Review

Angiogenesis by gene therapy: a new horizon for myocardial revascularization?

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Abstract

The concept of therapeutic angiogenesis is based on the premise that the potential for vascular growth inherent in vascular tissue can be utilized to promote the development of new blood vessels under the influence of the appropriate growth factors. Direct application of growth factors of the fibroblast (acidic, basic fibroblast growth factor, FGF-5), endothelial (vascular endothelial growth factor) and other series has been effective in preliminary studies. Angiogenesis by gene transfer provides an attractive alternative, with the advantage that the protein may continue to be secreted for a longer period of time and that the gene may be targeted to specific tissues to enhance efficacy and reduce systemic side effects. Angiogenesis by gene transfer is currently under investigation using a variety of growth factors and a wide array of potential delivery systems. These include application of the gene as naked DNA or by viral vector in the proximal vessel by direct intravascular injection, interventional cardiologic techniques (hydrogel coating on balloon, double balloon system, stent implantation) or by direct application to adventitia, pericardium or ischemic tissue distal to the site of arterial obstruction. As our understanding of the molecular and genetic processes underlying angiogenesis increases, and as we examine the results of preliminary animal and human protocols, we hope to develop the potential of angiogenesis by gene transfer for therapeutic use.

Keywords: Atherosclerosis; Myocardial ischemia; Angiogenesis; Vasculogenesis; Gene transfer; Growth factor; Animal; Human

1. Introduction

Although there have been major advances in the prevention and treatment of atherosclerotic vascular disease, a large number of patients suffer from disabling symptoms despite pharmacotherapy and mechanical revascularization by angioplasty or surgery. In such patients the challenge to improve blood flow to the ischemic organ has led to extensive research programs in the fields of molecular biology, pharmacotherapy and newer mechanical technologies. The application of techniques of gene transfer to vascular biology provides a tantalizing potential method for promoting vascular growth in an area of ischemia. Therapeutic angiogenesis – the promotion and generation of new blood vessel formation to a vascular bed – by gene transfer is a novel and very promising avenue of research currently being studied.

2. Angiogenesis – basic concepts and mechanisms

The concept of therapeutic angiogenesis is based on the premise that the existing potential for vascular growth inherent in vascular tissue can be utilized to promote the development of new blood vessels under the influence of
the appropriate growth factors. This potential for vascular growth in the heart may be divided into 2 types: (1) true angiogenesis (the formation of capillaries – a process involving matrix dissolution, cell migration, adherence, proliferation and tube formation) and (2) recapitulated vasculogenesis (transformation of preexisting arterioles into small muscular arteries, or the expansion of pre-existing collateral vessels). Vasculogenesis is the more important mechanism of vascular growth, since it is the most efficient mechanism to deliver bulk flow to an ischemic region by bypassing the site of arterial obstruction. Angiogenesis increases capillary density and decreases vascular resistance in the ischemic region, but is less effective. In animal species relying almost exclusively on angiogenesis (e.g. pigs), a large number of small caliber collateral vessels resembling giant capillaries link adjacent vascular regions and deliver bulk flow to ischemic tissue, but in terms of hemodynamics, the energy losses are large, distal perfusion pressure low and flow is readily altered by changes in ventricular diastolic pressure [1].

Angiogenesis and vasculogenesis both develop in response to coronary obstructions and chronic myocardial ischemia in humans (Fig. 1). The natural compensatory processes are often inadequate, or the time course too slow in view of the rapidity of development of a thrombotic occlusion. It appears feasible to stimulate the processes by use of the appropriate growth factors. The growth factor basic fibroblast growth factor (bFGF) is a potent stimulator of vasculogenesis whereas vascular endothelial growth factor (VEGF) is angiogenic specific [1].

Other mechanisms that may be operative regarding the benefits of angiogenic growth factors include the effect of basic FGF and VEGF on vasomotor function. Abnormal contractile response to acetylcholine decreases and endothelial relaxation in response to ADP increases. The mechanism is probably mediated by nitric oxide [2], although it is possible that the changes are simply related to increased flow.

The changes in vascular gene expression and the role of inflammation and apoptosis in the development of angiogenesis are not altogether clear [3]. Inflammatory cells and macrophages are abundant in atherosclerotic tissue, and necrosis is the hallmark of infarction. The inflammatory process may modulate angiogenesis, and there may be differences in the angiogenic effect of vascular endothelial growth factor because of its action with regard to increasing vascular permeability and to stimulating macrophage migration [4,5]. Infusion of bFGF, which does not increase permeability, produced little or no angiogenesis in normal tissue in the absence of arterial injury [6,7]. Local infusion of monocyte chemotactic protein-1 (MCP-1), a potent and specific chemoattractant for monocytes, can markedly increase collateral and peripheral conductance in a rabbit hindlimb model [8]. Gene expression and protein secretion of MCP-1 are upregulated by changes in shear stress and cyclic strain [9–11] which occur at the site of pre-existing

Fig. 1. Overview of the major steps in the sequence of events leading to angiogenesis. Ischemia, necrosis, inflammation and/or shear stresses are associated with initiation and activation of gene expression. Growth factors and changes in receptor expression produce a complex series of cellular interactions, leading to vasculogenesis and true angiogenesis.
arterial connections in the presence of arterial occlusion. Apoptosis may be important in the pronounced remodeling processes which create space for the newly developing vessels.

3. Growth factors involved in angiogenesis

Vascular growth factors are polypeptides originally isolated in studies of tumor growth [12] and more recently demonstrated to be responsible for natural as well as pathologic angiogenesis. A large variety of growth factors belonging to several groups may play a role in angiogenesis [13]. Basic (bFGF) and acidic (aFGF) fibroblast growth factor enhanced collateral flow in acute [14] and chronic [15] animal models of hindlimb ischemia. Treatment with bFGF was reported to increase the number of arterioles and capillaries [16] and the ratio of collateral to normal blood flow [17] in canine models of myocardial infarction, although late application of the growth factor was associated with borderline results in the study by Yanagisawa-Miya et al. [16]. Fibroblast growth factor-5 (FGF-5), which has the leader sequence of a secreted protein and possibly greater efficacy, resulted in increased blood flow and contractile function in an ischemic pig model after intracoronary gene transfer [18].

Vascular endothelial growth factor (VEGF) is currently the focus of major interest as a possible substance to promote therapeutic angiogenesis. VEGF is a 45-kDa dimeric glycoprotein, isolated initially as a heparin-binding factor secreted from bovine pituitary cells [19]. It was also purified as a tumor-secreted factor that induced vascular permeability [20,21]. Unlike bFGF, VEGF is secreted by intact cells, since the NH$_2$ terminus is preceded by a signal sequence [22]. Its high affinity binding sites are present on endothelial cells and VEGF has no mitogenic effect on smooth muscle cells and fibroblasts [19,23], unlike aFGF and bFGF [24,25]. This makes VEGF an excellent substance for study in the circumstances of endothelial disruption or myocardial ischemia, where rapid endothelialization and/or angiogenesis are desirable, while substances promoting smooth muscle or fibroblast growth may be
counter-productive. In tissue culture, human endothelial cells transfected with the gene for VEGF show increased growth, elongation and clustering as the onset of the process of new vessel formation (Fig. 2).

Myocardial VEGF production was significantly upregulated by hypoxia in vitro and ischemia in pigs [26] and in rats [27], suggesting that VEGF is a likely mediator in natural ischemia-induced neovascularization. The possible role of exogenous VEGF was examined in a canine model, where ameroid constriction of the circumflex artery was followed by daily intracoronary administration of 45 μg of the growth factor, commencing 10 days later [28]: treatment with VEGF was associated with a 40% increase in collateral blood flow. In a rabbit model of hindlimb ischemia, a single arterial bolus of 500–1000 μg VEGF [29] augmented collateral development and improved endothelial-dependent blood flow [30,31]. Direct daily intramuscular injection of 200–1000 μg of VEGF for 10 days [32] produced site-specific dose-dependent therapeutic angiogenesis in the thigh muscle of a rabbit ischemic hindlimb model.

It is likely that a number of yet to be discovered angiogenic growth factors will have advantages and effects superior to those presently under study. VEGF represents a family of growth factors which interact with different receptors to induce endothelial mitogenesis [33,34]. VEGF-B appears to be highly expressed in cardiac tissues [33], while VEGF-C may be involved in lymphatic formation. The regulation of growth factor expression in cardiac myocytes and message stability of these factors in response to hypoxia and other stimuli is being studied [35]. On the other hand, inhibitors of endothelial cell proliferation probably play a role in regulation of vascular growth in normal tissues [36] and deserve further study. Another aspect of angiogenesis where molecular intervention could be considered is the regulation of cellular growth factor receptors.

4. Growth factor receptors

A number of molecular tools have been developed for the investigation of the VEGF-receptor system [37–39] in endothelial cells as well as in animal tissues. Different molecular and cellular assays are established for analysis of different aspects of endothelial function [38–40] including various kinase assays.

Uprogulation or alteration of growth factor receptors by genetic engineering or other cellular manipulation to promote enhanced vascular growth is an interesting concept which could provide an additional strategy in the angiogenesis research program. Brogi et al. reported paracrine upregulation of the principal growth factor receptor (Kdr) in response to factors released from hypoxic skeletal myocytes [41].

5. Angiogenesis by gene transfer

Direct application of proteins produced by recombinant technology was effective in preliminary experiments of therapeutic angiogenesis [14,28–30] and in a variety of established clinical situations, such as the routine use of tissue plasminogen activator (r-tPA) as an effective thrombolytic drug for acute myocardial infarction. Angiogenesis by gene therapy, where the gene encoding the angiogenic protein is transferred to the host cells, provides an interesting alternative for producing and applying proteins capable of modifying vascular growth, and may be considerably cheaper. Following gene transfer, the protein may be secreted in vivo for a given period of time, rather than the ‘single-shot’ dosage achieved by injecting the protein itself. Gene transfer targeted to specific body tissues [42,43] could enhance efficacy and reduce side effects.

6. Vectors for gene transfer

Three major categories of gene transfer should be considered for therapeutic angiogenesis: use of naked plasmid DNA, DNA bound to liposomes or gene transfer using viral vectors (retrovirus or adenovirus). Naked DNA or liposome-DNA complex gene transfer have the advantage of simplicity, but efficiency is low and targeting is limited (Table 1). The duration of expression is transient.

Viral vectors are more efficient for gene transfer. Retroviral vectors have been used to transfer marker, selection and therapeutic genes to endothelial cells in vitro [44]. The use of a selection gene allows the growth of a homogenous population of endothelial cells, all expressing the gene of interest and the transgenes integrate into the genomic DNA. Cells dividing from the transduced cell continue to express the transgene and a prolonged period of transgene expression can be expected. However, efficiency of gene transfer using retroviral techniques is low, especially in vivo, since retrovirus is only effective for dividing cells.

Table 1

<table>
<thead>
<tr>
<th>Mode of gene transfer</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Efficiency</th>
<th>Duration of expression</th>
</tr>
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<tbody>
<tr>
<td>Naked DNA [49,56,61]</td>
<td>Simple</td>
<td>Lack of targeting</td>
<td>Low</td>
<td>Transient</td>
</tr>
<tr>
<td>Liposomes</td>
<td>Simple</td>
<td>Lack of targeting</td>
<td>Low</td>
<td>Transient</td>
</tr>
<tr>
<td>Retrovirus</td>
<td>Integration to genomic DNA</td>
<td>Dividing cells only</td>
<td>Low</td>
<td>Stable</td>
</tr>
<tr>
<td>Adenovirus [18,57]</td>
<td>All cells</td>
<td>Activation of immune system</td>
<td>High</td>
<td>Semi-transient (weeks–months)</td>
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Adenoviral vectors for gene transfer are more efficient in vitro and in vivo and their use shortens the period for achieving a cellular population that expresses the therapeutic gene. As the transgene is not integrated into the genomic DNA, cells that divide from the modified cells will not express the transgene. Although adenoviral vectors developed for gene transfer are replication deficient, an immunologic reaction triggered by expression of late viral genes may result in a host reaction which neutralizes the vector and the therapeutic effect. For short-term weeks to months local expression of therapeutic proteins, adenoviral based vectors for gene transfer may be appropriate, and the method may be well suited to vascular biology since a relatively short-term stimulus is presumably required with regard to promotion of angiogenesis.

7. Delivery systems for angiogenesis by gene transfer

Delivery systems being developed for angiogenesis by gene transfer can be considered under 4 major categories (Fig. 3): (1) **Intravascular injection** of the transgene. Using this approach, Giordano et al. [18] injected adenoviral vector coding for FGF-5 into the coronary arteries of pigs with stress induced myocardial ischemia. Residual blood flow directs the vector and gene to the site of obstruction and to the collateral vessels emanating from the proximal segment. Similarly, especially with regard to the coronary circulation, a gene to promote angiogenesis could be introduced into the contralateral artery, where such a vessel is the source of collateral blood supply to the distal segment of the obstructed artery. (2) **Gene transfer into the vascular wall**. In this system, plasmid DNA-phVEGF<sub>165</sub> applied to the hydrogel coating of an angioplasty balloon catheter entered the vessel wall after the balloon was inflated for several minutes in the proximal segment of the obstructed artery [45]. A variation of this method has been the instillation of a transgene into a segment of artery isolated by the simultaneous inflation of 2 balloons (double balloon technique). (3) The use of genetically engineered endothelial cells seeded on stents is currently being studied in our laboratory [46–48]. In this model gene transfer is performed in vitro by immersing seeded stents in a solution containing vectors encoding the appropriate gene. The stent, covered with genetically engineered cells, once deployed, acts as an intravascular mini-factory for production of growth factor(s), which are then released downstream into and around the area of arterial obstruction. (4) The fourth model involves direct application of the transgene to the ischemic region or artery, by direct injection into the ischemic tissue beyond the obstruction [49] or by perivascular (adventitial) application [50]. Pericardial instillation of bFGF has been used to enhance epicardial angiogenesis in a rabbit model [51].

8. Gene transfer using seeded stents

We are studying the feasibility of implanting a stent seeded with cells [46,52] genetically modified to over-express VEGF proximal to the site of arterial obstruction and region of ischemia. In this model, the modified cells secrete locally and intraluminally the selected therapeutic protein(s), which circumvents the problems presented by

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![Fig. 3. Possible delivery systems for angiogenesis by gene transfer: (1) Intravascular injection of the transgene (ipsilateral or contralateral artery). (2) Gene transfer into the vascular wall (coated on balloon catheter, instillation by double balloon technique). (3) Endothelial cells seeded on stents after ex vivo gene transfer. (4) Direct application of the transgene beyond the arterial obstruction (direct injection into ischemic tissue, perivascular (adventitial) application, pericardial instillation).](image-url)
anatomical barriers [53] in transferring relatively large size genetic material into the vessel wall. Gene transfer is limited to the cells seeded on the stent. The duration of protein secretion from seeded stents should continue for several days, depending on the vector used. An additional advantage of stent implantation is that stent deployment could itself be therapeutic in an area of arterial narrowing proximal to the site of major vascular obstruction.

A disadvantage of the seeded stent strategy is that endothelial cells from the patient must be harvested to prevent subsequent rejection and local inflammation. Autologous endothelial cells could be harvested from veins, arteries or adipose tissue [54,55]. Other possible ways to genetically modify stents include incorporation of nucleic acids (either DNA or RNA) into polymer coatings on stents. Hydrogel-coated balloon catheters in which plasmid DNA encoding therapeutic genes was incorporated produced a significant physiologic effect [56].

9. Preliminary basic and animal models of angiogenesis by gene therapy

Angiogenesis has been studied for several years in the molecular biology laboratory, and in small and large animals. In vivo angiogenesis was achieved by injecting subcutaneously into mice 750 μl of reconstituted basement membrane proteins (Matrigel) with recombinant adenoviral vector coding for acidic fibroblast growth factor (aFGF1–154) [57]. After 14 days, there was histologic evidence of neovascularization in the tissues surrounding the matrigel plugs. In vitro, secreted forms of aFGF produced a tenfold increase in cell number over control and was advantageous compared to the non-secreted form [57].

Giordano et al. [18] administered recombinant adenovirus expressing human fibroblast growth factor-5 (FGF-5) by direct intracoronary injection in a pig model of myocardial ischemia. Two weeks after gene transfer, regional abnormalities in stress-induced myocardial function and perfusion were improved, and the effects persisted for at least 12 weeks. Corresponding to the amelioration of stress-induced ischemia, polymerase chain reaction (PCR) and reverse transcriptase-PCR (RT-PCR) demonstrated transgenic FGF-5 DNA and mRNA in the myocardium, and immunoblotting showed FGF-5 protein in the myoccardium of animals that received FGF-5 gene transfer. The authors claimed direct evidence for angiogenesis by demonstrating that the number of capillaries surrounding each fiber was increased in both the ischemic and non-ischemic region, but other measures of capillarity such as capillary number per fiber cross-sectional area or per fiber number were not altered.

Transfer of the gene encoding for VEGF was achieved by an intravascular catheter based method in a rabbit model of hindlimb ischemia (femoral artery excised). Following the application of 400 μg of phVEGF165 (naked DNA) to the hydrogel outer coating of an angioplasty balloon catheter [56], the balloon was inflated proximal to the site of arterial obstruction, resulting in site specific transfection of phVEGF165 in the internal iliac artery confirmed by RT-PCR and sequencing the RT-PCR product. Augmented development of collateral vessels was documented by serial angiography and by an improvement in the calf blood pressure ratio. The same investigators reported a different approach to gene transfer in the same model by the direct intramuscular injection of naked DNA in the ischemic rabbit hindlimb [49]. After 30 days, angiographically recognizable collateral vessels and histologically identifiable capillaries were increased in VEGF transfectants compared with controls. This approach could provide a useful alternative to the catheter-based technique in patients with very severe vascular disease where intraluminal methods cannot be implemented.

10. Safety issues

Use of growth factors could promote intimal hyperplasia and neovascularization of atherosclerotic plaques as shown after gene transfer of FGF-1 in porcine arteries [58]. There could theoretically be increased plaque permeability following VEGF. These considerations raise concern that VEGF or FGFs could adversely affect plaque stability. Other problems relating to the therapeutic application of growth factors in humans include the possibility of blood vessel growth in patients with diabetic retinopathy, which is associated with the presence of VEGF in the vitreous humor [59]. Unwanted mitogenic effects in patients with clinically unrecognized neoplasms is another potential drawback. In vitro and in vivo studies showed that VEGF promotes adhesion of activated natural killer cells in the microvasculature of growing tumors, while bFGF inhibits adhesion through the regulation of cell-adhesion molecules [60]. The implications of such effects in distant organs and tissues need to be tested and examined further before growth factors are used freely to promote therapeutic angiogenesis in the circumstances of cardiac or other ischemia.

The safety of viral vectors needs to be examined carefully and specifically for each vector strain, and also for the delivery system and model used. Viral vector was not detectable by PCR in the liver, retina and skeletal muscle of the pig model used by Giordano et al. [18] (direct intracoronary injection of gene encoding FGF-5 using adenovirus), although present in the myocardium. It is hoped that with proper targeting using appropriate methods of gene delivery, these potential disadvantages will in practice not limit the application of the technique.

11. Human protocols and the potential for angiogenesis by gene transfer in humans

The first clinical trial involving the use of gene transfer and over-expression of growth factor to promote therapeu-
tic angiogenesis is a catheter based protocol conducted in Boston in patients with critical peripheral vascular disease, rest pain and/or non-healing ischemic ulcers [45]. The patients were not suitable candidates for non-surgical or surgical revascularization by conventional techniques and faced amputation. The program is examining the effect of escalating doses of phVEGF165 administered as naked DNA on a hydrogel coated angioplasty balloon. No major effect was achieved in the initial patients who received 100 and 500 µg. Doses of 1000 µg of phVEGF165 improved lower limb blood flow by magnetic resonance imaging in 3 of 5 patients and a dose of 2000 µg in another patient was associated with an increase in collateral vessels by digital angiography, but failed to prevent the need for limb amputation 5 months later [61]. Another worrying feature in this patient was the development of spider angiomata and edema (possibly due to the effect of VEGF on vascular permeability) in the limb distal to the site of gene transfer. Although these features provided strong support that gene transfer had taken place, they highlighted the potential for unwanted side-effects and the possibilities of excessive over-expression following gene transfer in tissues where receptor regulation and other non-controllable factors may be operative. Other clinical protocols for angiogenesis using growth factors rather than gene therapy plan to be operative. Other clinical protocols for angiogenesis over-expression following gene transfer in tissues where receptor regulation and other non-controllable factors may be operative. Other clinical protocols for angiogenesis using growth factors rather than gene therapy plan to examine the effects of bFGF applied locally at the time of bypass surgery in patients with incomplete revascularization, and the effects of bFGF and VEGF by intracoronary injection [13]. Therapeutic angiogenesis appears to be potentially feasible in humans, but more studies and more data are eagerly awaited.

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References


[51] Landau C, Jacobs AK, Haudenschild CC. Intrapericardial basic fibroblast growth factor induces myocardial angiogenesis in a rabbit model of chronic ischemia. Am Heart J 1995;129:924–931.


