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Combating cardiovascular disease with angiogenic therapy

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For over a decade we have lived with the promise that therapeutic angiogenesis, defined as the growth of new blood vessels in tissues damaged by poor blood perfusion, would provide a lasting clinical benefit to patients suffering from cardiovascular disease, the leading cause of death in the Western world. Numerous successful protein, gene and cell-based angiogenesis studies in animals with experimentally induced ischemia have not been followed by positive efficacy data in human trials. Armed with knowledge of the shortcomings of earlier clinical studies, emerging results from more recent trials indicate that protein-based angiogenesis therapy may provide a viable treatment option for patients suffering from advanced atherosclerotic disease.

Angiogenesis represents an excellent therapeutic target for the treatment of cardiovascular disease. It is a potent, physiological process that underlies the natural manner in which our bodies respond to a diminution of blood supply to vital organs, namely the production of new collateral vessels to overcome the ischemic insult [1–5]. A large number of pre-clinical studies have been performed with protein, gene and cell-based therapies in animal models of cardiac ischemia as well as models of peripheral artery disease (reviewed in refs. [2,4,5]). Reproducible and credible successes in these early animal studies led to high enthusiasm that this new therapeutic approach could be rapidly translated to a clinical benefit for millions of patients in the Western world suffering from these disorders. However, a decade of clinical testing of both gene and protein-based therapies designed to stimulate angiogenesis in underperfused tissues and organs, has led from one disappointment to another. What are the likely reasons for this unsuccessful transition from pre-clinical to clinical studies?

Pre-clinical animal studies in rodents, canines and pigs [2–4] have, in general, offered great hope that angiogenic growth factors and their genes could lead to success in humans. Efficacy in animal studies was typically documented by an increase density of new blood vessels, either histologically following sacrifice, or angiographically in the live animal. Improvements in heart function could often be demonstrated by telemetry readouts from implanted electrical devices in the animals' hearts. Finally, as more sophisticated laboratory instrumentation became available, increases in heart perfusion could be measured by CT and MRI imaging, and improvement in ventricular wall motion and electrical signaling in the cardiac tissue could be precisely measured by electromagnetic mapping instrumentation. Although all of these pre-clinical readouts, which offered great promise for the transition of angiogenesis therapy from animals to humans, were in one fashion or another, incorporated into early stage clinical trials, the FDA has, to date, insisted that the primary endpoint for approval of an angiogenic agent must be an improvement in exercise performance of treated patients.

If one reviews in detail the various published angiogenesis clinical trials, which are summarized in Table 1, it can be realized that most of these trials had success in achieving various secondary or supportive endpoints, but failed when attempting to demonstrate a statistically significant improvement in exercise performance, typically done by a treadmill exercise test. Perhaps the greatest reason for these trials' failure to achieve success is the high occurrence of the 'placebo effect' in studies employing a treadmill exercise test readout [2]. Thus, even though a majority of the treated patients in these trials experience relief of such clinical symptoms such as chest pain, and generally performed better on most efficacy readouts, there were enough 'responders' in the blinded placebo groups to render the trial inconclusive (see, for example ref. [6], the *Euroinject One Trial*, which utilized the VEGF

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TABLE 1

Myocardial angiogenesis clinical studies

Therapeutic agent	Delivery method	Comments	Refs
Gene therapy			
$VEGF_{165}$ (naked plasmid DNA)	Myostar® Injection Catheter	Phase II, placebo controlled, blinded study did not show efficacy over control group	[6]
$VEGF_{165}$ (naked plasmid DNA)	Intramyocardial injection via thoracotomy	Phase I; all five patients had significant reduction in angina and reduced ischemia	[19]
$VEGF_{121}$ (adenoviral vector)	Intramyocardial injection	Phase I; subjective improvement in angina in all 21 patients; most patients had improvement on exercise testing	[20]
$VEGF_{165}$ (naked plasmid DNA)	During surgery by myocardial injection	Patients with chronic stable angina; significant improvement in area of ischemic myocardium and in perfusion scores	[21]
$VEGF_{165}$ (plasmid DNA)	Intramyocardial transfection	Demonstrated increase in plasma VEGF level and subsequent return to baseline; all patients reported reduced angina and nitroglycerin use	[22]
VEGF-2 (naked plasmid DNA)	Direct myocardial injection via thoracotomy	Showed procedure is feasible and well tolerated	[23]
FGF4 (adenoviral vector)	Intracoronary administration	AGENT trial; greater improvement in exercise time among treated vs placebo group	[24]
Cell therapy			
Peripheral blood stem cells	Compare intracoronary infusion with mobilization alone by G-CSF	Greater improvement in cardiac function and remodeling with intracoronary infusion	[25]
PBSCs	Intracoronary infusion	Patients with acute MI; significant improvement in left ventricular function in treatment group	[26]
Recombinant proteins			
VEGF	Intracoronary	Phase I; some improvement in perfusion with low dose; five of six patients had perfusion improvement on rest and stress at higher doses	[27]
VEGF	Intracoronary/intravenous	VIVA trial; no significant different between placebo and low-dose group; by day 20, high-dose group had significant improvement in angina class and nonsignificant trends in exercise test time and angina frequency compared to placebo	[28]
VEGF	Intracoronary	Dose escalation trial; well tolerated up to 0.05 mg/kg/min; 7 of 14 patients had improvement in myocardial perfusion	[29]
FGF-2	Intracoronary or intravenous	Ascending dose trial; no control group; evidence of improved resting perfusion and attenuation of stress-induced ischemia	[30]
FGF-2	Intracoronary	Improvements in exercise tolerance and quality of life; MRI showed reduction in size of ischemic area	[31]
FGF-2	Intracoronary	Generally well tolerated; no signs of systemic angiogenesis	[32]

gene, or ref. [7], the *FIRST* clinical trial utilizing recombinant FGF-2 protein). In addition to the placebo effect, more recent animal studies have also highlighted various factors that may inhibit an angiogenesis response including certain drugs, smoking and hypercholesterolemia [3,8].

Although shown to be relatively safe therapies, not one angiogenic therapeutic has yet made it through the gauntlet of clinical testing required for drug approval. By capitalizing on the large database of what did and did not work in previous clinical trials, results from more recent studies with redesigned clinical protocols [8–11] give renewed hope that angiogenesis therapy will be a treatment choice for sufferers of cardiovascular disease resulting from occluded vessels.

Physiology of angiogenesis

In established blood vessels in mature organisms, the endothelial cells remain in a quiescent, non-proliferate state until stimulation of angiogenesis occurs by stimuli including wounding, inflammation, hypoxia or ischemia. As shown in Figure 1, the formation of new vessels can be considered as the result of several processes: (i) dissolution of the matrix underlying the endothelial cell layer; (ii) migration, adhesion and proliferation of endothelial cells; (iii) formation of a new three-dimensional tube, which then lengthens from its tip as circulation is re-established; and (iv), in larger vessels, vascular smooth muscle cells also migrate and adhere to the newly deposited matrix of the nascent vessel. Angiogenic growth factors induce, promote and/or interfere with all these steps of angiogenesis [12–14].

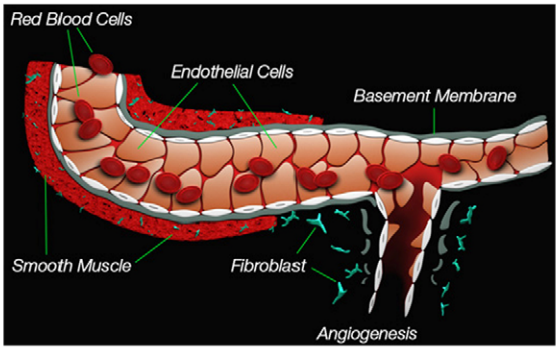

FIGURE 1

Schematic representation of angiogenesis and new vessel formation. Cell types are indicated and the initial stages of angiogenesis involve the migration of fibroblasts and endothelial cells to form a nascent blood vessel. This structure is subsequently remodeled with the addition of smooth muscle cells.

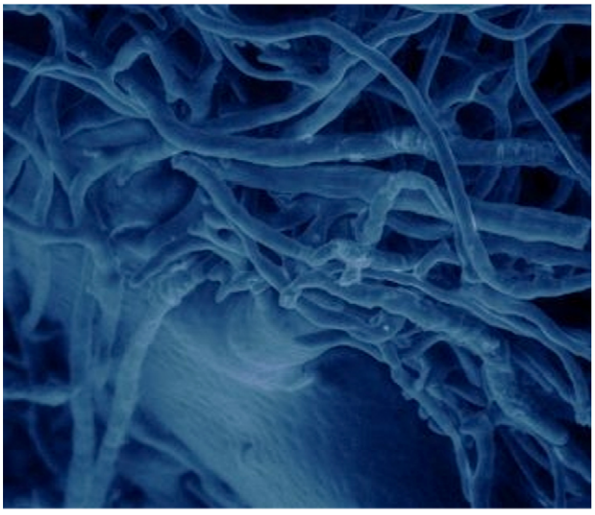

FIGURE 2

New blood vessel formation seen in an angiogenic response to growth factor administraion. Visualization of newly formed blood vessels during an angiogenic response. This response can be elicited with exogenous growth factor administration or by local production of growth factors by malignant tissues.

A variety of potent growth factors exist in the body that are capable of stimulating cellular proliferation, maturation, and differentiation of cells that comprise mature blood vessels. These factors typically act as signaling molecules between cells, and bind to specific receptors on the surface of their target cells. The best known growth factors with proven angiogenic potency are the family of fibroblast growth factors (FGFs) and vascular endothelial growth factors (VEGFs). These proteins [15,16] can be readily assayed for angiogenic properties in established bioassays. A typical angiogenesis response to growth factor stimulation is shown in Figure 2 where a dramatic stimulation of new blood vessel growth can be visualized.

Research in the field of angiogenesis began in earnest approximately 30 years ago, and was predominantly directed to the inhibition of angiogenesis to limit tumor growth. In the 1980s, the isolation, characterization and purification of the first angiogenic growth factors were reported [17]; subsequently, inhibitors of angiogenesis were developed. In 2004, the FDA approved the first angiogenesis inhibitor Bevacizumab (rhuMAb-VEGF, Avastin®) for use in metastatic colorectal cancer in combination with established chemotherapy. In contrast to the active research and development of commercially viable anti-angiogenic therapeutics, the commercial development of pro-angiogenic proteins as a therapeutic option in cardiovascular disease settings has lagged far behind, even in light of the importance, frequency and socioeconomic impact of cardiovascular disease in the Western world. Cardiovascular disease is the leading cause of death in the United States, with an estimated 71 million American affected [18]. The clinical spectrum of cardiovascular disease is broad, but occluded blood vessels because of the atherosclerotic process contribute to the disease pathology in most of the main types of cardiovascular disease including coronary heart disease, heart failure, stroke, and peripheral arterial disease. The estimated direct and indirect cost in 2006 of caring for Americans with cardiovascular disease is a staggering $400 billion [18]. Clearly, therapies that could stimulate the replenishing of an adequate blood supply to ischemic and damaged tissues would have enormous medical and economic benefits.

Angiogenesis and gene therapy

The advent of gene therapy, which received considerable scientific and medical attention, quickly found its way into gene-based angiogenesis trials in humans. Numerous Phase I trials with either adenovirus vectors carrying an angiogenesis gene, or 'naked' plasmid DNA vectors harbouring an angiogenic gene, demonstrated the safety of these new gene-based products (see Table 1 for a listing of angiogenesis gene therapy trials and refs. [6,19–24]). However, as these trials progressed to more tightly controlled, blinded Phase II clinical trials, efficacy with this approach could not be demonstrated. The most recent example of such a casualty is the GENASIS trial (Genetic Angiogenic Stimulation Investigational Study) in which 400 patients were to be enrolled in a Phase II trial in which the naked VEGF-2 gene was to be injected into the heart of patients with severe coronary artery disease. The trial was halted after 265 patients were enrolled because of the report of several severe adverse events involving edema in the heart area. Subsequent analysis of the efficacy data collected in this trial led to the recommendation of an independent monitoring committee not to continue the trial, as they stated that there was little chance of achieving the primary endpoint of the trial, which was increased exercise tolerance. One very important lesson learned from this trial was that the more severely affected patients responded better to angiogenesis therapy, and statistical significance could be observed when a subset of only those patients with severe angina symptoms were included in the data analysis. It should be noted that the company reported significant improvements in exercise tolerance and in reduction of anginal symptoms and the number of nitroglycerin tablets taken following completion of a Phase I study, which led them to conduct the subsequent Phase II study.

The FDA has not yet approved any human gene therapy product for sale. A total of 401 FDA- authorized clinical trials exploring gene therapy are currently underway, most of them addressing advanced tumor stages. Of the 23 gene therapy trials addressing cardiovascular disease, less than five are in advanced testing stages beyond Phase I safety studies (see U.S. National Institutes of Health: http://clinicaltrials.gov).

Angiogenesis and cell-based therapy

Cell-based angiogenesis therapy, although highly promising, is still many years away from large-scale clinical trials. It should be stressed that the majority of cell based clinical trials now ongoing are primarily directed at patients with heart failure and are aimed at regenerating cardiac muscle tissue. In a formal sense, these cannot be considered angiogenesis trials as new cardiac muscle cells, versus coronary vascular cells, are being formed. There have been some notable successes in early stage clinical studies and two such trials are listed in Table 1 and described in more detail in refs. [25] and [26]. In addition to these two studies, Bioheart Inc. is conducting a Phase I study in 15 no-option patients using cells isolated from patients' muscle tissue and injected into the heart via catheter. An expanded Phase IIa trial of the company's MyoCell

therapy is underway in Europe. Bioheart's MyoCath-SR-200 cell therapy, using local endocardial delivery and direct injection of myoblasts to deliver its MyoCell product is in two Phase I/II safety studies in Europe.

Although these smaller clinical trials are not sufficiently powered to show conclusive efficacy results, promising trends are appearing in which the precursor muscle cells appear able to differentiate and integrate into the heart wall giving rise to functional cardiac muscle cells.

In cell-based therapy which targets angiogenesis and the formation of new blood vessels, the approach that has proven successful in animals is to transplant progenitor or precursor endothelial cells, a primary blood vessel cell constituent, into the damaged heart. This leads to the proliferation and remodeling of the endothelial cells into functioning blood vessels, a process referred to as neovascularization. Several early stage clinical trials are planned with this approach including a trial by TheraVitae Ltd., that has not yet begun recruiting patients for its planned Phase I trial of Vescell™ – intracoronary injection of autologous angiogenic cell precursors – in patients with severe angina. In addition, the Texas Heart Institute is recruiting patients for a trial involving intramyocardial injection of autologous aldehyde dehydrogenase-bright stem cells for therapeutic angiogenesis.

Overall, the use of cell-based therapies will probably be largely directed at cardiac muscle regeneration in patients who have suffered myocardial infarctions, or in patients with severe congestive heart failure. One could argue that an increased blood vessel supply will be required to nourish this newly regenerated cardiac muscle, and it is unclear at present whether neo-angiogenesis will occur *de novo* in patients receiving muscle progenitor cells, or whether angiogenesis therapy will also be required to get fully functional myocardial tissue. Such a clinical trial, introducing at the same time both progenitor muscle cells and potent angiogenic growth factors is probably not acceptable because of safety concerns of potential inappropriate cellular proliferation. Nevertheless, the potential of tissue regeneration by stem cell therapy will only be answered by expanded, well-controlled clinical studies in which both the efficacy and safety of cell-based treatments can be carefully and quantitatively examined.

Protein therapy to stimulate angiogenesis

Early clinical studies with protein-based therapeutics [1–5,12–14] largely focused on the intravenous administration of a particular growth factor to stimulate angiogenesis in the affected tissue or organ. Table 1 lists a number of these earlier trials with recombinant proteins [27–32], and it can readily be seen that most of these trials relied on an intracoronary delivery of the angiogenic protein. Most of these trials did not achieve statistically significant improvements in their clinical endpoints. This ultimately led to an abandonment of this approach and a widespread belief in the field that protein therapy, especially with a single agent, was not a viable option to treat ischemic cardiovascular disease.

However, the failure of gene or cell-based therapy to deliver, as of yet, a suitable treatment choice for diseases resulting from poor blood flow, has led to a resurgence of interest in returning to protein-based therapy to stimulate angiogenesis. Lessons learned from earlier protein-based studies, which indicated that an intravenous or intracoronary delivery of the protein was not efficacious, have led to completed and ongoing clinical studies in which the angiogenic protein is injected directly into the beating ischemic heart. As will be discussed in more detail below, such localized administration of the potent angiogenic growth factor, human FGF-1, has recently given promising results in a clinical trial in no-option heart patients [8–11].

Two family of growth factors, the VEGF and FGF families of proteins, have been the most extensively studied angiogenic agents and have been involved in the greatest number of clinical trials.

VEGF growth factors and angiogenesis

The VEGF family of proteins has been shown to play a critical role in angiogenesis [16,33,34]. The VEGF superfamily is composed of seven members, but VEGF-A is believed to be the most important contributor to the angiogenesis process [33]. At least nine subtypes of VEGF-A have been described in the literature and the subtype composed of 165 amino acids has been most extensively utilized in ongoing angiogenesis trials [6,21,33]. VEGF is a potent endothelial cell mitogen and transmits its physiological signaling through the tyrosine kinase receptors, FLK-1 and FT1 [34]. VEGF-A, in addition to its stimulation of new capillary growth, is also known to increase the vascular permeability of blood vessels.

VEGF proteins are primarily endothelial cell mitogens involved in the proliferation of small capillaries. Although these growth factors produce a robust angiogenic response in ischemic tissues, they do not appear capable on their own to further mature the capillaries into larger arterioles or arteries. Because of this fact, there has been concern that the amount of increased blood perfusion seen in ischemic tissue after VEGF administration is not sufficient enough to hit the efficacy readouts in angiogenesis clinical trials, including statistically significant increase in SPECT perfusion and exercise performance on treadmill exercise testing. In addition, there is some concern that the durability of the VEGF-induced capillaries is not high enough to sustain a lasting clinical benefit, and would require the patient to be subjected to repeated therapeutic interventions. Finally, all growth factors have a different adverse event and safety profile when tested in animals and humans, and one potential adverse effect characteristic of VEGFs, as mentioned above, is their known ability to increase the permeability of the vessel wall. In the discontinued GENASIS trial with VEGF, the severe adverse event that was reported in this trial was edema or swelling around the heart. There was speculation that such an event could be because of the known property of VEGF to increase vascular permeability.

Fibroblast growth factors and angiogenesis

The FGF family with its prototype members FGF-1 and FGF-2 (basic FGF) consists to date of at least 22 known members [15,36]. Most are 16–18 kDa single chain peptides and display high affinity to heparin and heparan sulfate. In general, FGFs stimulate a variety of cellular functions by binding to cell surface FGF-receptors in the presence of heparin proteoglycans. Figure 3 depicts the three dimensional structure of FGF-1 and one of the FGF receptors to which this growth factor binds [37,38]. The FGF receptor family is comprised of seven members and all the receptor proteins are single chain receptor tyrosine kinases that become activated through autophosphorylation induced by a mechanism of FGF

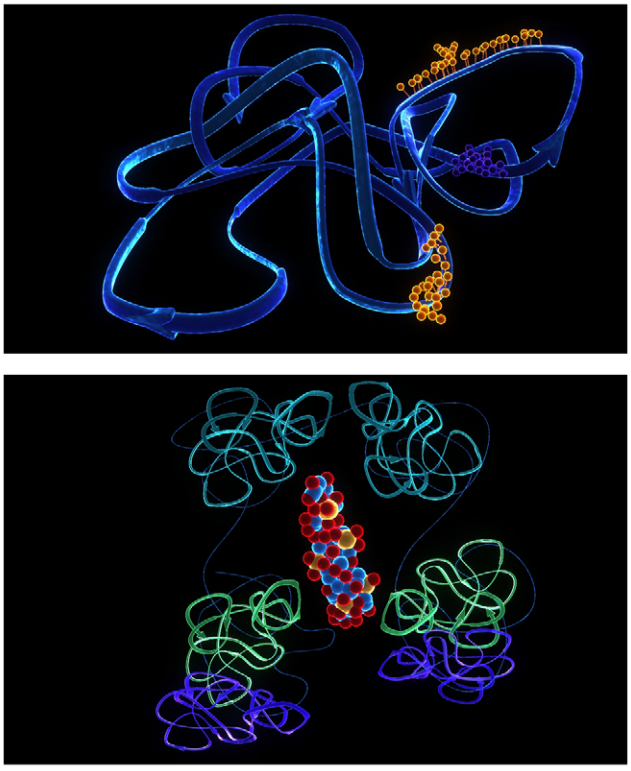

FIGURE 3

Three-dimensional structures of fibroblast growth factor 1 (FGF-1) and FGF-1 receptor (FGFR). The receptor binding sites are indicated in yellow, the Heparin binding sites in blue [38,39].

mediated receptor dimerization [15]. Receptor activation gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation, and matrix dissolution—thus initiating a process of mitogenic activity critical for the growth of endothelial cells, fibroblasts, and smooth muscle cells.

FGF-1, unique among all 22 members of the FGF family [15], can bind to all seven FGF receptor subtypes, making it the broadest acting member of the FGF family, and a potent mitogen for the diverse cell types needed to mount an angiogenic response in damaged tissues, where upregulation of FGF receptors occurs. FGF-1 stimulates the proliferation and differentiation of all cell types necessary for building an arterial vessel, including endothelial cells and smooth muscle cells, and this fact distinguishes FGF-1 from other pro-angiogenesis growth factors, such as VEGF which primarily drives the formation of new capillaries [15].

Three human trials [8–11,21] have been completed with FGF-1 in which the angiogenic protein was injected directly into the damaged heart muscle. Angiogenesis was documented by angiographically visible 'blushing', and functional exercise tests were also performed on a subset of patients (see Figures 4 and 5). The attractiveness of protein therapy is that relatively large amounts of the therapeutic agent can be injected into the ischemic area of interest, to pharmacologically 'jump start' the process of blood vessel growth and collateral artery formation. In addition, from pharmacokinetic data collected from the recent FGF-1 studies in

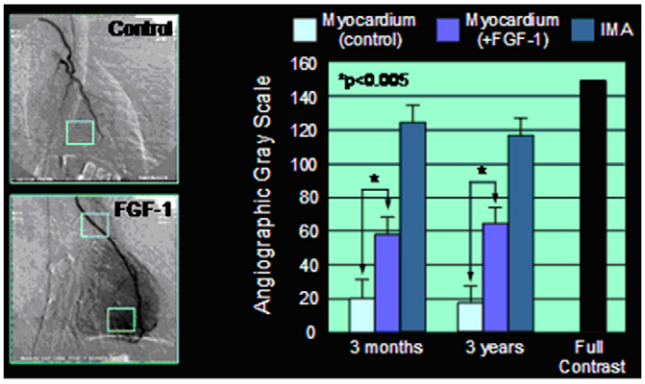

FIGURE 4

Angiographic 'Blushing' following FGF-1 injection into the human heart. Results from FGF-1 human trials performed in Germany [9–11]. Left, coronary angiography indicating an increased capillary density after FGF-1 treatment in the left descending coronary artery territory (FGF-1) compared to the control. Right, semi quantitative measurement of pixel density ('gray value') in angiograms, indicating a threefold increase of vessel density in the treated humans (myocardium + FGF-1) after three months, and three years [40], respectively. LAD: left anterior descending coronary artery; IMA: internal mammary artery.

the human heart, it appears that FGF-1, once it exits the heart is cleared in less than three hours from the circulation. This would presumably prevent FGF-1 from stimulating unwanted angiogenesis in other tissues of the bodies where it could potentially cause harm, such as the retina and in the kidneys. No serious adverse events have yet to be noted in any of the completed or ongoing clinical trials in which the FGF-1 protein is utilized as the therapeutic agent to stimulate angiogenesis.

In addition to studies utilizing VEGF and FGF proteins, other growth factors known to have a role in tissue repair and angiogenesis have been tested in clinical studies including colony granu-

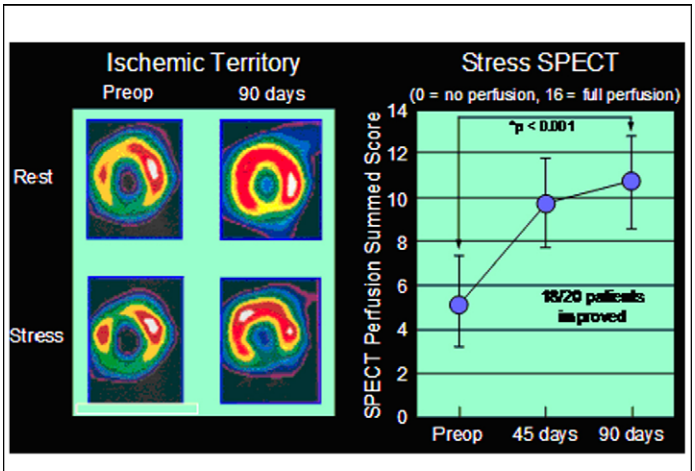

FIGURE 5

Improvement in blood perfusion in the heart as demonstrated by SPECT analysis following FGF-1 administration. ^{99m}tc-sestamibi stress SPECT imaging of the left ventricle preoperatively and 90 days after FGF-1 treatment as sole therapy for no option heart patients [10]. Significant post-treatment improvement of myocardial perfusion was seen in this trial. SPECT: single photon emission computed tomography.

locyte stimulating factor (CGSF), hepatocyte growth factor (HGF, see ref. [35]) and platelet-derived growth factor (PDGF). Although the therapy was deemed safe, statistically significant efficacy could not be consistently demonstrated in clinical trials involving these growth factors.

The outlook for protein therapy and angiogenesis

The goal of angiogenesis therapy is to safely and efficiently recreate the natural process in our bodies whereby new blood vessels are formed to nurture and replenish tissues that have been damaged by underperfusion and ischemia. Capitalizing on lessons learned from previous clinical trials, several high profile clinical studies are now advancing in patients with severe coronary artery disease include Cardium Therapeutic's Phase III FGF-4 gene therapy study in woman, and CardioVascular BioTherapeutics Phase II clinical trial with the FGF-1 protein, delivered by the Myostar® catheter (Cordis Corp., J&J family). Cell-based clinical trials in patients with heart failure continue in earlier stage trials with small patient numbers and the promise of this therapy will have to await expanded trials with appropriate control groups. Given the current limitations, both real and imagined, of gene and cell-based angiogenic therapy, the prospect of protein-based therapy becoming a dominant treatment option for patients with coronary artery disease appears achievable in the not too distant future.

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