Review

Therapeutic angiogenesis in cardiology using protein formulations

Mark J. Post, Roger Laham, Frank W. Sellke, Michael Simons

Angiogenesis Research Center and Cardiothoracic Surgery Division (FWS), Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

Received 6 June 2000; accepted 1 September 2000

Abstract

Therapeutic angiogenesis in cardiovascular disease aims at improving myocardial function by increasing blood flow to ischemic myocardium that is not amenable to traditional forms of revascularization. Preclinical data have provided proof of the concept that angiogenic growth factors such as fibroblast growth factor 2 (FGF-2) and vascular endothelium growth factor (VEGF) may indeed improve myocardial flow and function when administered in ways that ensure prolonged tissue exposure to these short-lived molecules. Although other cytokines have been shown to enhance angiogenesis in vivo, FGF-2 and VEGF have been most widely studied and may serve as prototype proangiogenic drugs. Currently, several delivery techniques that are clinically applicable are being studied with respect to tissue distribution and retention as well as angiogenic efficacy of FGF-2 and VEGF. Although tissue distribution and retention of FGF-2 after intramyocardial injection compares favorably with other routes of administration, efficacy studies are not yet conclusive. At the same time, different protein- and gene-based formulations are being investigated. Arguments for and against protein and gene therapy are presented, showing that protein-based therapy seems to have advantages over gene therapy at the present time, although continuous efforts should be made to increase the tissue exposure time after a single administration of protein. While delivery systems and growth factor formulations are being improved, double-blind, placebo-controlled trials designed with existing animal data in mind, are needed to firmly establish the utility of therapeutic angiogenesis in cardiovascular disease. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Angiogenesis; Coronary disease

1. Introduction

Current treatment options for patients with advanced ischemic heart disease include medical therapy or coronary revascularization by percutaneous coronary angioplasty (PTCA) or coronary bypass surgery (CABG) [1–4]. However, a significant number of these patients are not candidates for standard revascularization procedures or have incomplete revascularization with these procedures [1,5]. For example, in patients with 2- and 3-vessel disease, complete revascularization of hemodynamically significant stenoses was successful in 23 and 9% of cases, respectively [5]. As a result, many of these patients have residual symptoms of myocardial ischemia despite medical therapy. Furthermore, full functional recovery of the ischemic myocardium may not be achieved if revascularization is delayed [6]. Preliminary clinical experience with therapeutic angiogenesis suggests that this new treatment may provide additional blood flow to underperfused and incompletely revascularized areas and thus be valuable in the management of these patients [7–11].

2. Biology of cardiac angiogenesis in adult tissues

To evaluate different treatment strategies it is important to understand the basic pathophysiology of blood vessel formation in adult tissues. Three different processes may contribute to the growth of new blood vessels: vasculogenesis, arteriogenesis and angiogenesis [12–14].

Vasculogenesis denotes formation of new vasculature...
from pluripotent endothelial stem cells [15], which in the course of embryonic development, results in formation of primitive vascular plexus, and is followed by recruitment of other vascular cell types to complete the process of vessel formation. Preliminary evidence suggests that vasculogenesis may play a role in new vessel formation in mature adult tissues [16–18] but the significance and frequency of this event has not been established.

Angiogenesis is the process responsible for formation of new vessels lacking developed media that are thought to arise, in mature tissues, from postcapillary venules [12]. Examples of angiogenesis include capillary proliferation in the healing wound or along the border of myocardial infarction.

Arteriogenesis refers to the appearance of new arteries possessing fully developed tunica media [19]. This is a poorly understood process that may involve maturation of preexisting collaterals or may reflect de novo formation of mature vessels. Examples of arteriogenesis include formation of angiographically visible collaterals in patients with advanced obstructive coronary or peripheral vascular disease. All vascular cell types including smooth muscle cells and pericytes are involved.

The occurrence of both angiogenesis and arteriogenesis has been conclusively demonstrated in a variety of animal models [20,21] as well as in patients with coronary disease [22–24]. Although related, arteriogenesis and angiogenesis may be differentially regulated. Arteriogenesis generally occurs proximal from the ischemic territory where hemodynamic (e.g., shear stress) or hematologic (prothrombotic) changes dominate [25], whereas true angiogenesis is primarily driven by hypoxia or tissue ischemia [26,27].

These general considerations notwithstanding, the differences in molecular responses controlling angiogenesis and arteriogenesis are poorly understood. The occurrence of tissue ischemia is thought to increase cellular levels of hypoxia-induced factor (HIF)-1α protein that in turn upregulates expression of vascular endothelium growth factor (VEGF) and its receptors [28,29], leading to increased capillary density in the ischemic regions of the heart. However, the ability to respond to a hypoxic stimulus may also play an important role in arteriogenic response. A recent retrospective study documented higher incidence of HIF-1α response to hypoxia in monocytes of patients with advanced coronary disease and angiographically visible coronary collaterals (arteriogenesis) compared to patients who had no or minimal collaterals [30]. In addition, patients with unstable angina frequently demonstrate elevated levels of fibroblast growth factor 2 (FGF-2) in the serum [31] and the pericardial fluid [32]. The contribution of FGF-2 to the arteriogenic or angiogenic response however, has not been addressed in these studies.

A major difference between embryonic vasculogenesis and adult angiogenesis/arteriogenesis is the proinflammatory environment in which the latter usually takes place [19,33,34]. A number of inflammatory mediators such as interleukin 1α (IL-1α), substance P [35], insulin-like growth factor 1 (IGF-1) [36], CXC chemokines [37], tumor necrosis factor α (TNF-α) [38], proline/arginine rich peptide 39 (PR 39) [39] and various matrix proteases [14,40] are actively involved in adult angiogenesis. Inflammatory cells such as macrophages most likely propagate the angiogenic response by virtue of their capacity to produce and release angiogenic factors and matrix degrading enzymes [38,41,42]. Ineffective inflammatory responses, due to genetic make-up [43], pathophysiological processes or pharmacotherapy, may adversely affect the ability to induce new vessel growth [34,44].

Newly formed vessels, whether formed by natural processes or due to therapeutic application of growth factors, are typically of capillary or small arteriolar size (10–200 μm) and may or may not possess tunica media [45–47]. The maturation of vessels into multilayer structures may actually be important for their persistence. Neovascularization and subsequent regression of newly formed vessels has been observed after single stimuli such as the mast cell secretagogue 48/80 [48] and in tumor vessels [49]. Whether regression also occurs in growth factor-induced angiogenesis in the heart or peripheral vasculature and what is required to prevent it, is still unknown.

Candidates for pharmacological stimulation of therapeutic angiogenesis in cardiac or peripheral limb ischemia include angiogenic cytokines such as fibroblast growth factors (FGF) [50], vascular endothelial growth factors (VEGF) [51], hepatocyte growth/scatter factor (HGF/SF) [52], CXC chemokines such as interleukin 8 (IL-8) [53] and monocyte chemoattractant protein 1 (MCP-1) [54], growth factors involved in maturation of the vascular tree such as angiopoietins [55–57] and platelet derived growth factor (PDGF) [58,59], and transcription factors that stimulate expression of angiogenic cytokines and their receptors such as HIF1α [60].

3. Protein versus gene therapy

Theoretically, therapeutic angiogenesis can be achieved by employing either growth factor proteins or by introducing genes encoding these proteins [61]. Sustained local production and release of growth factors through gene therapy can overcome the inherent instability of angiogenic proteins (Table 1) [62–64] and may therefore be preferred. However, although prolonged presence of growth factors may be beneficial, there is no conclusive evidence to support this hypothesis. In fact, preliminary evidence suggests that prolonged local production of potent growth factors such as VEGF-A [65] and FGF-2 [66] may cause unwanted hemangioma formation [67] or fibromatosis [Post et al. unpublished data]. Moreover, the theoretical advantage of gene therapy approaches with respect to longer-term angiogenic factor exposure depends on effec-
Table 1
Differences between gene and protein therapy

<table>
<thead>
<tr>
<th></th>
<th>Gene therapy</th>
<th>Protein therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temporal exposure</td>
<td>Sustained presence</td>
<td>Finite</td>
</tr>
<tr>
<td>Dose response</td>
<td>Unpredictable</td>
<td>Defined</td>
</tr>
<tr>
<td>Administration</td>
<td>Single</td>
<td>Repeated?</td>
</tr>
<tr>
<td>Targeting</td>
<td>Possible</td>
<td>Possible</td>
</tr>
<tr>
<td>Slow release</td>
<td>Yes</td>
<td>Possible through formulation</td>
</tr>
<tr>
<td>Inflammatory response</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Foreign material</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Serum half-life</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>Tissue half-life</td>
<td>Unpredictable</td>
<td>Short, subject to engineering</td>
</tr>
<tr>
<td>Influence patient serology</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Various gene therapy vectors differ in their efficacy of cell transduction, the type of cells that get transduced (proliferating vs. non-proliferating), and duration and extent of transgene expression. Plasmid DNA and early generation adenoviral vectors mediate rather short-term (days to weeks) duration expression, while other viral vectors (e.g. retroviral, lentiviral, AAV) can result in a very long (months) duration of expression. The limited duration of transgene expression (~1–2 weeks) achieved in the heart with first generation adenovirus vectors makes them in some manner ideal for angiogenic gene delivery [63,68]. However, this limited duration of expression is at least partially attributed to an immune response against adenoviral proteins [69,70] and may be very short indeed in patients with pre-existing neutralizing antibodies. Thus, considerable concerns have risen over inflammatory responses to these vectors, although this remains controversial and inflammatory responses may be more likely in some tissues than others [71].

The issue of immune and inflammatory responses to viral antigens may be partially overcome with alternative viral vectors (e.g. AAV) [72]. However, these vectors may lead to longer-term transgene expression with the concomitant safety concerns associated with prolonged angiogenic stimulation. Thus the ‘gene therapy paradox’ is that ‘safer’ vectors result in potentially deleterious prolongation of therapeutic gene expression. To address this issue, vector systems capable of regulated therapeutic gene expression are currently under development [72].

The other drawback of the gene therapy approach is inconsistent level of expression achieved with the same dose in different patients. This is partly attributable to delivery issues (see below) and partly to variability in the presence and level of neutralizing antibodies. With regard to the latter, a screen of a consecutive series of patients referred for PTCA and/or CABG demonstrated that over 70% possessed neutralizing antibodies to type 5 adenovirus, which in 50% achieved very high titers [73].

The major limitation of the protein therapy approach is thought to be the limited tissue half-life of angiogenic proteins. However, a number of approaches are available to extend the tissue half-life. For instance, the serum half-life of FGF-1 in the presence of heparin can be increased from hours to days by a single amino acid mutation [74]. Furthermore, extended tissue exposure to the growth factors can be accomplished by a variety of slow release formulations (e.g. heparin–alginate beads) [75]. At the same time, the major advantage of the protein therapy approach lies in precise knowledge of delivered dose, the ability to combine several proteins into a single therapeutic formulation and a relatively well understood safety profile [61]. In the case of both gene and protein therapy approaches, delivery issues are critical to their therapeutic effectiveness and these will be addressed later in this review.

4. Efficacy of angiogenic growth factors in myocardial ischemia

Although preclinical evidence of in vivo efficacy has been obtained for all of the major angiogenic growth factors, studies of FGF-2 and VEGF-A are the most extensive to date (see [76] for a detailed review). FGF-2 belongs to the FGF family that currently numbers 22 members [77]. These proteins are distinguished by their pattern of expression and preference for different subclasses of FGF receptor (FGF-R) isoforms (FGF-R1–R4), and the presence of the leader sequence (absent in FGF-1 and 2). The ability of FGF-2 to induce angiogenesis in mature tissues was suggested by studies that documented significantly higher vessel counts following intracoronary injections of FGF-2 in the setting of acute coronary thrombosis in dogs and pigs [78,79]. These studies were followed by more detailed functional evaluation of therapeutic efficacy of FGF-2 in chronic myocardial ischemia that employed the ameroid constrictor model. The placement of the ameroid constrictor leads to gradual narrowing of the instrumented artery, which occludes completely over about 3 weeks. This gradual occlusion together with the
formation of a limited amount of native collateral vessels results in development of chronic myocardial ischemia accompanied by myocardial hibernation in the affected coronary territory with only limited subendocardial infarction [80,81].

Continuous administration of FGF-2 in the ameoboid dog model, either directly into the occluded coronary artery or into the left atrium, resulted in augmentation of coronary flow [82,83]. Similar studies in pigs showed that sustained release perivascular administration or intrapericardial delivery of FGF-2 not only improved myocardial blood flow in the ischemic myocardium, but also improved regional left ventricular function in the ischemic zone [84–86]. Interestingly, despite the relatively systemic nature of these deliveries, the angiogenic effect of FGF-2 was limited to the ischemic myocardium with no increase in the vessel number or changes in coronary blood flow noted in non-ischemic areas of the heart. Similarly, a single intracoronary injection of FGF-2, but not intravenous administration, resulted in improved perfusion [87,88] and function [89].

Experience with FGF-1 to date is much more limited. Early studies using a native form of FGF-1 protein delivered by several different modalities reported no angiogenic effect [90,91]. The significance of the studies, however, is limited given the very short half-life of the native FGF-1 protein [74]. Sustained release perivascular administration of the S117 mutant of FGF-1 that results in marked prolongation of the protein half-life [74] improved regional flow and function in chronically ischemic pig myocardium [92]. FGF-1, like FGF-2, also provides protection against ischemia–reperfusion injury, but this effect is more likely due to the vasodilatory effects of FGF-1 rather than its angiogenic effect, since a non-mitogenic FGF-1 mutant provided similar protection [93].

VEGF-A comes in at least five splicing forms as VEGF_{121}, VEGF_{145}, VEGF_{165}, VEGF_{189} and VEGF_{206}. In addition, there are currently four other closely related genes termed VEGF-B, -C, -D and -E [13]. Of these, VEGF-A_{165} has been studied most extensively in the cardiovascular system. In animal models of chronic myocardial ischemia, VEGF_{165} improved collateral blood flow [47,94]. Sustained perivascular administration, but also single bolus intracoronary injection of VEGF_{165} proved to be sufficient to improve myocardial flow and function in the pig ameoboid model [95]. Similar to FGF-2, single intravenous [89] or repetitive intra-atrial injection of VEGF_{165} were not effective [96].

Long-term side effects, such as induction of tumor growth or exacerbation of proliferative retinopathy [97], atherosclerosis [98] or bone or kidney disorders [99], did not occur in these small-scale efficacy studies. However, systemic hypotension proved to be a dose limiting side effect of both VEGF [100,101] and FGF-2 [102] although doses of FGF-2 leading to significant hypotension are substantially higher than for VEGF-A. Furthermore, other side effects of FGF-2, including proteinuria and CNS toxicity have not been observed in recent clinical studies [9].

FGF-4 and FGF-5 have been evaluated for their angiogenic potency in the form of gene (adenoviral) therapy [63] and for this reason will not be discussed further here.

5. Delivery techniques

Given that the therapeutic approach to induction of angiogenesis uses highly potent angiogenic growth factors that may have grave side effects, a high drug target level and low systemic exposure should be the ultimate goal. With the rapid growth of newly emerging delivery technologies, a continuous evaluation and re-evaluation of growth factor delivery methodology is clearly warranted. Currently, four clinically applicable catheter-based methods (intravenous, intracoronary, intramyocardial and transendocardial intramyocardial delivery) and two surgical methods (transepiperalial intramyocardial and slow release epicardial delivery) are being evaluated.

Since efficacy studies in large animals are expensive and time-consuming, a number of biodistribution studies with ^{125}I-VEGF-2 has been performed to compare the efficiency of these delivery strategies (Fig. 1) [103–105]. The results may be more of less specific to FGF-2 since this growth factor has a 5-fold higher affinity for extracellular heparan sulfates than FGF-1 [106], which in turn has a much higher affinity than VEGF-A [107]. Despite this relative FGF-2-specificity, these studies provide a valuable insight with regard to all heparin-binding growth factors.

Single intravenous or intracoronary administration of FGF-2 leads to myocardial deposition of <0.6 or 1.5%, respectively, of the total injected dose in the ischemic myocardium 1 h after administration [104]. Recovery from the normal myocardium is even less. Twenty-four hours after administration, retention in the myocardium drops to <0.1% of the initial amount administered. With intrapericardial deposition of FGF-2, <1% is recovered from the myocardium at 1 h but retention at 24 h is slightly better, with 0.5% in the normal myocardium and up to 8% (with a very high variability), in the ischemic myocardium [104].

Transendocardial or transepiperalial injections compare favorably, with 25–30% of the injectate being recovered from the myocardium and 5% retained up to 3 days after the injection [Laham et al., unpublished observations]. In all these cases, most of the ^{125}I FGF-2 activity was recoverable from the liver and the lungs. It is unknown whether the low recovery and short retention has functional consequences or whether the amount retained by the target tissue is still sufficient to exert a physiological effect. For instance, when 200 μg of FGF-2 is given intracoronary, 200 ng (0.1%) is retained up to 24 h in the ischemic myocardium. With a flow area of one coronary artery taken as 150 g of tissue and a 33% (50 ml)
specialized equipment and a higher skill level of the operator than needed for intracoronary injection [109]. Furthermore, if the ultimate goal of therapy is to induce arteriogenesis of the epicardial vessels, intramyocardial injection may not prove to be the most logical or ideal place of growth factor delivery. In this regard it should be noted that to date no conclusive data regarding physiological efficacy of this mode of administration has been presented.

Intrapericardial administration, despite its theoretical appeal, is limited due to very high (>90%) frequency of prior coronary artery bypass surgery in patients currently enrolling in angiogenesis trials [61] and by high operator skill level required for access of the normal pericardial space.

Taking all the evidence into account animal studies suggest that protein therapy can be effective with single administration if delivered through the intracoronary or pericardial route. This puts protein-based therapeutic angiogenesis into the realm of clinical feasibility in a wide range of patients.

6. Clinical trials of protein therapeutics

Therapeutic angiogenesis for treatment of ischemic cardiac disease is still in its infancy and to date, no phase III trials have been initiated with protein-based therapy in these patients. However, limited efficacy data were derived from ongoing and completed phase I/II trials.

Safety of FGF-1 (10 μg/kg) was first demonstrated in 20 patients with three-vessel disease undergoing CABG [110] in whom the growth factor was injected intramyocardially close to the internal mammary artery-LAD anastomosis. While angiography suggested the presence of increased capillary filling in the growth-factor treated compared to control patients, there was no other evidence of improved coronary perfusion or ventricular function. Seeking to address these issues, we performed a double-blind randomized trial of epicardially implanted FGF-2 protein in a sustained release (heparin–alginate) beads. Twenty-four patients undergoing CABG in whom one of the major arteries was not viable but ischemic myocardium was considered not bypassable for technical reasons, were randomized to receive ten heparin–alginate beads with a total dose of 10 or 100 μg FGF-2 or a placebo [8]. Nuclear and MRI perfusion scans were performed prior to hospital discharge and then again at 90 days. Two patients in the trial died at the time of CABG (one in the control, and one in the 100-μg FGF-2 group). At the time of the 90-day evaluation, all seven remaining patients in the 100 μg FGF-2 group were symptom-free while three of seven patients in the control group continued to experience angina and two required additional revascularization procedures. Both nuclear and MR perfusion imaging demonstrated a significant reduction in the size of the target zone.

estimated extracellular distribution phase, the concentration of FGF-2 will be in the order of 4 ng/ml, which is still in the range of the effective concentrations used for in vitro studies [108]. With the possibility of occluded or partially occluded epicardial coronary arteries, FGF-2 distribution will be much more heterogeneous and lower in non-perfused area. With direct intramyocardial injection with 10 μg injected per each injection site and a 5% retention, 500 ng will be retained by ~10 cc of tissue yielding an effective local concentration of 50 ng/ml. From these studies, it may be concluded that intramyocardial delivery of growth factors is preferred since it includes the possibility of targeting the desired areas of the heart, and has a higher efficiency of delivery and prolonged tissue retention.

This enthusiasm for the intramyocardial administration is tempered by its invasive nature, a requirement for highly...
in the 100-μg FGF-2 group but not in the 10-μg FGF-2 or control groups. Thus, this small study demonstrated the safety and feasibility of this mode of FGF-2 therapy.

The safety and feasibility of intracoronary and intravenous FGF-2 delivery was tested in an open-label dose-escalation Phase I study of 66 patients with severe coronary disease that were suboptimal candidates for conventional therapeutic approaches [9]. Fifty-two patients received intracoronary infusions of FGF-2 ranging in dose from 0.33 to 48 μg/kg and fourteen patients received intravenous infusions of 24 and 36 μg/kg FGF-2. FGF-2 infusions were well tolerated with systemic hypotension becoming the dose-limiting toxicity at 48 μg/kg. Clinical follow-up over 6 months documented mortality in four patients (two sudden deaths in patients with 22% and 30% EF, one death following a cardiac transplant and one from the non-Hodgkin’s lymphoma diagnosed 8 days after FGF-2 infusion) while no significant laboratory toxicity was observed. Angina frequency score and exertional capacity score were improved in the entire FGF-2 patient population at 2 and 6 months compared to the baseline. Furthermore, the FGF-2 patients as a group demonstrated a 2.4-min improvement in the treadmill exercise time while MRI perfusion imaging demonstrated a significant reduction in the size of ischemic territories although patients with ischemia on the rest nuclear imaging study (thus demonstrated the presence of hibernating myocardium) demonstrated a significant reduction in the size of this defect [Chronos and Simons, 49th ACC meeting presentation, Anaheim, CA, USA, March 2000]. Subgroup analysis of the study suggested that the benefit, defined as improvement in symptoms, exercise time and reduction in the size of nuclear-imaging determined ischemic zone defect, was most prominent in ‘sicker’ patients as defined by lower baseline exercise capacity, higher baseline symptom frequency and larger nuclear perfusion defects. The validity of these concepts, however, will require further testing in a double-blind study format.

The importance of this trial lies in the first delineation of patient subsets likely to positively respond to the growth factor therapy as well as a first demonstration of symptomatic improvement in a double-blind, placebo-controlled format. Another important lesson is the extent and prevalence of the placebo effect in this patient population. In fact, this placebo response clearly makes evaluation of efficacy possible only in the double-blind trial format. Additionally, this trial demonstrated relative safety of intracoronary FGF-2 in a considerable number of high risk patients. In particular, there was no excess mortality or sudden death in FGF-2 treated patients and this mortality (2%) was significantly lower than seen in laser revascularization trials [111,112].

The prevalence of the placebo response and the dangers of open label analysis were amply demonstrated in clinical trials of intracoronary and intravenous VEGF-A. Two small Phase I trials of intracoronary (n=16) and intravenous (n=14) VEGF infusions were interpreted to show a significant improvement in exercise capacity, symptoms (defined as angina class) as well as promising results with SPECT imaging [113,114]. However, a randomized, double-blind, placebo-controlled Phase II trial (VIVA trial) of two different dosages of VEGF was completely negative with regard to exercise time, symptom improvement and nuclear imaging [115]. Several unusual features of this trial make interpretation of therapeutic efficacy of VEGF-A somewhat problematic. In particular, the format for VEGF delivery — an intracoronary infusion followed by three intravenous infusion given over the next 7 days was never tested in either animal models or Phase I trials. Given that VEGF is not effective when delivered intravenously [116] and that repeat administrations of VEGF induce VEGF receptor tachyphylaxis [101], the regimen is clearly suboptimal and may even be counterproductive. Furthermore, the highest VEGF dose tested in the trial, 50 ng/kg/min, was not found to be effective in the porcine ameroid model study [116]. Finally, the VIVA trial enrolled a high proportion of patients with Class II angina that in the light of the FIRST trial data, are unlikely to benefit from angiogenic therapy.

7. Peripheral vascular disease

Although FGF-2 [117] and VEGF [118,119] have shown efficacy in animal models of hindlimb ischemia, most clinical attention has focused on gene therapy [120]. Therefore, clinical information on protein-based growth factor therapy in this field is rather scarce. A small Phase I study of intra-arterial FGF-2 suggested improvement with regard to lower limb perfusion in the treated patients [Lazarous et al., unpublished]. This claim is being tested in a currently ongoing double-blind placebo controlled TRAFFIC study that is evaluating single or double (30 day apart) intra-arterial infusions of FGF-2 in patients with claudication.
8. Future issues

The first and foremost task in cardiovascular therapeutic angiogenesis, whether by protein or gene therapy, is to show the clinical efficacy of a combination of growth factor and delivery strategy in randomized double blind, placebo-controlled trials. Current clinical experience in CAD trials suggest that three issues appear critical to successful evaluation of this mode of therapy: effective delivery, proper selection of patients and the choice and timing of outcome measures [61]. The delivery-related issues have already been addressed in this review. The choice of patients for these trials is critical, and must take into account the initial variability among patients due to disease severity, previous treatments like angioplasty and bypass surgery and ongoing atherogenesis. Apparently, more severely diseased patients respond better to angiogenic treatment than less sick patients. In the FIRST trial patients with baseline SAQ angina frequency score >40 not only showed no response to FGF-2 administration with regard to their symptom frequency, but also did not show a placebo response in the control group that was so prominent among patients with a baseline SAQ angina score <40. Finally, the choice of outcome measures and their timing are major challenges. For regulatory purposes, demonstration of clinical benefit, an improvement in life expectancy, some life-quality related benefit such as symptom status or improvement in exercise capacity is clearly desirable. While detailed review of these issues is outside the scope of this manuscript (please refer to [61] for the further discussion of these issues), the quality of life end-points may currently be the best choice in this field [121,122]. However, for the medical community to accept therapeutic angiogenesis, demonstration of physiological improvement such as improved myocardial perfusion or function will likely be required. This will require a sensitive imaging modality, and while all available choices have their drawbacks, MR imaging appears the most promising at the moment.

The best time for assessment of treatment effect has not been determined. Even after a single administration of growth factor, the process of angiogenesis and arteriogenesis is likely to take several weeks. The VIVA trial suggests improvements in patients’ symptom status continues at least until 6 months after treatment [115]. A 6-month follow-up of the FIRST trial will provide important information in this regard. Assessment of the duration of beneficial effects (if any) will require further trials.

In the meantime, parallel animal and clinical studies will investigate the feasibility and efficacy of various novel approaches such as targeted protein delivery, prolonging protein half-life, combination therapy of different growth factors or combinations of physical stimuli, such as laser revascularization, with growth factor therapy.

9. Summary and conclusions

The development of angiogenic growth factor therapy has potentially added new therapeutic alternatives for patients debilitated by serious cardiovascular diseases. While initial results are clearly exciting, we have yet to prove its clinical efficacy in the absence of serious toxicity and side effects. At the moment, protein-based therapy seems to have advantages over gene therapy, although continuous efforts should be made to increase tissue exposure time after a single administration of protein. Finally, double-blind, placebo-controlled trials designed with existing animal data as a guideline, are needed to firmly establish therapeutic angiogenesis or arteriogenesis in cardiovascular disease.

Acknowledgements

Supported in part by NIH grants HL53793, 56993, 63609 and RR01032. Dr. Simons is an Established Investigator of the American Heart Association.

References


Banai S, Jaklitsch MT, Shou M et al. Angiogenic-induced enhance-
ment of collateral blood flow to ischemic myocardium by vascular endothelial growth factor in dogs. Circulation 1994;89:2183±2189.


