Gene Therapy (2003) 10, 285–291

www.nature.com/gt

REVIEW

Gene therapy progress and prospects: therapeutic angiogenesis for limb and myocardial ischemia

TA Khan[1], FW Sellke[1] and RJ Laham[2]

[1]Division of Cardiothoracic Surgery, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA; and [2]Division of Cardiology and Angiogenesis Research Center, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA

After extensive investigation in preclinical studies and recent clinical trials, gene therapy has been established as a potential method to induce therapeutic angiogenesis in ischemic myocardial and limb disease. Advancements in viral and nonviral vector technology including cell-based gene transfer will continue to improve transgene transmission and expression efficiency. An alternative strategy to the use of transgenes encoding angiogenic growth factors is therapy based on transcription factors such as hypoxia-inducible factor-1α (HIF-1α) that regulate the expression of multiple angiogenic genes. Further understanding of the underlying biology of neovascularization is needed to determine the ability of growth factors to induce functionally significant angiogenesis in patients with atherosclerotic disease and associated comorbid conditions including endothelial dysfunction, which may inhibit blood vessel growth. The safety and tolerability of therapeutic angiogenesis by gene transfer has been demonstrated in phase I clinical trials. However, limited evidence of efficacy resulted from early phase II studies of angiogenic gene therapy for ischemic myocardial and limb disease. The utility of therapeutic angiogenesis by gene transfer as a treatment option for ischemic cardiovascular disease will be determined by adequately powered, randomized, placebo-controlled phase II and III clinical trials.
Gene Therapy (2003) **10,** 285–291. doi:10.1038/sj.gt.3301969

Keywords: *angiogenesis; gene transfer; ischemic heart disease; coronary artery disease; peripheral vascular disease; vascular endothelial growth factor; fibroblast growth factor*

In breif

Progress

- Improvements in adenoviral vector and plasmid DNA technology have improved transmission and expression efficiency in myocardial and skeletal muscle tissue.
- Newer techniques of gene transfer including AAV vectors and liposome complexes have been shown to be effective in preclinical studies of angiogenesis for myocardial and vascular disease.
- Studies in animal models of therapy based on HIF-1α have provided evidence of angiogenesis using an approach of transcriptional regulation.
- Preclinical studies of angiogenesis have identified disease processes that may contribute to attenuated angiogenesis such as endothelial dysfunction.
- Phase I and II clinical trials have demonstrated the safety and suggested the efficacy of gene transfer in therapeutic angiogenesis for CAD and PVD.
- Minimally invasive catheter-based and surgical techniques of delivery have been effective in clinical trials.

Prospects

- Further improvements in viral technology will increase transmission efficiency and reduce toxicity because of inflammatory and immune responses.
- The advancement of nonviral vector technology will allow for efficient gene transfer while avoiding the safety concerns associated with viral vectors.
- Refinements in cell-based gene transfer to allow multigene and sequential gene expression in addition to regulatable expression with organ and tissue specificity will be needed.
- Gene transfer using transcription factors that regulate the expression of multiple angiogenic genes may be preferable for the induction of angiogenesis.
- Current investigations of the molecular mechanisms of disease states that have been shown to inhibit angiogenesis will provide vital information to develop an effective therapeutic strategy of neovascularization.
- The method of delivery of angiogenic agents and timing of treatment will become essential factors in the strategy of therapeutic angiogenesis.
- Phase II and III clinical trials will be critical in determining the utility of angiogenic gene therapy in the treatment of ischemic limb and heart disease.

Correspondence: Dr RJ Laham, Angiogenesis Research Center, Beth Israel Deaconess Medical Center, 330 Brookline Ave., SL-423, Boston, MA 02215, USA
Received 10 September 2002; accepted 3 December 2002

Gene therapy is a potential method for therapeutic angiogenesis

Therapeutic angiogenesis has emerged as a promising investigational strategy for the treatment of patients with ischemic limb and heart disease. After over a decade of preclinical studies and recent clinical trials, gene therapy has been established as a potential method to induce therapeutic angiogenesis in patients with ischemic cardiovascular disease. Therapeutic angiogenesis using gene therapy vectors will be the subject of this review with emphasis on recent advancements and future directions in the treatment of ischemic limb and myocardial disease.

Neovascularization involves a complex series of events that likely include the coordinated action of several cytokines to produce new conduits of blood flow. Vasculogenesis, angiogenesis, and arteriogenesis are the three processes that may contribute to the growth of blood vessels.[1] Vasculogenesis is the formation of new vessels from pluripotent stem cells as seen in embryonic development. Increasing evidence suggests that vasculogenesis also occurs in the adult as seen in the mobilization of endothelial progenitor cells from bone marrow and the incorporation of these cells into foci of neovascularization. Angiogenesis describes capillary growth from enlarged venules that sprout capillary buds, become divided by periendothelial cells (intussusception), or are separated by transendothelial cell bridges (bridging) to form capillaries. The process involves vasodilation and increased permeability to allow extravasation of proteins to form an extracellular matrix, endothelial cell proliferation and migration, and vessel formation. Endothelial cell differentiation follows in response to the local tissue environment.[2] Angiogenesis is the manner by which capillaries proliferate in healing wounds and along the border of myocardial infarctions. Arteriogenesis is the process that produces arteries possessing a fully developed tunica media resulting in true collateral arteries. Smooth muscle cells may differentiate from various cell types including endothelial cells and bone marrow precursors. Arteriogenesis involves smooth muscle cell growth and proliferation, migration, and differentiation to a contractile phenotype.[2] An example of arteriogenesis is the development of angiographically visible collaterals in patients with advanced obstructive atherosclerotic disease (Figure 1).

In patients with coronary artery disease (CAD) and peripheral vascular disease (PVD), progressive occlusion of arteries often leads to the development of collateral vessels that supply the ischemic tissue. However, this natural compensatory process of neovascularization is often not sufficient as evidenced by the large number of revascularization procedures performed annually. The lack of an adequate angiogenic response in part may be related to reduced production of angiogenic factors. Therapeutic angiogenesis describes the method of improving blood flow to ischemic tissue by the induction of neovascularization by angiogenic agents administered as recombinant protein or by gene transfer. The administration of recombinant protein or the genes that encode these proteins both have been used as techniques of angiogenic therapy in preclinical and clinical trials. Advantages of gene transfer include persistent expression of the angiogenic factor providing prolonged, local exposure, potential for single-dose regimens, and cell-specific angiogenic therapy. However, low efficiency of gene expression and immune deactivation of the foreign material are limiting factors. Furthermore, induction of an inflammatory response, nonspecific gene transfer to other cell types, and lack of regulation of gene expression and the resulting uncontrolled level of growth factor are additional risks to the patient.[3,4] Protein formulations provide predictable pharmacokinetics and tissue therapeutic levels that allow for controlled dosing at the time of growth factor administration. In using protein-based angiogenic therapy, the administration of viral vectors and foreign genetic material is avoided. The short half-life of angiogenic proteins limits the duration of exposure and presents a possible need for additional doses. However, slow-release delivery systems may circumvent this issue and repeat dosing may be more effective because of the relative lack of significant inflammatory and immune responses to protein therapy.

As important as the type of angiogenic therapy is the delivery of the angiogenic agent. Intravascular delivery may lead to nonspecific and systemic exposure, while intramyocardial delivery techniques can allow for a local and sustained angiogenic effect.[5] In addition, therapy based on single growth factor agents may not be adequate to induce functionally significant angiogenesis in humans because of the complexity of the angiogenic process, particularly in the context of advanced atherosclerotic disease and associated comorbid conditions. Multi-agent therapy may be necessary to achieve angiogenesis and provide significant improvements in myocardial perfusion and function as well as clinical outcome in patients.

Viral and nonviral gene transfer agents have been successfully studied

Among the current clinical trials of therapeutic angiogenesis for CAD and PVD, adenoviruses and plasmids are the vectors most often studied likely because of the ease of production, reasonable transfection efficiency, and expression in nonproliferating cells. First-generation E1-deleted adenoviral vectors were limited in vascular gene transfer because of endothelial injury and the inflammatory response. The development of attenuated adenoviral vectors with further deleted elements of the viral genome has produced vectors that result in increased transgene expression and reduced inflammation in cardiovascular gene transfer. Deletions of the E1 and E4 regions to produce a second-generation adenoviral vector have been shown to result in improved transgene expression with reduced inflammatory response and preserved endothelium-dependent relaxation. However, lack of prolonged transgene expression at 28 days may have been representative of residual, although substantially less, inflammation induced by the second-generation adenoviral vector because of low-level, late gene expression.[6] Fully deleted adenoviral vectors may potentially eliminate this late expression and any associated inflammatory response. This technology may improve transgene expression despite the presence of antiadenoviral neutralizing antibodies.[7] Production of fully deleted vectors is made possible by a helper virus that provides viral proteins required for replication and packaging. A study of fully deleted adenoviral vectors, also known as gutless or helper-dependent, carrying a marker transgene, erythropoietin, demonstrated that intramuscular delivery resulted in efficient and prolonged expression in both immunocom-

Figure 1 Arteriogenesis in an animal model of myocardial ischemia. Batson casts were performed on explanted hearts following left circumflex coronary artery ameroid constrictor placement. After myocardial digestion, vessels were visualized (blue for occluded left circumflex, red for left anterior descending (LAD), and white for right coronary arteries). Arrows point to epicardial collateral vessels going from the LAD and right coronary arteries to the ischemic left circumflex territory.

petent mice and those immunized against the adenovirus serotype.[7] The results of the study are clinically important considering the significant proportion of the population with pre-existing immunity to adenovirus. In addition, recombinant adeno-associated viruses (AAV) are potential vectors for therapeutic angiogenesis. Advantages of the AAV vectors for gene transfer include the transduction of nonproliferating cells, lasting transgene expression, and reduced inflammatory response, while limitations involve difficulty with production and a small packaging capacity.

Nonviral methods of gene transfer studied in clinical trials include plasmid DNA and liposomal complexes. The use of nonviral techniques avoids the concerns over the toxicity associated with viral vectors. While the inflammatory response to adenoviral vectors is well described, both plasmid DNA and liposomal complexes also potentially induce inflammation.[8,9] Despite the ease of production and scale-up of plasmid and liposomal complexes, low transmission efficiency and transgene expression are limiting. Transmission efficiency of plasmid DNA may be improved by the use of ultrasound. Ultrasound exposure with microbubble echocontrast agents increase transgene expression significantly after naked DNA transfection by cell membrane permeabilization. This technique of membrane permeabilization, or acoustic cavitation, with microbubble echocontrast was reported to increase transgene expression by approximately 300-fold through the creation of transient small holes in the cell surface membrane through which naked DNA is rapidly translocated.[10] In a study of plasmid DNA transmission efficiency, luciferase plasmid transfection using ultrasound with microbubble echocontrast was increased approximately 10-fold compared to plasmid alone in cultured human skeletal muscle. In the same report, gene transfer of a hepatocyte growth factor (HGF) plasmid in a rabbit model of hindlimb ischemia produced increased angiographic score and capillary density in animals transfected using ultrasound with microbubble echocontrast *versus* transfection with plasmid alone.[11] The use of ultrasound with microbubble contrast also has been demonstrated to increase the transfection efficiency of plasmid DNA in human aortic endothelial and vascular smooth muscle cells without apparent toxicity.[12] This technique has the potential to improve the efficiency of plasmid DNA transfection in human myocardial tissue as well. The transmission efficiency of liposomes may be enhanced by improvements in cationic polymers.[13] Liposomes have been shown to be effective in the transfer of growth factors in animal models of angiogenesis. In a rabbit ischemic hind limb model, vascular endothelial growth factor (VEGF) gene transfer by cationic liposome resulted in neovascularization and improved blood flow in the ischemic limb.[14] Liposome carriers also have been demonstrated to be effective in angiogenesis based on HGF. Transfer of HGF with the hemagglutinating virus of Japan (HVJ)–liposome method has been shown to induce angiogenesis in normal and infarcted myocardium.[15]

Cell-based gene transfer is a novel strategy that utilizes autologous cells as vectors after *in vitro* transfection with a transgene of interest.[16] Such a system is able to circumvent the inflammatory response by using autologous cells and achieves prolonged expression by stable transfection using various measures including electroporation and *in vitro* retroviral or lentiviral transfection. In addition, complex constructs can be synthesized that would allow stable, regulatable expression and multiple transgene expression. Recent investigations have focused on gene transfer by cellular transplantation to induce neovascularization in ischemic tissue using skeletal myoblasts and angioblasts.[17–19]

VEGF and FGF are the most widely studied growth factors

Numerous growth factors and transcription factors have been associated with physiologic and pathologic angiogenesis. Among the transgenes under investigation in clinical trials for CAD and PVD, genes that encode growth factors predominate. VEGF and fibroblast growth factor (FGF) are the agents most widely studied in clinical trials, specifically the 121 and 165 amino-acid isoforms of VEGF1, VEGF2, FGF1, and FGF4. Gene transfer of VEGF and FGF has been shown to induce functionally significant angiogenesis in numerous preclinical studies of angiogenic therapy for ischemic heart disease[20] and peripheral arterial disease.[21] Another strategy under investigation in clinical trials is a therapy based on the transcription factor hypoxia-inducible factor-1α (HIF-1α). The expression of many angiogenesis-related genes, including VEGF and the VEGF receptor FLT-1, is regulated by HIF-1α. A hybrid, constitutively active form of HIF-1α, has been synthesized from the DNA-binding and dimerization domains of the HIF-1α subunit and the transactivation domain of the VP16 protein of the herpes simplex virus. Angiogenic gene transfer of this hybrid form has been reported in preclinical studies as described below. Another potential factor that regulates angiogenesis is the peptide PR-39. This peptide increases the cellular levels of HIF-1α by inhibiting its degradation in the ubiquitin–proteasome complex. PR39 has been shown to increase the expression of VEGF, the VEGF receptors KDR and FLT-1, and the FGF receptor 1.[22] Concerns over a nonspecific action of PR39 related to charge are being investigated.

Preclinical and clinical trials in PVD have demonstrated safety and suggested efficacy

Angiogenic responses to growth factor gene transfer using plasmid and adenoviral vectors have been well documented in animal models of chronic limb ischemia. VEGF gene transfer techniques using nonviral and adenoviral vectors have been the more common methods in preclinical studies. Recently, angiogenesis has been induced in animal models of limb ischemic using AAV vector-mediated therapy with VEGF. In rat hind limb models of ischemia, VEGF-based therapy via AAV vectors produced increased blood flow and capillary growth in treated, ischemic limbs compared to controls.[23,24] HGF also has emerged as a potential agent in therapeutic angiogenesis. In a rabbit ischemic hind limb model, intramuscular injection of human HGF plasmid resulted in enhanced collateral development by angiography and increased blood flow and blood pressure in the ischemic limb.[25] Furthermore, evidence has recently been published that suggests FGF-2 contributes to the regulation of HGF expression.[26]

Recent results of clinical trials of therapeutic angiogenesis for peripheral arterial disease have provided further information on the clinical response to angiogenic gene therapy including measures of neovascularization as well as known side effects such as edema formation. Lower extremity edema was evaluated in 90 patients that were treated with $VEGF_{165}$ by intra-arterial or intramuscular gene transfer. Edema was observed in 34% of patients, being more common in those patients with rest pain and ischemic ulcers as compared to those with claudication only. The increased vascular permeability was an effect attributed to the VEGF therapy.[27] Recently, the results of a phase II clinical trial of VEGF-1 gene therapy for chronic limb ischemia were published. In the randomized, placebo-controlled, double-blinded study of catheter-based VEGF-1 gene therapy after percutaneous transluminal angioplasty (PTA), patients in the treatment groups received intra-arterial VEGF-1 by adenoviral vector or liposome/plasmid carrier while those in the control group received crystalloid solution. Digital subtraction angiography (DSA) revealed increased vascularity in both treated groups. Both the VEGF-adenoviral and VEGF-liposome/plasmid groups showed increased vascularity distal to the site of gene transfer. In addition, the VEGF-adenoviral group demonstrated significantly increased vascularity in the clinically most severe region of ischemia. However, the ratio of lower extremity compared to upper extremity blood pressure, or ankle-brachial index (ABI), was not significantly different between treated and control groups. In addition, antiadenoviral antibodies increased in 11 of 18 patients administered VEGF-1 by adenoviral vector.[28]

Phase I/II clinical trials in patients with myocardial ischemia have been completed

Several preclinical studies have demonstrated efficacy of angiogenic gene therapy for myocardial ischemia. FGF- and VEGF-based protocols have been the most widely studied. A recent report of $VEGF_{121}$ gene transfer by an adenoviral vector in a porcine model of myocardial ischemia demonstrated that intramyocardial delivery resulted in transient, focal VEGF expression in the target, ischemic area of the myocardium. The localized VEGF expression was 10-fold greater than in intracoronary delivery and produced regional improvement in myocardial blood flow.[29] Other studies of late have reported alternative strategies producing favorable results in animal models of myocardial angiogenesis. In a mouse model of myocardial ischemia, an AAV-VEGF vector injected around ischemic myocardium resulted in neovascularization without evidence of inflammation.[30] Gene transfer of the HIF-1α/VP16 hybrid in a rabbit model of hind limb ischemia was associated with increased regional blood flow and capillary density.[31] In a rat model of acute myocardial ischemia, intramyocardial delivery of the HIF-1α/VP16 hybrid plasmid was able to reduce the size of myocardial infarction and increase capillary density in the border zone of the infarct area. The induction of angiogenesis was similar to the neovascularization that resulted from VEGF therapy by plasmid gene transfer in the same model.[32] In a study of transgenic mice with porcine PR39 cDNA, angiogenesis was induced in the PR39 mice compared to age-matched controls as seen by increased CD-31-stained vascular structures.[22] These results suggest that agents that produce a multifactorial angiogenic response such as HIF-1α and PR39 are effective in neovascularization and may represent a more effective strategy in therapeutic angiogenesis.

Results of several clinical trials of gene transfer for therapeutic angiogenesis have been reported. These trials include uncontrolled, open label designs primarily investigating safety and feasibility. The results of these studies should be interpreted with caution considering the significant placebo effect observed in patients with CAD. In a study of 129 patients enrolled in control groups of phase I and II clinical trials of therapeutic angiogenesis and laser myocardial revascularization, the mean CCS angina class was 3.0 ± 0.5 at baseline and 2.1 ± 0.6 at 6 months ($P < 0.001$), with 24.8% of patients improved by two or more angina classes. Mean follow-up was 30 ± 6 months and at last follow-up, mean CCS angina class was 2.3 ± 0.8 ($P < 0.001$). The results of this study underscore the significance of the placebo effect in this patient population of severe CAD.[33]

Two studies of surgical angiogenic therapy using gene transfer have been published recently. A phase I study evaluated the surgical delivery of $VEGF_{165}$ plasmid DNA through a mini-thoracotomy in seven patients. As previously reported, both nitroglycerin intake and CCS angina class were significantly reduced, while improved myocardial perfusion was suggested by single-photon emission computed tomography (SPECT) and coronary angiography.[34] A study of intramyocardial gene transfer through a limited thoracotomy was initiated using VEGF-2 plasmid DNA in patients with chronic stable angina. An initial report on 11 patients revealed reductions in angina episodes and nitroglycerin use as well as improvement in exercise tolerance testing.[35]

Two trials of catheter-based myocardial gene transfer of plasmid DNA encoding for VEGF-2 have been reported of late. An initial randomized, single-blind, placebo-controlled study investigated the safety and feasibility of VEGF-2-based therapy in six patients. Patients that received VEGF-2 plasmid DNA experienced reduced angina compared to control patients at 90 days of follow-up. Reduction in ischemia and improvement in myocardial perfusion were suggested by electromechanical mapping and SPECT imaging, respectively.[36] A multicenter, randomized, double-blind, placebo-controlled phase I/II clinical trial followed. VEGF-2 plasmid DNA was administered as part of a dose-escalating protocol to patients with Canadian Cardiovascular Society (CCS) class III or IV angina. The catheter-based delivery to the endocardial surface of the left ventricle resulted in no hemodynamic alterations, sustained ventricular arrhythmias, or electrocardiographic evidence of infarction. A recent interim report after enrollment of 19 patients described a significant reduction in CCS angina class in the treated group compared to controls.[37] Although these results as a whole suggest the therapeutic efficacy of angiogenesis by gene transfer, a study of intramyocardial gene transfer of plasmid DNA encoding VEGF-A and VEGF-B as an adjunct to CABG in 24 patients was published recently that provided only modest evidence of improved perfusion.[38]

The angiogenic gene therapy (AGENT) study was the first randomized, double-blind, placebo-controlled trial of therapeutic angiogenesis by gene transfer for myocardial ischemia. FGF-4 carried by an adenoviral vector was given intracoronary to 79 patients with chronic stable angina randomized in a 1:3 ratio to produce 19 in the control group and 60 in the treatment group. Overall, FGF-4 therapy using an adenoviral vector was well tolerated without significant safety concerns. No significant difference was observed in stress-induced wall motion by echocardiography between groups. The results of the primary end point, exercise treadmill testing (ETT), after 4 and 12 weeks of follow-up demonstrated a nonsignificant trend towards improvement in the FGF-4-treated group. Subgroup analysis revealed a significant improvement in those patients with baseline ETT of less than 10 min.[39]

Patient selection and end-point evaluation are important factors in clinical trial design

Therapeutic angiogenesis has provided a new treatment strategy for patients with end-stage CAD and PVD. Currently, results of adequately powered, randomized, double-blind, placebo-controlled trials are lacking. For enrollment in clinical trials, randomization should include baseline angiogenic response manifest as collateralization. Patients selected for trials of therapeutic angiogenesis often previously have had multiple percutaneous and surgical revascularization attempts. These individuals may possess resistance to stimulation of neovascularization, considering they likely suffer from failure of natural angiogenic responses. Thus, patients enrolled in current trials may represent the group least likely to respond. Ideal candidates are those with ischemic but viable myocardium and diffuse multivessel disease. Exclusion criteria generally include a history of malignancy or proliferative retinopathy because of concerns of pathogical angiogenesis. Patients with abnormal baseline renal function and proteinuria are excluded from trials of FGF therapy because of the risk of renal toxicity.

End points of cardiac morbidity and mortality including myocardial infarction and death may provide objective measures of outcome. However, the low frequency of these events in clinical trials of treatment of myocardial disease indicate that a prohibitively large study population may be required to show significant reductions in these outcomes. Limb salvage from amputation may be used in a patient population with advanced peripheral vascular disease. Other end points in cardiac trials include exercise tolerance testing and measures of myocardial perfusion using single-photon emission computed tomography (SPECT) and magnetic resonance imaging (MRI), while end points in peripheral vascular trials may include vascularity by DSA or magnetic resonance angiography, lower extremity blood pressure by ABI, and healing of ischemic ulcers. The response to angiogenic gene therapy for limb ischemia may be assessed through improvements in claudication and rest pain. Relief of myocardial ischemic symptoms and improvements in quality of life may be determined by methods such as the Seattle Angina Questionnaire or the Canadian Cardiovascular Society angina classification. However, the placebo effect has been shown to be very powerful in studies of patients with severe CAD and PVD.

Prospects

Recent advances in gene transfer have allowed therapeutic angiogenesis to become a potential treatment for CAD and PVD.

Continued development of viral and nonviral vector technology will improve transgene transmission and expression efficiency. Refinements in cell-based therapy may provide for regulatable and multiagent therapy. The attenuated angiogenic response in patients enrolled in clinical trials may be related to comorbid pathophysiology that is associated with CAD and PVD. Endothelial dysfunction is associated with atherosclerotic disease, and may play a role in a reduced angiogenic response. We investigated the effect of endothelial dysfunction secondary to hypercholesterolemia on therapeutic angiogenesis by perivascular delivery of FGF-2 through a mini-thoracotomy.[40] In our pig ameroid constrictor model of chronic myocardial ischemia, hypercholesterolemic animals showed significant endothelial dysfunction and impaired angiogenesis manifest as decreased perfusion compared to the control, normal diet group. Thus, endothelial dysfunction may represent one of many factors that prevent a significant angiogenic response in patients with CAD and PVD. The future development of treatment options for endothelial dysfunction may allow for an improved angiogenic response to growth factor therapy in humans with atherosclerotic occlusive disease.

Improvements in delivery modalities with increased local distribution and retention and reduced systemic circulation are needed. Intramyocardial and intramuscular delivery techniques currently under investigation in phase I and II clinical trials may provide evidence of efficacy that was lacking in studies using intravascular routes of administration. Delivery optimization studies also should be conducted for specific agents prior to preclinical and clinical investigation. Multiagent therapy may be needed to achieve the complex process of angiogenesis in humans. The natural angiogenic response is likely a result of the actions of multiple growth factors at various points in time. The sequential or concomitant administration of the agents may be an important factor as well. In addition, a synergistic mechanism of action between growth factors in angiogenesis has been suggested. Furthermore, it has become clear that the timing of growth factor therapy may be critical in inducing an angiogenic response. A recent study of VEGF-based angiogenesis has demonstrated that a critical duration of growth factor exposure is required to prevent regression of the newly formed vasculature.[41] Thus, therapeutic angiogenesis using methods with prolonged presence of growth factors over a few weeks may be necessary, as suggested by the promising results of the clinical trial using surgically implanted heparin-alginate microcapsules that release FGF-2 over a course of 3–4 weeks.[42]

Conclusions

Therapeutic angiogenesis is a promising therapy for patients with CAD and PVD not amenable to current revascularization techniques. Clinical trials of gene transfer for therapeutic angiogenesis in the treatment of ischemic limb and heart disease have been limited to predominantly uncontrolled phase I studies that have demonstrated safety and preliminary reports of phase II trials that have provided modest evidence of clinical efficacy. As further investigation of gene transfer proceeds, monitoring of potential toxicities must be continued to maximize the benefit and minimize the risk of angiogenic therapy. Overall, the role of therapeutic angiogenesis by gene transfer as a potential treatment option for ischemic limb and heart disease will be determined by adequately powered, randomized, placebo-controlled phase II and III clinical trials.

Acknowledgements

Supported in part by NIH Grants MO1-RR01032 and HL63609 (RJL), and HL46716 and HL69024 (FWS). Dr Khan is supported by an NIH Individual National Research Service Award, HL69651-01.

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