,159]. The disadvantage of these vectors is the inconsistent and low transfection rate[147]. Recombinant viruses have generally been highly efficient vectors. Retroviruses, although they produce stable transfection, require dividing cells for infection[148,149]. The experience with vectors based on human

Table 2 Potential modes of delivery of gene therapy for angiogenesis in the ischaemic heart[146,149]

Site of injection	Method of delivery	Vectors used	Select references
Intra-coronary		Adenovirus	[107]
Intramyocardial injection	Epicardial (open chest)	Adenovirus	[161]
		Plasmid	[154]
	Intracavitary (percutaneous)		
Intra-arterial wall transfection	Hydrogel balloon	Plasmid	[144]
	Double balloon	Plasmid,	[186]
	Vector coated stents	Liposome	
Pericardial application	Transfer of cells	Native	[187]

adenoviruses has been more promising. Adenoviruses can transfer large amounts of recombinant genes into a wide variety of dividing and non-dividing cells[148]. Several animal models[107,160] and clinical trials have effectively utilized adenoviral vectors for intramyocardial and intracoronary delivery[149]. Recently, the completion of a phase I assessment of direct intramyocardial administration of a vascular endothelial growth factor-expressing adenovirus vector has been described in individuals with severe coronary artery disease[161].

Dosing and frequency

Another issue that also requires further attention, without reference to the specific strategy for drug delivery, is the determination of the optimal dosing, timing, and frequency of factors administered. No comparative studies have been performed to address this specific concern. As discussed earlier, the optimal angiogenic effect is theoretically achieved with prolonged, yet time-limited exposure to the growth factors administered. Administration of a single dose of the growth factor, whether systemically or locally, takes into account the assumption that binding of the factors to the extracellular matrix can provide a reservoir for their sustained delivery at the targeted area. Preliminary results of a recent clinical trial demonstrate that single intracoronary dosing of fibroblast growth factor-2 over a wide range of drug concentration (0·33–36 $\mu g \cdot kg^{-1}$) is safe[162].

An alternative method to a single dosing strategy is to employ frequent, repeated administration of the growth factors. In the VIVA study, patients received a single intracoronary dose of vascular endothelial growth factor at doses of either 17 or 50 $ng \cdot kg^{-1}\ min^{-1}$ on the first day followed by repeated intravenous dosing on days 3, 6, and 9[163]. The lack of favourable preliminary results in this study is currently unexplained, but may be related to dosing and frequency of the drug used. Most studies of therapeutic angiogenesis, however, utilize some method for slow and sustained delivery of the factors to avoid frequent dosing. In one study, use of microcapsules containing 100 μg of fibroblast growth factor-2 implanted in the subepicardial fat at the time of CABG surgery was not associated with any ill effects and may have improved the patients' angina symptoms[164].

Outcome assessment: how to quantitate the effectiveness of therapeutic angiogenesis?

In animal studies, direct study of the treated tissues facilitates the assessment of treatment efficacy. Histochemical evaluation of the myocardial tissue and the demonstration of enhanced endothelial cell proliferation

Table 3 Proposed end-points to document therapeutic angiogenesis in ischaemic heart disease

Clinical end-point	Functional end-point (stress vs rest)	Angiographic end-point
Angina class	Nuclear medicine	Angiograms (TIMI flow)
CHF class	Assess perfusion	Morphometric vessel counting
Exercise tolerance	Echocardiogram	MRI (myocardial perfusion)
Mortality	Assess LV function	
	Regional vs global	
	MRI	
	Assess LV function	
	Treadmill	

CHF=congestive heart failure; LV=left ventricular; TIMI=Thrombolysis in Myocardial Infarction; MRI=magnetic resonance imaging.

provide objective documentation for new vessel formation. Clearly, analogous assessment in the human clinical context is impossible. Unfortunately, diagnostic tools to identify effective human angiogenesis are, as yet, largely unavailable. Table 3 summarizes the objective clinical end-points utilized in the clinical studies to date.

Angiographic methods can be considered for direct demonstration of increased vascularity both in animal models and in human clinical trials. Angiographic methods suffer from the implicit failure to discriminate between true angiogenesis and collateral recruitment. Furthermore, existing radiographic methods may not be sensitive enough to detect collateral vessels of less than 200 μm in diameter[165]. Newer microangiography systems, with a spatial resolution of 30 μm, have been utilized to investigate the development of collateral arteries in the rat ischaemic hindlimb[166].

A therapeutic increase in vascularity should ideally also result in an improvement in myocardial perfusion. This can be objectively documented through myocardial scintigraphic methods. Here, again, the evidence is indirect and, in addition, changes in vascular reactivity, induced by the growth factors, may alter coronary flow. This may be difficult to differentiate from an absolute increase in vessel number or density. Functional studies that assess left venticular function, such as echocardiography, do not purport to quantify new vessel formation, but may provide important prognostic parameters to assess the success of treatment with angiogenic factors.

Obviously, the ultimate goal of therapeutic angiogenesis is the induction of clinical improvement within the cohort of treated patients. Therefore, the effectiveness of therapy within any regimen for therapeutic angiogenesis must include an assessment of the response of such clinical variables as angina class, heart failure class, exercise tolerance and time, and cardiovascular event rates, particularly in comparison to the response of placebo recipients.

Human clinical trials

Because of the encouraging results in animal models using individual growth factors, several clinical trials have been designed to study the effect of these angiogenic growth factors in humans. The first clinical trials were done in ischaemic limbs[167].

Peripheral vascular insufficiency

Pre-clinical findings in a case report suggested that intra-arterial gene transfer of a plasmid that encodes for $VEGF_{165}$, using a hydrogel polymer coating of an angioplasty balloon, was able to improve blood supply to the ischaemic limb[144]. In another case report, a patient treated with six consecutive, weekly intravenous infusions of recombinant fibroblast growth factor-2 demonstrated a beneficial clinical response by week 4 of therapy. Clinical responses were characterized by an improved walking distance, relief of ischaemic pain, a marked reduction in analgesic consumption, and healing of persistent, unresponsive, painful inflammation of the hallux[168]. In a larger study, intramuscular injection of naked plasmid DNA encoding $VEGF_{165}$ was performed in 10 limbs of nine patients with critical limb ischaemia. The majority of the treated limbs demonstrated increased collateral formation on angiography and improved flow on magentic resonance imaging along with significant improvement of the ankle–brachial index[155]. Large clinical trials are currently underway to address therapeutic angiogenesis in human peripheral vascular disease.

Chronic coronary insufficiency

The clinical trials that are currently underway address only those patients with severe ischaemic heart disease who are refractory to conventional medical treatment and who are not candidates for the conventional mechanical revascularization procedures. The ongoing clinical trials to evaluate the safety and efficacy of therapeutic angiogenesis are summarized in Table 4.

Native protein

The first study to report efficacy for angiogenic therapy of human coronary heart disease examined the role of basic fibroblast growth factor in 20 patients with three-vessel coronary disease undergoing CABG surgery[114]. In this study fibroblast growth factor ($0{\cdot}01\ mg\ .\ kg^{-1}$ body weight) was injected close to the vessels after the completion of internal mammary artery to left anterior descending coronary artery anastomosis. All the patients had additional peripheral stenoses of the left anterior descending coronary artery or one of its diagonal branches. Twelve weeks later, formation of capillaries could be demonstrated angiographically around the sites of injection and a capillary network sprouting from the proximal part of the coronary artery could be shown to have bypassed the stenoses and rejoined the distal parts of the vessel. No association was made between the radiographic finding and clinical improvement. Other studies are currently underway to further investigate the efficacy of fibroblast growth factor-2 in treating chronic coronary ischaemia[162,164,169].

The VIVA trial represents the first large placebo-controlled trial to investigate the efficacy of vascular endothelial growth factor treatment in ischaemic myocardium[163]. In this study patients with viable, underperfused myocardium who were not candidates for other interventions, were randomized in double-blind fashion to receive recombinant human vascular endothelial growth factor or placebo. The effects of therapy were measured clinically as well as objectively with exercise treadmill time, ejection fraction, and nuclear perfusion. The trial, although showing encouraging safety and

Table 4 Current list of clinical trials in evaluating angiogenesis in the ischaemic heart

Authors	Year	Trial phase	Growth factor	Drug delivery	Patient selection	Outcome measure	Sponsor	Reference
Schumacher *et al.*	1998	I/II	FGF-2	IM	CABG	Subtraction angiography		[114]
Sellke *et al.*	1998	I	FGF-2	Slow release, epicardium	CABG	Nuclear		[169]
Laham *et al.*	1999	I	FGF-2	IC	CAD	Nuclear, clinical TM, EF, MRI	Chiron	[162]
Laham *et al.*	1999	I	FGF-2	Slow release, subepicardium	CABG	Nuclear, MRI		[164]
Losordo *et al.*	1998	I	$VEGF_{165}$	Plasmid, IC via mini-thoracotomy	CAD	Nuclear, angiography		[154]
Henry *et al.*	1999	I/II	$VEGF_{165}$	IC & systemic	CAD	Nuclear, clinical treadmill, EF	Genentech	[163]
Vale *et al.*	1999	I/II	$VEGF_{165}$	Plasmid, IM via mini-thoracotomy	CAD	Nuclear, clinical	Vascular genetics	[170]
Rosengart *et al.*	1999	I	$VEGF_{121}$	Adenovirus, IM	CABG, CAD	Nuclear, clinical angiography, TM	Genvec/Parke-Davis	[161]

CABG=at the time of coronary bypass graft surgery; CAD=patients with severe coronary artery disease who were suboptimal candidates for standard revascularizaion procedures; EF=ejection fraction; IC=intracoronary injection; IM=intramyocardial injection; MRI=magnetic resonance imaging; Nuclear=any nuclear perfusion study including nuclear SPECT, rest/exercise perfusion, and gated sestamibi-determined ejection fraction; Slow release=slow release polymer; TM=treadmill exercise test.

tolerability reports, has not yet shown favourable results compared to placebo.

Gene therapy

To date, there are no final results on controlled trials demonstrating consistent angiogenesis in the ischaemic heart through gene therapy. However, given the results in the animal models for chronic coronary and peripheral ischaemia, several Phase I and II clinical trials for angiogenic gene therapy are underway. In a small phase I study, naked plasmid DNA encoding $VEGF_{165}$ was injected directly into the ischaemic myocardium of five patients via a mini left anterior thoracotomy. All patients had significant reduction in angina, and post-operative left ventricular ejection fraction was slightly improved in two of the patients. Objective evidence of reduced ischaemia was documented using dobutamine single photon emission computed tomography (SPECT)-sestamibi imaging and, with coronary angiography, showed improvement[154]. Similar results were recently reported for a larger cohort of patients as well[170]. In a separate Phase I study of 21 patients with clinically significant coronary artery disease, an adenovirus vector, expressing human $VEGF_{121}$ cDNA, was administered by direct myocardial injection into an area of reversible ischaemia either as an adjunct to conventional coronary artery bypass grafting or as sole therapy via a minithoracotomy[161]. While no systemic or cardiac-related adverse events were reported, clinical improvement was noted in all patients. Coronary angiography and stress sestamibi scintigraphy suggested improved wall motion in the area of vector administration, while in the group in which gene transfer was the only therapy, treadmill exercise assessment also suggested some improvement[161].

Transmyocardial laser revascularization and percutaneous myocardial revascularization

Any discussion of therapeutic angiogenesis should consider the insights derived from these laser-based revascularization approaches. Transmyocardial laser revascularization, and the catheter-based form of the procedure, percutaneous myocardial revascularization, have recently been explored as potential therapeutic options in patients with severe, refractory myocardial ischaemia[171]. The initial rationale for the development of these procedures was simple[172]. In transmyocardial laser revascularization, a high energy laser is used to surgically create 12 to 18 channels, about 2–3 mm deep and 1 cm apart, within the myocardium[173–176]. The intervention is intended to simulate the reptilian heart and allow communication of oxygenated blood from the left ventricle with myocardial sinusoids. Although the intent is to mechanically facilitate additional blood flow to the ischaemic myocardium, it has subsequently been observed that the mechanical benefit may be short-lived, because the newly created channels quickly occlude after the procedure. The exact mechanism of long-term benefit of these procedures, therefore, remains poorly understood[177,178]. More recently it has been suggested that these procedures stimulate angiogenesis by a non-specific reaction to tissue injury[179], perhaps by inducing an inflammatory response and the release of certain angiogenic factors in the targeted myocardium[177]. Recent animal studies have used transmyocardial laser revascularization in combination with vascular endothelial growth factor gene therapy to elicit complete reversal of ischaemic wall motion abnormalities in ischaemic myocardium[180].

Regardless of the mechanism of action, the results of initial observational studies of transmyocardial laser revascularization have been noteworthy. Transmyocardial laser revascularization was shown to reduce the angina class in these patients, with some improvement in their exercise tolerance[175,176,181]. The procedures were, however, associated with substantial peri-operative mortality and morbidity and with an inconclusive objective improvement in myocardial perfusion[182]. Recent published clinical studies demonstrated similar subjective symptomatic relief for those patients undergoing the procedure, with little objective evidence for such improvement[183,184]. The surgical procedure of transmyocardial laser revascularization was also associated with a high peri-operative morbidity and mortality, particularly among patients with depressed left ventricular ejection fractions[185]. The complication rates can theoretically be minimized with percutaneous myocardial revascularization, the percutaneous, catheter-based form of the procedure. Another limitation in these studies[183,184] has been the lack of placebo controls. More research, and possibly placebo-controlled clinical studies, will be necessary to fully evaluate the indications for these procedures and to understand their mechanism of action.

Summary and conclusions

Angiogenesis has generated tremendous enthusiasm for its promise as a novel therapeutic modality for ischaemic heart disease, especially for patients who do not have good therapeutic alternatives. Augmentation of physiological neovascularization in ischaemic cardiovascular disease is based on an intuitively rational scientific concept and, thus far, numerous lines of investigation have demonstrated the validity of this concept. Despite some mixed results, that serve, chiefly, to emphasize the plethora of as yet unanswered questions in this young field, there is a little doubt that stimulation of angiogenesis by growth factors, drugs, gene therapy, or mechanical manipulation, will have a place in future therapy of ischaemic heart disease. The future of therapeutic angiogenesis will be defined through further basic research into the molecular mechanisms of the cellular response to angiogenic stimulation. In addition, well-designed clinical studies will be required to delineate the long-term benefits of such therapy on the morbidity and mortality of chronic human ischaemic disease.

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