Review

Growth factor-induced therapeutic angiogenesis in the heart: protein therapy

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Received 18 June 2004; received in revised form 2 September 2004; accepted 3 September 2004
Available online 6 October 2004
Time for primary review 22 days

Abstract

Therapeutic angiogenesis, stimulated growth of new vasculature to compensate for tissue ischemia, remains an unfulfilled promise. After nearly a decade of trials, the development of drugs capable of stimulating revascularization of underperfused tissues remains an exciting but unrealized goal in cardiovascular therapeutics. This review will summarize experiences in therapeutic angiogenesis studies employing protein therapies and will draw conclusions based on our current understanding of biological processes of new vessel growth.

Keywords: Angiogenesis; Arteriogenesis; Growth factors; Clinical trial; Protein therapy

1. Introduction: scope of clinical problem

Ischemic heart disease is the leading cause of morbidity and mortality in the Western world and afflicts more than 10 million persons in the United States and hundreds of millions worldwide [1]. It has a broad spectrum of manifestations ranging from patients with only effort-induced angina without myocardial damage, through stages of myocardial ischemia that are associated with reversible and irreversible impairment in left ventricular function, to states of irreversible myocardial injury, and necrosis resulting in congestive heart failure (CHF). The rate of progression is predominantly determined by the growth and/or episodic rupture of atherosclerotic plaque that may lead to chronic stable angina or the development of acute coronary syndromes including myocardial infarctions. Currently, there are a large number of invasive and noninvasive treatment options for patients with coronary artery disease. Mechanical revascularization techniques such as coronary bypass surgery and angioplasty physically restore flow to the compromised myocardium, while medical therapies such as nitrates and β-blockers restore the perfusion supply/demand balance by reducing myocardial oxygen requirements. Finally, cholesterol lowering drugs influence further progression, although at present not regression, of coronary atherosclerosis.

Notwithstanding these options, there is a large and apparently increasing number of individuals who remain symptomatic despite maximum treatment at the present time. A subset of this population is the population of so-called no-option “end-stage” coronary artery disease patients who cannot be effectively revascularized by currently available mechanical techniques [2,3]. These patients typically have diffused coronary disease, small distal vessels, or other comorbidities making them poor candidates for traditional methods of treatment. The number of patients...
in this group can be expected to increase with an aging population and the increasing incidence of concomitant diseases such as obesity and diabetes mellitus.

Importantly, coronary atherosclerosis is also the leading cause of congestive heart failure (CHF), a disease process which affects approximately 1% of adults in the United States with 550,000 new cases per year diagnosed annually and an incidence of 10 cases per 1000 population after the age of 65. In many cases, these patients have a relatively large amount of viable myocardium, and only a moderately age of 65. In many cases, these patients have a relatively large amount of viable myocardium, and only a moderately impaired left ventricular function but are not candidates for complete revascularization [2]. CHF admissions rose from 377,000 in 1979 to 999,000 in 2000 (165% increase), and the number of affected individuals is expected to increase with the continued aging of the general population [1]. Consequently, novel therapies are needed to improve the outlook for this patient population as well.

In as much as the fundamental problem that underlies advanced ischemic heart disease is the inability of coronary perfusion to match the myocardial oxygen demand, a logical strategy would be to improve the coronary perfusion. To date, that can only be accomplished with mechanical interventions such as coronary bypass surgery or coronary angioplasty. Therapeutic angiogenesis seeks to improve perfusion to the compromised myocardium by stimulating new blood vessel growth. We will review approaches designed to promote myocardial perfusion using therapeutic angiogenesis strategies while emphasizing approaches that use protein-based therapies.

2. The process of neovascularization in the adult myocardium

Neovascularization in the chronically ischemic adult heart is a combination of several processes including angiogenesis, arteriogenesis, and potentially, vasculogenesis. Angiogenesis refers to the sprouting of new capillaries from the postcapillary venules [4] and, in the adult, is mainly stimulated by tissue hypoxia via activation of hypoxia-inducible factor (HIF-1α) expression, which serves to increase transcription of vascular endothelial growth factor (VEGF) and VEGF receptors flt-1 and neuropilin-1 among other target genes [5]. It results in a large increase in the capillary bed size but is relatively ineffective in increasing overall blood flow to the tissue in the presence of flow-limiting lesion in the proximal arterial conduit. In contrast, arteriogenesis refers to the process of maturation or de novo growth of collateral conduits and produces vessels capable of carrying significant blood flow [6,7]. These vessels are of a sufficient diameter to be visualized with angiography [8]. The primary arteriogenic stimuli are thought to be the shear stress and accumulation of blood-derived mononuclear cells at the sites of arterial narrowing, resulting in release and production of a number of angiogenic growth factors including fibroblast growth factors (FGF), platelet derived growth factors (PDGF), and VEGF [6,9]. To further complicate the picture, it has been suggested that neovascularization in adults is not only restricted to angiogenesis and arteriogenesis but may also involve vasculogenesis. Vasculogenesis is the process of an in situ formation of blood vessels from systemically derived endothelial and vascular progenitor cells (EPCs and VPCs) [10,11]. The functional significance of vasculogenesis in the coronary or peripheral circulation in the setting of ischemic disease has not been established, and the very occurrence of this process has been challenged [12]. Therefore, angiogenesis, arteriogenesis, and potentially, vasculogenesis contribute to neovascularization in the adult heart, with arteriogenesis being the most critical process necessary for significant improvements in myocardial blood flow.

3. Growth factors mediating neovascularization in the adult myocardium

Molecular mechanisms that regulate neovascularization are tightly regulated by numerous pro- and antiangiogenic soluble polypeptides such as VEGF, the angiopoietins, FGF, PDGF, transforming growth factor-β (TGF-β), tumor necrosis factor-α (TNF-α), the colony stimulating factor such as G-CSF and GM-CSF, CXC chemokines as well as many others that can regulate the process in both a positive and negative direction [13]. In addition, a number of membrane-bound proteins play critical in neovascularization, including members of the integrin, cadherin, syndecan, and ephrin families. Finally, mechanical forces can also transmit pro- and antiangiogenic signals [14,15].

Despite the potential for the myocardium to stimulate neovascularization in response to progressive decline in the arterial blood flow, in the overwhelming majority of cases, this response is fairly limited and does not fully compensate for the loss of the arterial blood supply. In situations where the native neovascularization response is sufficient to avoid myocardial necrosis but insufficient to even permit myocardial contractility even at rest, myocardial hibernation may ensue, whereby regional cardiac contractility is downregulated to restore the balance between myocardial oxygen supply and demand [16]. Hibernating myocardium is by definition viable, but over time may result in myocyte apoptosis, ventricular remodeling, and structural alterations including the loss of contractile material within cardiomyocytes as well as increases in the amount of interstitial connective tissue occurrence, eventually resulting in irreversible myocardial fibrosis.

Studies in genetically engineered mice lacking VEGF164 and VEGF188 isoforms demonstrated progressive cardiac failure over time due to insufficient angiogenic growth factor availability [17]. Similarly, cardiac myocyte-specific HIF-1α deletion results in reductions in contractility, vascularization, high-energy phosphate content [18]. The availability of these models is vital to further studies of a
The VEGFs are perhaps the most extensively studied family of angiogenic growth factors, and VEGF-A is the most well known form of VEGF. At least four isoforms of VEGF-A are produced as a result of alternative splicing containing 121 (VEGF121), 165 (VEGF165), 189 (VEGF189), and 206 (VEGF206) amino acids [19]. Other isoforms such as VEGF145 have been reported as well, but their significance remains unknown [20]. The VEGF isoforms, which differ in their heparin binding capacity, appear to have different angiogenic potency, with VEGF165 being which differ in their heparin binding capacity, appear to contain 121 (VEGF121), 165 (VEGF165), 189 (VEGF189), and 206 (VEGF206) amino acids [19]. Other isoforms such as VEGF145 have been reported as well, but their significance remains unknown [20]. The VEGF isoforms, which differ in their heparin binding capacity, appear to have different angiogenic potency, with VEGF165 being significantly more potent than VEGF121. VEGF121 and VEGF165 are secreted into the extracellular environment, whereas VEGF189 and VEGF206 remain cell- or matrix-associated because of their high affinity for heparan sulfates. The other members of the VEGF family are VEGF-B (VEGF-3), VEGF-C (VEGF-2), VEGF-D, VEGF-E, and placental growth factor (PIGF). VEGF-E is a viral protein that does not have a mammalian homologue. All forms of VEGF bind with differing affinities to one, two, or all three VEGF receptor tyrosine kinases: flt-1 (VEGFR-1), KDR/flk-1 (VEGFR-2), and flt-4 (VEGFR-3). VEGFR-2 is thought to the most active of the VEGF receptors that transduce angiogenic signals, although VEGF-R1 is also involved as demonstrated by PIGF and VEGF-D activity. In fact, VEGF-D was the strongest of the VEGF family members in inducing angiogenesis and lymphogenesis in the skeletal muscle [21].

VEGF exerts an array of effects on endothelial cells that include enhanced migration, increased permeability, enhanced survival and the production of plasminogen activators, and interstitial collagenase, all of which contribute to angiogenesis [22,23]. VEGF does not induce proliferation of other cells types such as smooth muscle cells or fibroblasts, although it increases smooth muscle cell migration [24]. The precise role of VEGFR-3 is less defined, but it appears to be involved in lymphangiogenesis [25].

The fibroblast growth factors include not only acidic FGF (FGF1) and basic FGF (FGF2) but also 21 other structurally related polypeptide growth factors [26,27]. The biologic functions of FGF are mediated primarily by specific cell surface receptors of the tyrosine kinase family and a nontyrosine kinase receptor syndecan-4 [28,29]. Like VEGF, FGF stimulate endothelial cell proliferation, migration, and production of plasminogen activator and collagenase [30]. Unlike VEGF, FGF stimulate proliferation of most cells derived from embryonic mesoderm and neuroectoderm, including pericytes, fibroblasts, myoblasts, chondrocytes, and osteoblasts [30].

Among other polypeptides with vessel growth promoting activities, PDGF-BB has been implicated in stimulating pericyte covering of the newly formed vascular structures thereby promoting arteriogenesis [31], while G-CSF, GM-CSF, and MCP-1 promote mononuclear cell influx thereby also stimulating arteriogenesis.

One poorly explored area is the effect of the state endothelium on the ability of growth factors to stimulate neovascularization. Most animal studies demonstrating physiologic effectiveness of angiogenic therapies have been performed in normal young animals, while clinical trials typically enroll older patients with advanced atherosclerosis. The decreased effectiveness of growth factor therapy due to age and concomitant disease could be a principle reason for failure of these trials [32]. This is substantiated by a poor track record of angiogenic growth factor effectiveness in ApoE<sup>−/−</sup> mice [33].

4. Angiogenic growth factor formulations

While the ultimate result of any delivery strategy is to change the levels of angiogenic growth factors at the critical site of action, different methods can be used to achieve this goal. Perhaps the most straightforward method uses recombinant proteins (human or mammalian) delivered locally or systemically. A known quantity of protein can be administered using intravenous, intra-arterial (specifically intracoronary), intramyocardial, or intrapericardial approaches. Regardless of the mode of delivery, a precise dose-response relationship in theory can be attained. Protein therapy does not require the transfection of cells, transcription of virus or plasmid, and the translation of DNA genes into mRNA. The most obvious limitation of protein therapy is the necessity to maintain therapeutic concentration sufficient to induce the desired angiogenic response for the necessary length of time. What that necessary time is remains a matter of conjecture. For proteins that act by directly stimulating new vessel growth, that time could potentially measure in weeks, as the continuous presence of the growth factors appears to be necessary not only to initiate the new vasculature growth but also to stabilize and “mature” the newly formed vessels [34]. On the other hand, for proteins that act by stimulation of release of circulating mononuclear cells, such as GM-CSF, G-CSF, PIGF, or by promoting their attraction to the site of new vessel growth such as MCP-1, this time may be significantly less. In this regard, it is interesting to note that single dose intravascular growth factor therapy is effective in young healthy pigs and rodents but not in patients [35,36].

The major alternative to protein therapy to date has been gene therapy. Gene therapies at present include two major forms that use either plasmid or viral-based gene transfer. Under optimal conditions, gene therapy can lead to sustained local growth factor production that should result in prolonged elevation of tissue protein levels [37]. However, currently available plasmid-based and adenoviral-based gene therapy platforms have a limited (1–2 weeks)
duration of expression that may not be adequate as discussed above [37]. Other disadvantages of the gene therapy techniques include the inflammatory response to adenoviral proteins and inconsistent level of gene expression in different patients with a given dose of vector [37].

Beyond the issue of protein compared to gene delivery, consideration must be given for the route of administration. Proteins can usually be considered to be deliverable by any route, although the ability to define the kinetics of delivery of any angiogenic protein in the human myocardium is obviously somewhat limited. In the case of gene transfer approaches, local delivery techniques will likely be required to achieve effective local concentrations [38]. This is in part related to impermeability of the endothelial barrier to adenoviruses [39] and to very low levels of transfection following intravascular plasmid injections as well as prompt degradation of naked DNA in the circulation [40].

5. Clinical studies using angiogenic growth factor protein

Although the majority of studies of therapeutic angiogenesis in the myocardium has been gene therapy studies, a number of trials have employed protein therapy (Table 1). Safety of FGF1 (10 μg/kg) was first demonstrated in 20 patients with three-vessel disease undergoing CABG, in whom the growth factor was injected intramyocardially close to the internal mammary artery-LAD anastomosis [41]. 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. In another “CABG-plus” trial, 24 patients undergoing CABG, in whom one of the major arteries supplying viable but ischemic myocardium was considered not bypassable for technical reasons, were randomized to receive 10 heparin-alginate beads with a total dose of 10 or 100 μg FGF2 or a placebo [42]. Three months later, the size of the ischemic defect was significantly reduced in patients receiving 100 μg FGF2 dose compared to placebo. All patients in this group were symptom-free, while three of seven patients in the control group continued to experience angina, and two required additional revascularization procedures. Three years later, the symptomatic improvement still persisted in the high-dose FGF2 group [43].

The safety and feasibility of intracoronary FGF2 infusion were tested in two open-label dose-escalation trials [44,45]. In both cases, the infusions were safe up to a high dose of FGF2 without evidence of systemic hypotension, and a number of patients exhibited symptomatic improvement as well as objective evidence of improved myocardial perfusion by magnetic resonance and nuclear imaging [46].

These suggestions of therapeutic efficacy were tested in a 337-patient double-blind phase II trial that examined three different intracoronary dosages of FGF2 (0.3, 3, and 30 μg/kg) versus placebo controls [47]. Ninety-day follow-up data demonstrated a nonsignificant improvement in the treadmill time in FGF2-treated patients. At the same time, there was a significant improvement in the Canadian Cardiovascular Society (CCS) angina scale and Seattle Angina Questionnaire (SAQ) angina frequency scale. Nuclear imaging demonstrated no overall improvement in the size of ischemic territories. Subgroups 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 that demonstrates 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 FGF2 in a considerable number of high-risk patients.

The prevalence of the placebo response and the dangers of open-label analysis were also amply demonstrated in clinical trials of intracoronary and intravenous VEGF-A165. 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 [48,49]. These trials were followed by the VIVA (Vascular endothelial growth factor in Ischemia for Vascular Angiogenesis) trial [50] that randomized 178 “no-option” patients to placebo, low-dose VEGF165, or high-dose VEGF165 protein with follow-up out to 120 days. Patients in the VEGF group received an intracoronary infusion on day 0, followed by intravenous infusions on days 3, 6, and 9. Overall, the trial showed no efficacy for VEGF in this population. There was no significant improvement in exercise treadmill time (ETT), the primary end point of the study from baseline to 60 days in VEGF-treated patients compared to placebo. By 120 days, there was a trend towards improved ETT in the high-dose VEGF group versus placebo. Similarly, at 60-days posttreatment, there was no difference in angina class improvement from baseline in VEGF versus control patients, whereas at 120 days, a statistically significant improvement in angina class for high-dose VEGF-treated patients compared with placebo was present. Myocardial perfusion studies performed at day 60 demonstrated no significant improvement in VEGF-treated patients versus placebo-treated patients.
A somewhat different strategy was pursued in a small trial that examined the effectiveness of a single intra-coronary injection of GM-CSF followed by a 2-week period of subcutaneous administration in patients with advanced CAD [51]. The authors demonstrated a small but significant increase in collateral flow in GM-CSF but not saline-treated patients. This beneficial effect of GM-CSF and a related protein G-CSF, likely mediated by increased recruitment of mononuclear cells to sites of arterial narrowing in the heart as well as a potential release of bone marrow stem cells, is in keeping with our current understanding of arteriogenesis [52]. Recent trials, however, raised concerns about the safety of these kinds of cytokines in patients with CAD [53,54].

The safety of growth factors in protein formulations has not been a serious problem to date. Although a number of theoretical dangers exist, including stimulation of occult tumor growth, promotion of atherosclerosis, and generation of unstable plaque via stimulation of coronary plaque angiogenesis, none of these have so far been observed in trials. The only recognized side effects to date include profound hypotension and edema with VEGF [50] and CNS side effects (vivid dreams, nightmares, and restlessness) with very high dose (over 48 \( \mu \)g/kg) of FGF2 [44].

### 6. Current trial status and future directions

Results of clinical trials of proangiogenic therapies using protein or other formulations have generally been disappointing, and the trials have failed to consistently demonstrate improvements in treated patients over placebo. One potential explanation is that the end points are wrong, or the methods used to assess the end points are inadequate or invalid. Nuclear imaging techniques (i.e., thallium, sestamibi) used in these clinical trials have low spatial resolution and may have a limited ability to detect small changes in myocardial perfusion in patients with end-stage CAD [55]. In addition, there appears to be a large variability in the measured percent myocardial ischemia (up to 50% variability) over time, which may limit the utility of scintigraphic techniques to detect improved myocardial perfusion in response to therapy [56]. Magnetic Resonance Imaging (MRI) with its high spatial resolution and potential for quantification of myocardial perfusion [57] may be better suited to detection of perfusion changes within regions of the heart treated with proangiogenic therapies [55,58]. Thus, in future studies evaluating the utility of the various novel therapies outlined in this chapter, consideration should be given to the use of more sensitive techniques such as MRI when assessing myocardial blood flow pre- and post-treatment.

Given the complex and highly ordered physiological regulation involved in the processes of angiogenesis, it is difficult to envision that any single growth factor, stem cell, or mechanical therapy will be the "magic bullet" to cure end-stage coronary disease. Rather, combination therapy with multiple growth factors may be necessary to achieve consistent clinical benefits in order to advance therapies for patients with CAD.

### References

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