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Gene Therapy For Ischemic Heart Disease

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Abstract

Current pharmacologic therapy for ischemic heart disease suffers multiple limitations such as compliance issues and side effects of medications. Revascularization procedures often end with need for repeat procedures. Patients remain symptomatic despite maximal medical therapy. Gene therapy offers an attractive alternative to current pharmacologic therapies and may be beneficial in refractory disease. Gene therapy with isoforms of growth factors such as VEGF, FGF and HGF induces angiogenesis, decreases apoptosis and leads to protection in the ischemic heart. Stem cell therapy augmented with gene therapy used for myogenesis has proven to be beneficial in numerous animal models of myocardial ischemia. Gene therapy coding for antioxidants, eNOS, HSP, mitogen-activated protein kinase and numerous other anti apoptotic proteins have demonstrated significant cardioprotection in animal models. Clinical trials have demonstrated safety in humans apart from symptomatic and objective improvements in cardiac function. Current research efforts are aimed at refining various gene transfection techniques and regulation of gene expression *in vivo* in the heart and circulation to improve clinical outcomes in patients that suffer from ischemic heart disease. In this review article we will attempt to summarize the current state of both preclinical and clinical studies of gene therapy to combat myocardial ischemic disease.

Keywords

gene transfer; cardiovascular therapy; ischemic heart disease

Introduction

Coronary heart disease (CHD) is the single leading cause of death in adults in the US, accounting for 1 in 5 deaths. According to the American Heart Association (AHA), prevalence of total cardiovascular disease in the US in 2006 was 81.1 million people, out of which 17.6 million people had coronary heart disease (myocardial infarction and angina pectoris). Among the people with CHD, the prevalence of heart failure (HF) was 5.8 million [1]. According to the AHA, estimated direct and indirect costs of coronary heart disease for the year 2010 is 177.1 billion dollars. Pharmacologic therapies for CHD and HF have multiple systemic side effects and are pre-disposed to several adverse drug interactions since

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polypharmacy is frequently involved in the treatment of such patients. In addition, compliance is a major issue in these patients. Importantly, pharmacologic therapies aim to reduce symptoms and halt progression of disease but do not necessarily reverse the pathophysiology associated with CHD and HF. Additionally, interventional or surgical coronary revascularization procedures in patients with multiple stenotic lesions or disease in multiple vessels do not provide long term relief and frequently patients remain symptomatic despite maximal anti-anginal therapies and may require repeat revascularization procedures. The treatment of end stage HF is orthotopic heart transplantation and patients have to wait for a year or more due to unavailability of donor hearts.

Gene therapy offers an attractive solution due to the aforementioned limitations of current therapies. It provides continuous delivery of therapeutic proteins locally at the site of disease after a single application, and can potentially lead to reversal of pathophysiology associated with acute myocardial infarction. Gene modification using novel gene constructs can allow genes to be switched on and off depending on the intracellular milieu and minimize unwanted side effects from unrestricted protein synthesis or inhibition. Stem cell-based therapies promote cardiac regeneration and have a higher rate of success when combined with gene therapy [2] [3,4]. Gene therapy can therefore potentially delay the need for heart transplantation or may even obviate the need for one by reversing the pathology, improving cardiac function and alleviating symptoms.

Gene therapy results in synthesis or inhibition of specific proteins leading to alterations in structure or function of the cells in the target tissues. The first step in gene therapy is to design the gene construct with the promoter/enhancer and other stabilizing sequences targeting the gene of interest. The construct is then integrated into the viral genome or a plasmid producing a vector or a delivery vehicle for the gene. The next step is delivering the transgene into the target cells to promote gene expression. Naked plasmid DNA by itself has low transfection efficiency and hence, delivery vectors in the form of adenoviruses, adenoassociated viruses, retroviruses and non-viral vector systems are needed to transfect target cells significantly. Specialized catheters deliver the vectors carrying transgenes into either coronary vessels or into the myocardium. In addition, pericardial administration and invasive surgical intra myocardial approaches have been employed.

In this review we discuss the current preclinical gene therapy studies in *in vivo* models of myocardial ischemia. We describe various gene therapy modalities that in animal models have shown to be of benefit while targeting different dysfunctional aspects of ischemic heart disease. These models are critical in determining which therapies are suitable for clinical investigation.

Gene Therapy for Angiogenesis to Combat Ischemia

VEGF Gene Therapy

VEGF is perhaps the most highly investigated growth factor that has been studied to induce angiogenesis in the ischemic heart. Isoforms of VEGF bind to specific receptors on endothelial cells and play an essential role in angiogenesis [5]. The mammalian genome encodes five isoforms of the VEGF family, which are VEGF-A, VEGF-B, VEGF-C, VEGF-D and placental growth factor [6]. VEGF-A and VEGF-B signal via VEFG receptor-1 and VEGF receptor-2 and regulate blood vessel physiology [7–10].

VEGF-A plays a key role in angiogenesis in the heart [6], especially during hypoxia and nutrient deprivation [11,12]. Transcripts encoding its isoforms VEGF-121 and VEGF-165 are detected in the majority of cells and tissues expressing the VEGF gene. VEGF-121 lacks the amino acids encoded by exon 7 of the VEGF gene, which is present in VEGF-165 and

enables VEGF-165 to bind to heparin and heparin sulfate. Gene therapy of VEGF-165 has been found to be highly potent for promoting angiogenesis [13]. VEGF-165 gene therapy mediated through plasmids in rats [14,15] or through non-viral delivery systems in rabbits [16] induces significant neovascularization and improves fractional shortening after myocardial infarction (MI). In porcine models of MI, VEGF-165 has been shown to increase myocardial blood flow, increase vasodilation with adenosine [17], improve wall thickening and strain [18], improve wall motion[19], increase ejection fraction [20] and increase myocardial viability [21] thereby leading to significant overall improvement in cardiac function. Additionally, VEGF-121 gene therapy augments collateral circulation following MI in rats [22], and in a porcine model of chronic myocardial ischemia [23]. Efficacy of VEGF-121 and of VEGF-165 gene therapy is accentuated with the use of transmyocardial laser, which results in increased capillary formation [24], and improved wall motion [25] in pig models of cardiac ischemia.

VEGF-B is highly expressed in tissues rich in mitochondria, such as the heart, skeletal muscle and brown adipose tissue [26] and plays an important role in revascularization of the ischemic myocardium [27]. Overexpression of VEGF- B186 after cardiac ischemia in pigs and rabbits leads to improved myocardial perfusion and ejection fraction [28]. Similarly VEGF-C gene therapy demonstrates increased collateral formation and reduced wall thickening after myocardial ischemia in piglets. [29] VEGF-D in normal porcine heart has also proven to improve perfusion when administered through the catheter mediated intramyocardial gene transfer [30]. Taken together these studies show that expression VEGF via gene therapy in animal models significantly promotes angiogenesis and improvements in cardiac function following myocardial injury. Angiogenic therapies are not without drawbacks however and unregulated expression limits the efficacy and safety of VEGF gene therapy [31–33]. To circumvent this hurdle, novel gene constructs have been developed whose expression can be switched on or off depending on cellular environments. These constructs have shown to achieve increased VEGF levels during cardiac ischemia [34,35], lead to improved infarct size [36] and induce angiogenesis [37].

Hepatocyte Growth Factor Gene Therapy

Hepatocyte growth factor (HGF) is secreted by mesenchymal cells and acts as a multi-functional cytokine targeting cells of epithelial origin. HGF binds to a tyrosine kinase receptor on vascular endothelial cells thus affecting their migration, proliferation, protease production, invasion and neovascularization [38]. Human HGF (hHGF) gene therapy has been shown to induce angiogenesis in rats and dogs after MI [39–41]. Additionally, it has been shown to improve remodeling [42,43], decrease apoptosis [44,45], improve mobilization of stem cells for cardiac repair [46], decrease fibrotic scar formation [47,48], and improve contractility of the heart [49,50]. HGF gene therapy has proven effective when combined with ultrasound mediated microbubble destruction in an effort to improve gene transfection in a rat model of MI [51] and is currently being evaluated in clinical trials.

Fibroblast Growth Factor Gene Therapy

Fibroblast growth factors (FGFs) bind to tyrosine kinase receptors and mediate mitogenic and cell survival activities. FGFs promote tissue growth and affect cellular proliferation and migration [52,53]. FGF family members differ from one another in their ligand affinities and tissue distribution. FGF-1 and FGF-2 promote endothelial cell proliferation and the physical organization of endothelial cells into tube-like structures. FGF-2 gene therapy has been shown to consistently improve arteriogenesis and echocardiographic parameters of left ventricular (LV) function in chronic ischemia in pigs [54,55]. FGF-2 dependent pathway is activated following the delivery of a “master switch” gene called PR39, which when overexpressed also activates VEGF dependent pathways and leads to improved myocardial

perfusion in a porcine model of chronic ischemia [56]. FGF-4 and FGF-5 are secretory FGFs that have further advantages and may cause paracrine and endocrine effects when compared to FGF-1 and FGF-2, which are primarily intracellular. FGF-4 gene therapy results in increased perfusion and decreased dysfunction in stress induced MI in pigs [57] while FGF-5 gene therapy not only improves blood flow [58] but also reduces pacing induced regional myocardial dysfunction by stimulating the mitotic replication of myocytes, leading to an increase in LV mass [59].

Angiogenesis through Gene Modified Cells

Gene modified cells act as transgene carriers when they have been transfected with the gene of interest. These cells have the ability to overexpress the transgene and lead to increased levels of therapeutic proteins in target tissues. The potential benefit of cell-based therapy lies in the ability of the myogenic cells to differentiate into a cardiac phenotype, become a part of the myocardium and prevent adverse LV remodeling [60]. The transfected cells also express the transgene to produce sustained growth factor release, which then promotes angiogenesis and improves cell implantation and survival. Cells derived from a variety of sources have been employed. Vascular smooth muscle (VSMC) cells modified to overexpress VEGF, when administered via intra coronary route in an intermittent repetitive LAD occlusion model increase collateral circulation in the ischemic heart [61]. Fibroblasts, gene modified to overexpress bFGF gene, when administered by coronary injections in a swine model of chronic ischemia lead to improved collateral formation and myocardial contraction as measured by coronary angiography and electromechanical mapping [62].

Skeletal myoblasts (SkMs) modified to overexpress VEGF, when transplanted in MI models, not only increase angiogenesis and improve cardiac contractile function [2,63,64] but also decrease the amount of apoptosis in the ischemic heart [3]. In a rat model of MI-induced by cryogenic injury, delivering SkMs overexpressing the growth factor VEGF leads to an improvement in the survival of these transplanted SkMs making them available in the ischemic tissue for extended periods [65]. In addition, transplantation of SkMs transfected to overexpress angiopoietin [4] and SDF-1alpha [66] also enhanced angiogenesis and improved LV function in the ischemic heart. Furthermore, SkMs transfected with adenovirus to overexpress HIF-1alpha improved transplanted cell survival, cell engraftment and angiogenesis after rat MI [67]. Thus, SkMs have been used to produce both angiogenic and pro-survival factors in the ischemic heart.

Some advantages of MSCs are their ability to be transduced by vectors easily, ability to be delivered systemically and their capacity to home in to damaged tissues. MSCs also possess low immunogenicity and hence can be used allogenically [68]. As transgene carriers, MSCs have been used to overexpress angiogenic growth factors such as VEGF-165 [69], FGF-1 [70], HGF [71] leading to increased vessel density and improved perfusion of the ischemic heart. Transplanted MSCs modified to express Akt were resistant to apoptosis [72], attenuated remodeling [73] and were found to have anti-apoptotic actions on distant ischemic myocardial cells away from their sites of injection[74]. Other single genes used in transfection of MSCs for treating the ischemic heart were angiogenin [75], HSP20 [76], BCL-2 [77], HO-1 [78], Shh [79] which showed neovascularization, decreased fibrosis, improved contractile function, improved remodeling and improved graft cell survival of the ischemic heart respectively. All the therapies mentioned so far have utilized the overexpression of a single gene. Multiple gene expression by transplanted cells has also been achieved. MSCs have been modified to express a combination of angiopoietin-1 and Akt [80], which led to improved survival of transplanted cells, increased blood vessel density and improved fractional shortening and ejection fraction in the ischemic heart. Other combinations achieved through cell based gene delivery were VEGF with IGF-1 [81] and VEGF with bFGF [82], resulting in improvement in EF and decreased scar formation after

MI. Apart from the above-mentioned cells, cord blood stem cells after gene modification to overexpress VEGF with PDGF [83] and angiopoietin-1 [84] have also been used leading to angiogenesis after rat MI. Thus we see cell based gene delivery improves angiogenesis in the ischemic heart through expression of angiogenic factors while the transplanted cells perform myogenic and regenerative functions in the ischemic heart.

Gene Therapy for Myocardial Reperfusion Injury

Gene Therapy and Oxidative Stress

Oxidative injury plays a critical role in several cardiovascular diseases including myocardial infarction, myocardial ischemia/reperfusion (MI/R) injury, atherosclerosis, endothelial dysfunction, restenosis, hypertension and cardiomyopathies and heart failure [85–89]. Reactive oxygen species (ROS) formed during I/R injury not only cause lipid peroxidation and protein oxidation but also affect several calcium handling proteins such as ryanodine receptors (RyR), SR Ca –ATPase (SERCa) and Inositol 1,4,5-triphosphate (IP_3)-induced Ca release channel thus increasing calcium entry into cardiomyocytes and triggering widespread cellular injury [90]. Gene expression of most antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (Gpx), catalase or hemeoxygenase-1 (HO-1) is inducible under inflammation, or other stressful conditions as a protective mechanism to clear ROS. There are three SOD isoforms that catalyze the dismutation of superoxide anion O_2 thereby attenuating oxidative stress in various cellular compartments [91].

Antioxidant gene therapy offers promise in combating MI/R injury in the heart [91] and has been primarily utilized prior to the MI/R insult to increase levels of tissue anti oxidants so that they are available for cardioprotection at the time of MI/R. Gene therapy to induce overexpression of extracellular superoxide dismutase (Ec SOD) leads to decreased stunning [92] and decreased infarct size following MI/R injury [93,94]. The intracellular counterparts (Mn-SOD and Cu/Zn-SOD) have been proven to decrease apoptosis, decrease infarct size and to delay induction of NF-kappaB [95]. Mn-SOD and eNOS genes when delivered together lead to decreased infarct size following MI/R as well [96]. Human hemoxygenase-1 (hHO-1) gene therapy administered 6–8 weeks *prior* to MI/R leads to decreased mortality at one year [97], decreased lipid peroxidation [98] and reduction in ventricular thinning [99]. HO-1 has also been suggested to induce formation of VEGF [100]. Hypoxia-regulated gene therapies have been designed to circumvent the problem of excess expression under normoxic conditions. One such therapy involves plasmid mediated hHO-1 gene transfer. Gene therapy in this case *pre-emptively* administered results in gene expression following hypoxia and subsequent decrease in infarct size, lipid peroxidation and attenuated remodeling [101].

Thioredoxins (Trx) are proteins that act as potent antioxidants in mammalian cells and provide protection against oxidative stress by decreased p38MAPK signaling [102] and decreased superoxide anion generation [103]. Trx-1 gene therapy administered post MI leads to angiogenesis, decreased apoptosis, reduced ventricular remodeling, and improved ejection fraction in diabetic rats [104]. Most gene therapy using anti-oxidant genes has been in the form of *preemptive* therapies where protein expression has been enhanced by the time MI occurred. In reality this therapy may not be practical in an acute MI setting but may be beneficial as a preventive strategy in patients who have known coronary artery disease and other cardiovascular disease states to protect them from future ischemic events.

Endothelial Nitric Oxide Synthase (eNOS) Gene Therapy

Nitric oxide (NO) is a signaling molecule with multiple protective functions in the heart especially during I/R injury [105]. It is synthesized from L-arginine by the action of three

isoforms of nitric oxide synthase in mammals of which endothelial nitric oxide synthase (eNOS) is primarily responsible for cardioprotection. Adenovirus mediated human eNOS gene therapy administered in animals four days *before* MI leads to decreased infarct size, increased capillary density, improved contractility and decreased MAPK phosphorylation [106,107]. Gene transfer of eNOS *after* MI in rats leads to decreased fibrosis, lower levels of TGF-β1, p27 and NF-kappaB protein levels and lesser apoptosis [108]. eNOS S1177D (an activated form of eNOS) has been utilized in liposome based gene delivery and results in decreased NF-kappaB activation and decreased polymorphonuclear cell infiltrate after MI [109]. In the same study the authors concluded that VEGF gene transfection affords cardioprotection in MI/R via phosphorylation of eNOS. Inducible nitric oxide synthase (iNOS), viewed traditionally as a deleterious enzyme, given *pre-emptively* before MI/R leads to decrease in infarct size [110], induction of COX-2 [111], and upregulation of HO-1 mRNA [112]. Thus NOS gene therapy has the capability of inducing angiogenesis, reducing apoptosis and decreasing inflammation after an ischemic insult to the heart.

Gene Therapy and Proteins Involved in Apoptosis

Heat shock proteins (HSPs) function as molecular chaperones responsible for protein folding, intracellular trafficking of proteins, and modification of proteins denatured by heat or other stresses. HSP 70 gene delivered in myocardium of rabbits *prior* to MI decreases infarct size following MI [113]. HSP 20 also given *prior* to MI/R injury reduces apoptosis, improves LV end systolic and end diastolic pressures and decreases infarct size [114]. HSP 72 gene transfer during rat MI/R decreases apoptosis, improves respiratory index, increases Mn SOD activity, increases Bcl-2 level and inhibits upregulation of caspase-3 [115]. Hence gene therapy of the ischemic heart with HSPs reduces apoptosis and has proven to be cardioprotective after MI in animal models.

Mitogen-activated protein kinase (MAPK) cascade consists of extracellular signal-regulated protein kinase (ERK), p38 kinase and c-jun N-terminal protein kinase (JNK). MAPK is a critical regulator of cell survival and death. Adverse postinfarction remodeling is associated with reduced p38 signaling. Transfection of wild-type (WT) p38 kinase combined with that of active MAP kinase kinase 3b (which is an upstream activator of p38 kinase) during rat MI/R results in reduction in infarct size, lesser apoptosis, increased capillary density, decreased fibrosis and improvement in ejection fraction [116].

Protein kinases are important regulators of angiogenesis [117]. Troponin I type 3 interacting kinase (TNNI3K) is a mitogen activated protein (MAP) kinase, which apart from being angiogenic, is also myogenic. Gene transfection of P19CL6 pluripotent progenitor cells with TNNI3K gene and administration in mice after MI leads to reduced MI induced injury, inhibits remodeling and induces myogenesis in the ischemic heart [118]. Thus targeting the MAPK/p38 signaling cascade by gene therapy of ischemic heart leads to reduced apoptosis and promotes angiogenesis as well.

Insulin like growth factor -1 (IGF-1) is produced primarily by liver as an endocrine hormone but is produced in target tissues in a paracrine/autocrine fashion. Production is stimulated by action of growth hormone and inhibited by malnutrition and growth hormone insensitivity. IGF-1 while inhibiting programmed cell death, activates AKT signaling pathway and stimulates growth and proliferation of cells. IGF-1 overexpressing mesenchymal stem cells help accelerate bone marrow stem cell mobilization via activation of SDF-1alpha/CXCR4 signaling which leads to growth and proliferation of cells and decreased apoptosis thereby promoting myocardial repair and improving fractional shortening and ejection fraction in a rat MI model [119]. Hence gene therapy with IGF-1 and SDF-1alpha reduces adverse remodeling and is cardioprotective.

Gene Therapy for other proteins involved in Apoptosis

Tumor Necrosis Factor (TNF) is a cytokine produced by activated macrophages and acts via the TNF receptor triggering apoptosis, inflammation and suppression of tumorigenesis. Soluble TNF-alpha receptor 1 (sTNFR1) has the ability to act as an antagonist to TNF. sTNFR1 gene therapy after MI in mice reduces infract size and improves cardiac function [120].

Leukemia inhibitory factor (LIF) is an interleukin 6-related cytokine that regulates differentiation, growth and regeneration, both during embryogenesis and in adult tissues [121,122] including the myocardium [123,124]. Gene delivery of LIF and subsequent overexpression leads to decreased fibrosis, increased LV thickness, fewer apoptotic nuclei in the border zone of ischemia, decreased ventricular dilatation and hence preservation of rat myocardium post MI [125].

Sonic hedgehog homolog (Shh) is a protein involved in mammalian hedgehog signaling pathway that plays a key role in organogenesis. Gene transfer of naked DNA encoding the human Shh gene in myocardial ischemia has been shown to preserve ventricular function by enhancing neovascularization, reducing apoptosis and reducing fibrosis [79,126].

The kallikrein-kinin system is a system of proteins that controls blood pressure and induces pain and inflammation. The system is mediated through bradykinin and kallidin that are liberated from their precursor kininogens by the protease action of kallikreins. Kallikrein gene delivery has been shown to increase capillary density, decrease apoptosis, decrease endothelial dysfunction and preserve cardiac output post MI in rats [127].

Cluster of Differentiation 151 (CD151) is a gene encoding for a cell surface protein that plays a role in cell development, growth, activation and cell motility. CD151 gene delivery after myocardial infarction promotes neovascularization and improves cardiac function in pigs [128] and rats[129].

The Akt family of protein kinases is involved in cell survival pathways and its members inhibit apoptosis. Akt1 has also been implicated in angiogenesis. Adenovirus-mediated Akt gene transfer pre emptively in rat hearts limits infarct size following ischemia-reperfusion injury [130]. Adenoviral gene transfer of Akt in wild-type rat hearts enhances myocardial contractility and intracellular calcium handling [131]. Hence, Akt gene therapy has potential to be used in heart failure as well.

Bcl-2 protein, functioning in the mitochondria is anti-apoptotic by counteracting Bax and Bak and inhibiting cytochrome c release. Overexpression of Bcl-2 promotes cell survival and inhibits cell death [132]. Overexpression of human Bcl-2 in ischemia/reperfusion injury in a *transgenic* mouse model over expressing the Bcl2 cDNA led to reduction in infarct size and improvement in ejection fraction over wild type controls [133]. Gene therapy with Bcl2 gene in rabbits after MI/R shows reduced apoptosis, lesser ventricular dilatation and decreased wall thinning [134].

Apoptosis repressor is another protein utilized as anti apoptotic therapy in ischemic heart disease. Post ischemic cardiomyopathy and remodeling following MI/R injury in rabbits is delayed with viral gene transfer of an apoptosis repressor with caspase recruitment domain, which results in inhibition of apoptosis, lesser LV dilatation, preserved EF [135].

Cardiotrophin-1 (CT-1), a member of the interluekin-6 family of cytokines protects the heart against ischemia/reperfusion injury and plays a substantial role in cardiac repair and hypertrophy [136,137]. Gene therapy of CT-1 in mouse hearts after MI results in decreased

apoptosis, decreased infarct size, decreased caspase-3 activation and improved ventricular pressure indices [138].

Sphingosine kinase (SPHK) is a lipid kinase involved in sphingolipid metabolism. SPHK 1 leads to formation of sphingosine-1-phosphate (S1P), which, acting through its receptor S1P1 activates eNOS, induces endothelial cell chemotaxis and maintains vascular integrity [139]. SPHK1 gene therapy in rats after MI preserves systolic and diastolic functions of the heart and leads to improved peak contraction velocity [140].

Thus gene therapy overexpressing TNF, LIF, Shh protein, Kallikrein, CD151, Akt, Bcl-2, apoptosis repressor gene, CT-1 and SPHK improve various aspects of pathophysiology associated with ischemic heart disease.

Clinical Trials on Gene Therapy for Ischemic Heart Disease

Controlled clinical trials for gene therapy in ischemic heart disease that have been published include: VEGF-165, VEGF-121, VEGF-C and FGF-4. Gene therapy with VEGF-165 has been proven successful in the Kuopio Angiogenesis Trial (KAT trial) and encouraging in the EUROINJECT trial. In the KAT trial, adenovirus mediated VEGF-A165 gene therapy when administered by intracoronary injection in patients with class 2–3 angina undergoing PTCA showed improvement in coronary perfusion with no difference in the rates of vascular stenosis [141]. The study followed patients for 8 years and demonstrated its safety and efficacy in patients with coronary artery disease [142]. VEGF-A165 gene therapy administered intramyocardially in plasmid form in patients with Canadian class 3–4 angina with no other therapeutic options showed little improvement in perfusion defects after 3 months in the EUROINJECT trial [143]. However, subsequent analysis revealed improvement in wall motion and LV function suggesting that these benefits may be mediated by factors others than an increase in angiogenesis [144]. VEGF-165 plasmid mediated gene therapy followed by injection of G-CSF to mobilize stem cells has been tried in 16 patients who failed conventional therapeutic strategies [145]. Gene therapy in this small trial showed no improvements in myocardial stress perfusion and may have been due to inadequate homing of stem cells at zones of infarction. Other factors responsible for the lack of response may have been poor timing of G-CGF injections or inadequacy of SPECT scanning in demonstrating minor changes in regional perfusion. Similarly, the NORTHERN trial, involving VEGF-A165 gene therapy through transfection with naked plasmid DNA did not show any improvement in perfusion and exercise time in class 3–4 chronic angina [146]. Interestingly, both the EUROINJECT and NORTHERN trials clearly demonstrated that gene therapy was safe and despite the lack of clear benefit was not detrimental and did not worsen clinical outcomes. Since naked plasmid DNA was used in both these studies, low gene transfection could be one of the reasons why these trials did not lead to significant clinical improvement and future trials may require the use of viral vector mediated gene transfer.

Several other clinical trials have demonstrated symptomatic improvements with gene therapy. Preliminary results with VEGF-121 and VEGF-2 have been promising. In the REVASC trial, VEGF-121 gene therapy through adenoviruses, administered via intramyocardial injection following a mini-thoracotomy in patients with severe refractory angina not amenable to standard medical therapy resulted in improved exercise time before electrocardiographic changes of ischemia, increased total exercise time and improved anginal symptoms at 26 weeks of follow up [147]. Plasmid VEGF-2 (VEGF-C) therapy in patients with chronic class 3–4 angina was safe and resulted in improvement of symptoms in initial pilot studies [148] and in long term follow up [149].

In the AGENT (Angiogenic GENe Therapy) [150] and AGENT 2 trials [151], FGF-4 gene therapy administered to patients with class 2–3 angina resulted in improvement in exercise time at 4 weeks. But larger trials the AGENT -3 and -4, trials of a low and high dose of Ad5FGF-4 for chronic angina involving 532 patients in a randomized, double-blind, placebo-controlled fashion in multiple countries were halted when an interim analysis of the AGENT-3 trial indicated that the primary end point change from baseline in total ETT time at 12 weeks did not reach significance. Sub-group analysis in these trials have however shown significantly improved exercise time in women and may have been due to increased severity of CAD in women compared to men. Therefore gender based differences in response to gene therapy is an important factor which may need to be investigated further to appropriately identify target populations that would benefit from these therapies [152]. There is an ongoing bFGF gene therapy clinical trial in women with ischemic heart disease (AWARE trial), for the same reason. A gene therapy trial with VEGF-165/bFGF bicistronic plasmid been completed but the results are unavailable. This therapy might prove superior since a double treatment strategy with the use of two angiogenic factors is being employed. If successful this approach would demonstrate a change in myocardial perfusion on SPECT scans as the primary outcome measure.

Current Problems Limiting Application of Gene Therapy

As with most experimental therapies, safety of gene therapy for ischemic heart disease is of paramount importance. Though clinical trials have shown short-term safety, long-term surveillance over a period of decades is lacking. The question still remains as to which therapy benefits what subpopulation of patients. Inclusion of a wide selection of patients in studies over time may lead to improvement in subgroups of patients if not the entire population. Confounding factors such as use of concurrent medications and concurrent medical conditions lead to difficulty in standardizing groups of patients. Objective end points of assessment need to be used uniformly as exercise testing may be subjective and is victim to high variability in the same patient on different days. Frequency of testing for objective improvements may need to be ramped up as the effects of therapeutic gene may have abated at the time of a single test. Another surprising factor that confounded results of clinical trails was a strong placebo effect. This might be minimized when objective and not subjective end points are used when assessing outcomes. Drug related issues such as the dose, gene transfection efficiency, pharmacokinetics and pharmacodynamics of individual therapies are valid as these may differ in different populations of patients. Also cost-effectiveness analysis has to be considered, as production of gene therapy vectors itself is cumbersome requiring specialized equipment and personnel and administration of gene therapy is invasive in nature. Besides, specific gene therapy may not compare favorably to available pharmacological agents in use to treat ischemic heart disease in terms of cost: benefit ratio. It is possible that extensive use of small animals for preclinical research may have led to excessive enthusiasm too early. Gene therapy testing on larger animals may provide a better insight into the true efficacy of specific therapies.

Conclusion and Future Directions

The vast amount of preclinical research attests to the enormous interest currently shown by researchers and clinicians in developing gene therapies for ischemic heart disease. Several improvements in gene therapy technology have improved efficacy and led to marked success in animal models of ischemic heart disease. Importantly, these improvements will likely augment the successes of clinical trials on gene therapy that have already demonstrated an excellent safety profile in this setting.

One such improvement is the design of novel gene constructs used to regulate gene expression that lead to controllable protein levels in target tissues [153] [154]. Another improvement is the inclusion of tissue specific promoters in some gene constructs which promotes therapeutic gene expression restricted to target tissues and limits gene expression in non target tissues [155] [156].

Use of specialized catheters (such as the NOGA catheter) and improved gene transfection techniques are making gene therapy more efficacious. Ultrasound-targeted microbubble destruction is one such technique that employs myocardial contrast echocardiography to deliver therapeutic plasmids to the myocardium. Plasmids bind to microbubbles, which are then delivered to the myocardium possibly after endothelial injury induced by the microbubbles [157]. Novel bubble liposomes which are smaller than conventional microbubbles have been developed, and could prove better than microbubbles for gene transfection [158].

In a few patients, understandably, infusion of viral vectors may be of concern due to fear of infectious disease from infused viral vectors or to insertional mutagenesis. Several non-viral vectors with good transfection efficiency such as biodegradable lipid modified polymers [159] and nanoparticles [160] have been developed in the recent years. Despite the promising results seen in clinical trials and good initial safety profile there are several challenges that need to be overcome before gene therapy becomes a mainstream therapy for ischemic heart disease. With the current pace of rigorous preclinical research to delineate various targets in the diseased pathways, constantly improving gene transfer techniques, design of novel regulated gene expression mechanisms and, improving safety profiles, gene therapy may soon become an accepted treatment for ischemic heart disease in man.

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Table 1

Summary of Published Clinical Trials on Gene Therapy for Ischemic Heart Disease

Therapeutic Agent	Clinical Trial	Phase	Target Population	Outcome Summary	Ref
Ad-VEGF-A165	Kuopio Angiogenesis Trial (KAT trial)	2/3	Class 2–3 angina patients undergoing PTCA	Improvement in coronary perfusion	141,142
Ph-VEGF-A165	EUROINJECT Trial	2/3	Class 3–4 angina with no other therapeutic options	Improvement in wall motion and LV function. No improvement in perfusion defects	143,144
Ph-VEGF-A165 followed by G-CSF injection		1	Class 3–4 angina patients who failed conventional therapeutic strategies	No improvement in myocardial stress perfusion	145
Ph-VEGF-A165	NORTHERN Trial	2/3	Class 3–4 angina patients who have reached maximal medical therapy	No change in myocardial perfusion despite improved revascularization	146
Ad-VEGF-121	REVASC Trial	2	Patients with severe angina (>75% had class 3–4 angina) not amenable to revascularization	Improved exercise time and improved anginal symptoms	147
Ph-VEGF-2 (or Ph-VEGF-C)		1/2	Patients with class 3–4 angina refractory to maximal medical therapy or multivessel occlusive coronary artery disease (CAD) not amenable to percutaneous or surgical revascularization	Improvement in anginal class and exercise time at 2 years	148,149
Ad5-FGF4	AGENT Trial	1/2	Patients with stable class 2–3 angina	Improvement in exercise time at multiple doses	150
Ad5-FGF4	AGENT 2 Trial	2	Patients with class 2–4 angina on maximal anti anginal therapy and not optimal candidates for revascularization	Improved stress induced myocardial perfusion defect	151
Ad5-FGF4	AGENT 3 Trial	3	Patients with class 2–4 angina on maximal anti anginal therapy who did not require immediate PCI or CABG	Female patients with significant improvement in exercise time and anginal class	152
Ad5-FGF4	AGENT 4 Trial	3	Patients with class 2–4 angina on maximal anti anginal therapy who were not candidates for PCI or CABG	Female patients with significant improvement in exercise time and anginal class	152

Ad = adenovirus; VEGF = vascular endothelial growth factor; FGF = fibroblast growth factor; Ph = plasmid