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Gene Transfer for Congestive Heart Failure: Update 2013

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Abstract

Congestive heart failure is a major cause of morbidity and mortality with increasing social and economic costs. There have been no new high impact therapeutic agents for this devastating disease for more than a decade. However, many pivotal regulators of cardiac function have been identified using cardiac-directed transgene expression and gene deletion in preclinical studies. Some of these increase function of the failing heart. Altering the expression of these pivotal regulators using gene transfer is now either being tested in clinical gene transfer trials, or soon will be. In this review, we summarize recent progress in cardiac gene transfer for clinical congestive heart failure.

Keywords

Gene Transfer; Adenovirus; AAV; LV Contractility; Ca^{2+} Handling; β-adrenergic receptor

INTRODUCTION

Congestive heart failure (CHF) is a condition in which the heart cannot pump enough blood to meet the body's needs. It is a leading cause of morbidity and mortality in the world. In the US, there are approximately 300,000 deaths every year which are due to CHF.[1] There are >23 million CHF patients worldwide, and this number is projected to double by 2030. Even with optimal pharmacological and device therapy, the outcome for subjects with CHF remains poor with 50% mortality within 4–5 years. Heart transplantation has an 80% 5-year survival, but is a solution for only 2,500 patients annually in the US.

This unmet medical need demands more effective options for patients with CHF. Stem cell delivery shows some promise in treating acute myocardial infarction;[2] however, it has not been shown to be effective in randomized clinical trials. Gene transfer is simpler and easier to apply clinically than is stem cell delivery, and two recent clinical gene transfer trials have been initiated. Here, we first review general considerations for selection of vector,

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TT and HKH wrote and approved the final draft of the manuscript.

transgene, and delivery methods for cardiac gene transfer, and summarize recent progress in cardiac gene transfer for clinical CHF.

GENERAL CONSIDERATIONS FOR CARDIAC GENE TRANSFER

The chief impediment to successful CHF gene therapy is in obtaining sufficient cardiac transgene expression to provide a therapeutic effect. There are three requirements that must be met for successful CHF gene therapy: (1) a therapeutic transgene that can increase function of the failing heart; (2) a suitable vector with an appropriate promoter that will ensure long-term and sufficient transgene expression with minimal toxicity; and (3) a delivery method that safely provides effective cardiac gene transfer and yet is easy to deploy.

Cardiac myocyte-targeted transgene expression and gene deletion studies in mice have identified pivotal regulators of cardiac function that are deficient or dysfunctional in CHF. Many of these proteins are suited to gene transfer, providing new potential treatments for CHF. New virus vectors have been engineered and novel delivery methods have been tested to enable long-term and higher level expression. Virus vectors encoding a variety of therapeutic transgenes appear to increase function of the failing heart in preclinical studies,[3–7] and thus a focus of this review. Indeed, clinical CHF trials using intracoronary delivery of an adeno-associated virus (AAV) vector encoding SERCA2a (ClinicalTrials.gov NCT01643330) and adenovirus encoding adenylyl cyclase 6 (AC6) (ClinicalTrials.gov NCT00787059) are currently in progress (Table 1). Other transgenes including βARKct and S100A1, which have shown efficacy in preclinical studies, may advance to clinical trials soon.[8]

VECTORS AND METHODS FOR CARDIAC GENE TRANSFER

Plasmid Vectors

Plasmid vectors were used in previous gene transfer trials in angiogenesis, and this approach is being used in a current gene transfer trial in subjects with symptomatic CHF. The gene, stromal cell-derived factor 1 (SDF-1), is injected into the LV wall using a catheter-based system. Results from an open-label Phase 1 study (ClinicalTrials.gov NCT01082094; Table 1),[9] supported initiation of a randomized, double-blind, placebo-controlled Phase 2 study (ClinicalTrials.gov NCT01643590). Results of the randomized trial are not available.

Virus Vectors

Adenovirus and AAV are the most commonly used vectors for cardiac gene transfer. E1,E3-deleted adenovirus provides reasonable gene transfer efficiency,[10] particularly when used with mechanical or pharmaceutical adjuvants following intracoronary delivery. Newer generation adenovirus, including so-called gutless adenovirus that are engineered by deleting all regions encoding virus proteins, may have lower immunogenicity than previous vectors.

AAV, with an insert capacity <5 kb, provides potential long-term expression and, like adenovirus, is not associated with risk of insertional mutagenesis. Persistent transgene expression has been shown in rodents and larger mammals years after a single injection of AAV.[11] Although recent clinical trials have found that some AAV serotypes incite immune responses after intramusclar injection,[12, 13] other AAV vectors (AAV5, 6, 8 and 9) do not appear to have similar problems in non-human primates.[14] Previous exposure to AAV, with subsequent generation of neutralizing antibodies, impairs the effectiveness of AAV vectors in cardiac gene transfer. Pre-existing anti-AAV8 antibodies are present in 19% of human

subjects. AAV1 and AAV2 have a 50–59% prevalence of neutralizing antibodies, making these serotypes not useful in clinical applications.[15]

Self-complementary AAV vectors (scAAV) may provide more rapid and perhaps higher transgene expression than their single stranded (ssAAV) analogs.[15] Transgene expression using ssAAV vectors is delayed four weeks until the complementary DNA strand is synthesized. By encoding for the complementary DNA strand within the vector, scAAV (insert capacity 3.3 kb), enables transgene expression in two weeks.[15]

Promoters

The CMV (cytomegalovirus) promoter is widely used for cardiac gene transfer. It provides strong transgene expression in cardiac myocytes. However, the CMV promoter is susceptible to methylation and subsequent inactivation in the liver and skeletal muscle.[16, 17] RSV (Rous sarcoma virus), CBA (chicken β-actin), and EF1α (elongation factor 1α) provide less robust transgene expression in cardiac myocytes, but are less susceptible to methylation. None of these promoters provide cardiac-specific transgene expression.

The α-MHC (α-myosin heavy chain) promoter is used for cardiac-directed transgene expression in transgenic mice.[18] However, it provides less robust transgene expression in virus vectors. More relevant, its size (~5.5 kb) is over the packaging capacity of AAV and prevents its use in the AAV-mediated cardiac gene transfer. A 2.1 kb fragment of the MLC-2v (myosin light chain 2v) promoter may be a suitable option.[19] It is intriguing that a 418 bp fragment of chicken cardiac troponin T (cTnT) promoter provides 100-fold more transgene expression in the heart than liver after AAV-mediated gene transfer, although transgene expression is lower than that provided by CMV and CBA promoters.[20]

Gene Delivery Methods

Delivery methods are often determined by vector selection. For example, intravascular delivery is not suitable for lentivirus, a vector that is unable to cross the capillary endothelium.[21] Direct intramyocardial injection appears to be the best delivery route for lentivirus for cardiac gene transfer. However, intramusclular injection provides transgene expression limited to the area adjacent to the needle tract. Direct intramyocardial injection is less efficient than intravascular delivery of adenovirus or AAV.

There are three effective delivery methods for cardiac gene transfer: direct intracoronary, indirect intracoronary (indirect IC), and IV.[22, 23] In mice, the coronary arteries are too small for direct intracoronary delivery. Indirect IC delivery, instead, has been used to deliver virus vectors to the LV in mice and rats. In this procedure, the aorta and pulmonary arteries are cross-clamped and virus vectors are delivered into the LV chamber. Continued LV contraction then forces the vector into the coronary arteries. This method is used to deliver adenovirus and AAV vectors in rodents. Since vector exposure time promotes gene transfer, hypothermia is used to prolong dwell time but preclude brain injury. Pharmacological agents, such as histamine[24, 25], serotonin,[26] nitroprusside[27, 28], sildenafil[29], and substance P[30], are also administrated to increase adenovirus-mediated gene transfer efficiency.[31] IV delivery of AAV vectors (especially AAV6, AAV8, and AAV9) has been used with success in cardiac gene transfer,[6, 8, 32] although indirect IC delivery provides superior cardiac gene transfer efficiency compared with IV delivery, regardless of AAV serotype.[33]

Regulated Transgene Expression

Long-term expression vectors require, for safety in clinical trials, the ability to turn off transgene expression in the event that untoward effects develop. Regulated expression also enables the flexibility of intermittent rather than constant transgene expression. There are

four regulated expression systems currently available: ecdysone, tamoxifen, tetracycline, rapamycin.[34–37] The size of the ecdysone system requires a two-vector strategy and tamoxifen presents difficult to resolve issues with toxicity. Tetracycline and rapamycin regulation systems (Table 2) have been tested in large animal models.[5, 11, 38–42]

Tetracycline-Regulated Expression—The tet-regulation system has been extensively studied.[16] Unlike previous rtTA constructs, newer rtTA variants, such as rtTA2^S-M2, provide robust tet-dependent expression with no basal activity (ie. no "leak") and 10-fold higher sensitivity to tetracycline (maximum transgene expression activation at 0.1 μg/ml).[43] A single daily dose of doxycycline of 10–20 mg may suffice for complete activation of transgene expression in human subjects.[44] Doses of 200 mg/d are well tolerated by patients using oral doxycycline chronically for acne and chronic infections.[44, 45] Tetracyclines may attenuate matrix metalloproteinase (MMP) activity and affect LV remodeling when administered in the first few days after MI.[41] In clinical settings, tetracycline should be avoided in the acute phase of MI.

Immune responses to components of the rtTA system did not occur when AAV4.tet and AAV5.tet gene transfer (intraretinal) were used in non-human primates,[38, 44] where tetracycline-dependent transgene expression persisted for the 2.5 year duration of the study. We do not see inflammation in mouse hearts expressing high levels of rtTA,[41, 42, 45] or in rats after AAV5-mediated regulated expression of IGF-I using the rtTA2^S-M2 regulation element.[46] It appears that intramuscular delivery of AAV in nonhuman primates, unlike intraretinal or vascular delivery, does lead to attenuation of regulated expression, owing to immune responses to the bacterial and virus components of the transactivator fusion protein.[47] The rapamycin-regulation system, which does not possess bacterial or virus proteins, and is not associated with provocation of the immune response,[11] may be a suitable alternative.

Rapamycin-Regulated Expression—In the rapamycin regulation system, transgene expression is triggered by nanomolar concentrations of rapamycin or a rapamycin analog, which is dose-dependent and reversible.[37] Rapamycin is used clinically to suppress immune response, forestalls deleterious effects of aging in mice[40] and inhibits glioblastoma multiforme[48] by blocking the mammalian target of rapamycin (mTOR) signaling pathway.[49] The oral rapamycin analog AP22594, which activates transgene expression as effectively as rapamycin, exhibits minimal immune suppression, and does not inhibit mTOR.[11, 49–51] Additional preclinical studies directly comparing tetracycline-regulated and rapamycin-regulated expression will be required before using these systems in clinical trials.

Alternative Methods for Cardiac Gene Transfer

Paracrine-based gene transfer is an alternate to cardiac-targeted gene transfer and may be applicable for CHF and other cardiovascular diseases. A prerequisite for this approach is the selection of a transgene that has cardiac effects after being released to the circulation from a distant site. We have tested this concept using skeletal muscle injection of AAV5 encoding IGF-I (insulin growth factor I) under tet regulation (AAV5.IGFI-tet).[46] In this study, AAV5.IGFI-tet was injected in the anterior tibialis muscle in rats with severe CHF induced by myocardial infarction. Activation of IGF-I expression by addition of doxycycline to the drinking water increased serum IGF-I levels and improved function of the failing heart. This new approach enables transgene expression at a remote site and circumvents the problem of attaining high yield cardiac gene transfer.

CANDIDATES FOR CHF GENE TRANSFER

CHF is associated with several cell signaling pathways that are dysfunctional. Consequently, several potential therapeutic targets have been identified. We will summarize here only those strategies that have proven to be effective in preclinical studies and have advanced or may soon advance to clinical trials for CHF. It is not surprising that these potential targets influence β-adrenergic receptor (βAR) and Ca^{2+} signaling.

βAR Signaling

A hallmark of clinical CHF is impaired left ventricular (LV) βAR signaling.[52] The molecular basis for impaired βAR signaling includes decreased βAR density, βAR desensitization, uncoupling of βAR and Gαs, deficits in adenylyl cyclase (AC) expression, and subsequent defective cAMP production.[53] Clinical use of βAR antagonists (beta-blockers) reduce symptoms and prolong life somewhat in CHF.[54] Correcting impaired βAR signaling safely has been a focus for CHF research for many years, and presents considerable challenges.

βARKct—GRK2(G-protein-coupled receptor kinase 2) is a protein kinase that phosphorylates the βAR.[53] This phosphorylation promotes βAR binding to β-arrestin, which in turn promotes Gαs uncoupling and attenuation of βAR signaling. β-arrestin binding also leads to β_1AR internalization. The human failing heart is associated with increased expression and activity of GRK2.[55] Conversely, in preclinical studies, deletion of GRK2 increases survival, attenuates LV remodeling, and reduces the extent of CHF after myocardial infarction.[56]

βARKct (C-terminal domain of GRK2) was engineered to block GRK2 membrane translocation and activation. Expression of βARKct after virus-mediated gene transfer increases βAR density, cAMP production, and LV contractile function in myocardial infarction-induced CHF in rats, rabbits, and pigs.[7, 57, 58] βARKct expression in cardiac myocytes from failing human hearts also increases cAMP production, cell shortening, and relaxation.[59] Importantly, βARKct may work addictively with βAR antagonists.[58, 60]

AC6—AC is the effector molecule that links βAR stimulation with cAMP production.[61–63] In the heart, it plays a pivotal role in LV contractile function and relaxation in response to βAR stimulation.[24, 43, 64, 65] The failing heart is associated with decreased expression and activity of AC6, a major AC isoform in cardiac myocytes.[52, 66–68] Cardiac-directed expression of AC6 in a genetic animal model of CHF increases impaired LV function and prolongs life.[69, 70] Associated with these beneficial effects in the failing heart are increased cAMP generating capacity in response to βAR stimulation, normalized PKA activity, increased phospholamban phosphorylation, and increased sarcoplasmic reticulum (SR) Ca^{2+} uptake.[70, 71] AC6 expression is not associated with increased heart rate or development of arrhythmias in the failing heart.[72] Increased LV AC6 expression is not associated with changes in contents of βAR, Gαs, or Gαi2 have been observed.[64] Deletion of AC6 is associated with reduced SR Ca^{2+} uptake and decreased LV function in response to βAR stimulation.[73] In addition, AC6 expression normalizes prolonged action potential duration and attenuates ventricular arrhythmias.[74]

AC6 expression also has a pronounced favorable effect on cardiovascular function in CHF induced by myocardial infarction. Although AC6 has no effect on infarct size, it prevents deleterious LV remodeling, and reduces mortality in acute myocardial infarction.[75] When AC6 expression is activated 5 weeks after myocardial infarction, at which time severe CHF is evident, both LV systolic function and diastolic function are increased.[5] Intracoronary delivery of adenovirus encoding AC6 increases LV function in the failing pig heart.[27] A

clinical trial of AC6 gene transfer for CHF is in progress (ClinicalTrials.gov NCT00787059). AC6 expression increases LV Ca^{2+} handling and LV function in aged mice suggest a potential role in elderly subjects with CHF with preserved ejection fraction.[76]

Ca^{2+} Handling

Calcium plays a crucial role in controlling LV contraction and relaxation. During every heartbeat, Ca^{2+} is taken up and then released from SR. The failing heart is characterized by defective excitation-contraction coupling (E-C coupling) and dysfunctional SR Ca^{2+} uptake and release.[77]

SERCA2a—SERCA2a (SR Ca^{2+}-ATPase 2a) is the Ca^{2+} pump responsible for cardiac SR Ca^{2+} uptake. CHF is associated with abnormal SERCA2a expression and activity.[78, 79] Cardiac-directed SERCA2a expression increases SR Ca^{2+} uptake and LV contractile function and relaxation.[80, 81] Homogenous SERCA2a deletion is lethal. Mice with deletion of only one SERCA2a allele show decreased SR Ca^{2+} uptake and are more prone to CHF after pressure overload.[82, 83] These data suggest a role of SERCA2a in mediating Ca^{2+} handling and LV function.

SERCA2a gene transfer increases contractile function *in vitro* in cardiac myocytes isolated from CHF patients.[84] In pressure-overloaded hearts, expression of SERCA2a by adenovirus-mediated gene transfer increases contractile function and relaxation in rats.[85, 86] SERCA2a gene transfer also increases LV function in aged hearts.[87] In volume-overloaded pigs, AAV-mediated SERCA2a gene transfer attenuated LV dysfunction and remodeling.[3] SERCA2a gene transfer shows similar beneficial effects in sheep with pacing-induced CHF.[88] A clinical trial of SERCA2a gene transfer with 39 CHF patients was conducted, which indicated that intracoronary delivery of AAV1.SERCA2a was safe. The trial was insufficiently powered to determine efficacy although some indications of improvement were reported. A larger clinical trial of SERCA2a gene transfer is in progress (ClinicalTrials.gov NCT01643330).

S100A1—S100A1, a family member of EF-hand Ca^{2+}–binding proteins, is expressed in cardiac myocytes.[89] The subcellular locations of S100A1 include SR, mitochondria, and sarcomere. S100A1 can bind to RyR2, the SR Ca^{2+} release channel, and to SERCA2a, the SR Ca^{2+} uptake pump,[90, 91] suggesting that S100A1 is not solely a Ca^{2+}-binding protein but also a regulator for Ca^{2+} homeostasis in cardiac myocytes. There is evidence that ischemic cardiomyopathy is associated with decreased S100A1 expression.[92]

Cardiac-directed S100A1 expression is associated with improved Ca^{2+} handling, decreased deleterious LV remodeling, and reduced mortality after myocardial infarction in mice.[93] AAV-mediated gene transfer of S100A1 in CHF increases Ca^{2+}-transients and LV function.[94] S100A1 gene transfer also improves Ca^{2+} handling and contractile function *in vitro* in cardiac myocytes isolated from human failing heart.[95] Recent data demonstrate that intracoronary delivery of AAV9 encoding S100A1 normalizes SR Ca^{2+} handling, attenuates LV remodeling, and increases contractile function.[6]

Although βARKct, AC6, SERCA2a, and S100A1 likely operate via effects on βAR and Ca^{2+} signaling, favorable cardiac effects may be mediated by additional mechanisms. For example, both βARKct and AC6 expression activate Akt – a kinase that promotes cell survival.[96, 97] S100A1 inhibits 2-deoxyglucose and oxidative stress-induced apoptosis in neonatal cardiac myocytes *in vitro*.[98] SERCA2a and S100A1 appear to bind to eNOS in endothelial cells, suggesting a role in modulating production of NO – a pivotal molecule for blood flow regulation.[99, 100] Further exploration of the underlying mechanisms may help improve the efficacy of these CHF therapy candidates.

CONCLUSION

Preclinical studies have identified potential therapeutic genes for treatment of CHF. SERCA2a and AC6 have advanced to clinical gene transfer trials. The optimization of virus vectors, regulated expression systems, gene delivery methods, and identification of new therapeutic candidates will move gene transfer for CHF to more importance in the next 10 years.

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Table 1

Clinical Trials Using Cardiac Gene Transfer for CHF

Transgene	**SDF-1**	**SDF-1**	**SERCA2a**	**SERCA2a**	**AC6**
Trial Identifier	NCT01082094	NCT01643590	NCT00454818	NCT01643330	NCT00787059
Phase	1	2	1 and 2	2	1/2
Vector	plasmid	plasmid	AAV1	AAV1	Adenovirus
Delivery Method	Endomycardial injection	Endomycardial injection	Intracoronary injection	Intracoronary injection	Intracoronary injection
Enrollment	17	90	39	200	72
Sample Size	5 mg dose: 4 15 mg dose: 6 30 mg dose: 7	Placebo: 30 15 mg dose: 30 30 mg dose: 30	Placebo: 14 Low dose: 8 Middle dose: 8 High dose: 9	Placebo: 100 Treatment: 100	Placebo: 18 Treatment: 54 in 6 doses
Design	Open label	Randomized Double-blind Placebo-controlled	Randomized Double-blind Placebo-controlled	Randomized Double-blind Placebo-controlled	Randomized Double-blind Placebo-controlled
Status	Completed	Recruiting	Completed	Recruiting	Recruiting
Outcome	Apparently safe; Trends in improving symptoms and functions	N/A	Apparently safe; Trends in improving symptoms and functions	N/A	N/A

Table 2

Tetracycline vs Rapamycin Regulation

Feature	Tetracycline	Rapamycin
Activator	Doxycycline	AP22594
Basal Expression (“leak”)	Very low/none	None
Linear Dose-Response	Yes	Yes
Activator Side-effects	Low (avoid in pregnancy)	Immunosuppressant
Bacteria/Virus Proteins	Yes	No
Used in Clinical Trials	Not yet	Not Yet

AP22594, oral rapamycin analog with $1/100^{th}$ immune suppression *vs* rapamycin.