AAV-mediated gene therapy for heart failure: enhancing contractility and calcium handling

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Abstract

Heart failure is a progressive, debilitating disease that is characterized by inadequate contractility of the heart. With an aging population, the incidence and economic burden of managing heart failure are anticipated to increase substantially. Drugs for heart failure only slow its progression and offer no cure. However, results of recent clinical trials using recombinant adeno-associated virus (AAV) gene delivery offer the promise, for the first time, that heart failure can be reversed. The strategy is to improve contractility of cardiac muscle cells by enhancing their ability to store calcium through increased expression of the sarco(endo)plasmic reticulum Ca²⁺-ATPase pump (SERCA2a). Preclinical trials have also identified other proteins involved in calcium cycling in cardiac muscle that are promising targets for gene therapy in heart failure, including the following: protein phosphatase 1, adenylyl cyclase 6, G-protein-coupled receptor kinase 2, phospholamban, SUMO1, and S100A1. These preclinical and clinical trials represent a “quiet revolution” that may end up being one of the most significant and remarkable breakthroughs in modern medical practice. Of course, a number of uncertainties remain, including the long-term utility and wisdom of improving the contractile performance of “sick” muscle cells. In this regard, gene therapy may turn out to be a way of buying additional time for actual cardiac regeneration to occur using cardiac stem cells or induced pluripotent stem cells.

Introduction

Heart failure is a leading cause of death in the United States and is mentioned in one out of nine death certificates [1]. Survival after diagnosis has improved over time; however, the death rate remains high with ~ 50% mortality within 5 years. There is no cure for heart failure short of a heart transplant, which is an option only for a few select individuals. Thus, there is a great need for new therapies. Heart failure, and here we focus on systolic heart failure or heart failure with reduced ejection fraction, results from damage to the heart that prevents it from meeting its primary function to deliver oxygenated blood to the body. The most common causes of heart failure are coronary artery disease and hypertension, which can compromise contractility by reducing oxygen delivery to the heart muscle (or by adverse remodeling of the heart in the case of hypertension) or cause muscle damage outright through infarction. Ironically, cardiac muscle damage leading to heart failure may also result from reperfusion-injury following percutaneous coronary intervention (PCI), which is performed to increase oxygen delivery to cardiac muscle [2]. With reduced contractility, increased neurohormonal drive kicks in, causing genetic and structural changes to the heart that further reduce the ability of the heart to function as a pump [3,4]. Moreover, the increased metabolic and oxidative stress and inflammation that develop during the progression of heart failure elicit additional structural damage to the heart that also compromises pump function [5,6]. Thus, the trajectory of heart failure is multipronged and exponential,
which makes therapeutic treatment difficult. At best, medicines today slow the progression of heart failure. An alternative strategy to treat heart failure is to restore the function of the heart as a pump using gene therapy. Here, we focus on successful preclinical trials that used viral gene delivery to enhance cardiac myocyte contractility by improving calcium handling, some of which have already progressed to clinical trials.

**Calcium handling in the failing heart**

Contraction of cardiac muscle is initiated by the influx of Ca\(^{2+}\) via L-type Ca\(^{2+}\) channels that open as a result of membrane depolarization brought about by the action potential. This Ca\(^{2+}\) activates the sarcoplasmic reticulum Ca\(^{2+}\) release channels known as ryanodine receptor 2 (RyR2), leading to a large increase in sarcoplasmic Ca\(^{2+}\).

The rise in sarcoplasmic calcium causes contraction by Ca\(^{2+}\) binding to troponin C, which relieves constraints on actin-myosin interaction and cross-bridge formation. With relaxation, 60%-80% of the sarcoplasmic Ca\(^{2+}\) is actively transported into the sarcoplasmic reticulum lumen by the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase pump (SERCA2a), while the remainder exits the cardiac myocyte by way of the Na\(^+\)-Ca\(^{2+}\) exchanger [7]. With heart failure, Ca\(^{2+}\) uptake into the sarcoplasmic reticulum is impaired due to decreased expression of SERCA2a [7]. Activity of SERCA2a is decreased as well, due to an increased association of SERCA2a with its inhibitory regulator phospholamban for two reasons: first, normally, phosphorylation of phospholamban relieves SERCA2a inhibition by causing its dissociation from SERCA2a, but, in heart failure, there is less phosphorylation of phospholamban due to increased protein phosphatase 1 (PP1) activity [8]; second, the decreased SERCA2a/phospholamban ratio means that there is more phospholamban relative to SERCA2a, thus favoring SERCA2a inhibition. In addition, the sarcoplasmic reticulum becomes leaky in heart failure due to CaMKII-dependent phosphorylation of RyR2 [9]. Thus, in the failing heart, the sarcoplasmic reticulum does not function as well in removing Ca\(^{2+}\) from the sarcoplasm, which has two consequences: (1) there is an increase in resting sarcoplasmic Ca\(^{2+}\) that contributes to reduced relaxation and diastolic dysfunction; and (2) there is less Ca\(^{2+}\) released from the sarcoplasmic reticulum during contraction, which means that the force of contraction is reduced [7]. Strategies to improve or restore sarcoplasmic reticulum function would be predicted to reverse heart failure.

**Viral gene therapy for heart failure**

**AAV delivery**

Recombinant AAVs (adeno-associated viruses – serotypes 1, 6, 8, and 9 in particular) are currently the most widely used vectors for gene delivery to the heart, mainly due to their relative selectivity for cardiac myocytes, efficient long-term transgene expression, and low immunogenicity and rates of insertional mutagenesis [10-14]. However, restricted packaging capacity and inability to evade neutralizing antibodies limit their potential therapeutic effect and usage. Strategies are currently underway to overcome these issues. Cell-specific promoters can be used to further enhance expression of the transgene in cardiac myocytes [15]. While numerous vector delivery techniques have been developed, intravascular is the least invasive but effective approach for AAV delivery to the heart, largely due to the ability of the virus to cross the capillary endothelium. To avoid post-intravenous (IV) systemic neutralization and non-cardiac tissue transduction and subsequent toxicity and titer dilution, direct and indirect intracoronary injections are preferred and proven to be more efficient [16], and these are the current mode of administering AAV in heart failure clinical trials. Finally, although AAV-mediated gene expression is relatively long lived, the recombinant vectors used do not integrate into the genome, making it likely that follow-up injections will be needed [17]. For more information about the different gene delivery technologies the reader is referred to an excellent recent review by MG Katz et al. [18].

**SERCA2a on trial: a new era for heart failure management**

Impaired SERCA2a activity is associated with high diastolic but low systolic Ca\(^{2+}\) levels, which correlate with poor disease prognosis [19]. Overwhelming pre-clinical evidence from animal models has shown the importance of SERCA2a in heart failure and the power of treating heart failure by increasing SERCA2a expression by means of viral transduction of a SERCA2a transgene [20-23]. In 2007, Celladon Corporation sponsored the first Phase I/II human clinical trial ([24] CUPID; NCT00454818) in which a SERCA2a transgene was transferred, via an AAV1 Vector (MYDICAR), by percutaneous intra-coronary infusion. No safety concerns were revealed by the Phase I open label and sequential dose escalating part of the trial involving nine heart failure patients. A small Phase II, 3-doses, randomized, double-blind, placebo-controlled trial enrolling 39 heart failure patients. A small Phase II, 3-doses, randomized, double-blind, placebo-controlled trial enrolling 39 heart failure patients. In addition to supporting the earlier safety findings, this trial reported significant clinical improvement of cardiac remodeling and heart failure symptoms. These results were accompanied by a marked reduction in cardiovascular hospitalization and clinical events that persisted for 6 to 12 months post high-dose treatment [25]. A larger Phase II, randomized, double-blind, placebo-controlled, multinational and multicenter clinical trial A Study of Genetically Targeted Enzyme Replacement Therapy for Advanced Heart Failure (CUPID-2b) is currently underway (NCT01643330). This trial will hopefully overcome the limitations of the small studies and

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confirm the efficacy of gene therapy in the management of heart failure. Details on the clinical trials involving AAV-mediated SERCA2a gene therapy are summarized in Table 1.

**Other potential targets**

In addition to SERCA2a, many calcium cycling proteins are potential therapeutic targets in heart failure (Figure 1) [17]. Preclinical gene therapy studies targeting these molecules, directly or indirectly, support their safety and efficacy in improving cardiac function (Table 2). Their assessment as targets for heart failure management in clinical trials is likely.

**Protein phosphatase 1**

Protein phosphatase (PP1)β is the catalytic subunit isoform of PP1 that primarily suppresses sarcoplasmic reticulum Ca2+ uptake by dephosphorylating phospholamban. Knockdown of the PP1β using short-hairpin (sh)RNA improved cardiac function and reduced cardiac remodeling and interstitial fibrosis in the muscle LIM protein-deficient mouse model of heart failure [26,27]. shRNA was delivered with an AAV9 vector under the control of a B-type natriuretic protein (BNP) promoter to achieve heart failure-inducible gene expression.

**Adenylyl cyclase 6**

β-Adrenergic enhancement of contraction of the heart is mediated in part through adenylyl cyclase 6 (AC6), which converts ATP to cAMP and, thus, activates protein kinase A (PKA). PKA phosphorylates phospholamban to enhance SERCA2a activity and cardiac troponin I to enhance the force of contraction and relaxation [28-30]. In AC6 knockout hearts, phospholamban phosphorylation and SERCA2a activity are reduced [28]. Viral delivery of an AC6 transgene has been shown effective in alleviating heart failure symptoms in both small and large animal models of heart failure, due to improved sarcoplasmic reticulum Ca2+ function and storage resulting from increased phospholamban phosphorylation and decreased PP1 expression [31-34]. AC6 may also decrease phospholamban expression independently of cAMP formation [28].

**GRK2**

The serine/threonine kinase GRK2 (G-protein-coupled receptor kinase) attenuates β-adrenergic signaling in the heart due to receptor desensitization and downregulation [35]. Levels and activity of GRK2 are increased in heart failure due to over-activation of the compensatory sympathetic drive. Viral-mediated knockdown of GRK2 in cardiac muscle is, thus, a viable strategy for enhancing cardiac contractility, while its knockdown in the adrenal gland helps reduce the increased circulating levels of catecholamines observed in heart failure [35].

**Phospholamban**

Phospholamban is a small, 52 amino acid protein that binds SERCA2a as a monomer and inhibits its activity [36]. Phosphorylation of phospholamban causes dissociation from SERCA2a and favors the formation of pentamers that are functionally inactive towards SERCA2a. Several strategies have been used to target phospholamban in models of heart failure using viral delivery, including expression of a chicken antibody-derived protein targeting phospholamban, an inhibitory phospholamban peptide, antisense, a zinc-finger transcriptional repressor, and a pseudophosphorylated mutant of phospholamban. Improvements in contractility and Ca2+ handling of cardiac myocytes have been reported.

### Table 1. Clinical trials on SERCA2a for heart failure

| Clinical Trial | NCT00454818 | NCT00534703 | NCT00454818 | NCT01643330 |
|---------------|-------------|-------------|-------------|-------------|
| Acronym       | CUPID       | NA          | CUPID       | CUPID-2b    |
| Sponsor       | Celladon Corporation | Imperial College London | Celladon Corporation | Celladon Corporation |
| Study design  | OL/SDE      | R/DB/PC     | R/DB/PC     | R/DB/PC     |
| Outcome measures | Safety | Efficacy | Safety | Efficacy |
| Drug/Vector   | MYDICAR (AAV1-CMV-SERCA2a) | AAV6.-CMV-SERCA2a | MYDICAR (AAV1-CMV-SERCA2a) | MYDICAR (AAV1-CMV-SERCA2a) |
| Doses (DRP)   | 1.4 × 10^{11} | 5 × 10^{12} | 6 × 10^{11} | 3 × 10^{12} |
| Delivery      | AECAI       | AECI        | AECAI       | AECAI       |
| Phase         | Pi          | P/Pi        | Pi          | Pi          |
| N-Status      | 9 patients-completed | 16 patients-unknown | 39 patients-completed | 200-recruiting |
| Results       | Positive    | NA          | Positive    | NA          |
| PMID          | 19327618    | 21709064    | NA          | NA          |

AECI, antegrade epicardial coronary artery infusion; DB, double-blinded; DRP, DNase resistant particles; NA, not applicable; OL, open label; Pi, Phase I; PII, Phase II; PC, placebo-controlled; R, randomized; SDE, sequential dose escalation.
Influx of Ca\(^{2+}\) via L-type Ca\(^{2+}\) channels (LTC) that open with membrane depolarization activates the sarcoplasmic reticulum (SR) Ca\(^{2+}\) release channels known as ryanodine receptor 2 (RyR2), leading to a large increase in sarcoplasmic Ca\(^{2+}\) and muscle contraction. With relaxation, most of the sarcoplasmic Ca\(^{2+}\) is actively transported into the SR lumen by the sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase pump (SERCA2a) and bound to calsequestrin 2 (Casq2), while the remainder exits the cardiac myocyte by way of the Na\(^+\)-Ca\(^{2+}\) exchanger (NCX). Sumoylation by SUMO1 increases SERCA2a activity and protein levels. Phospholamban (PLN) inhibits SERCA2a and this inhibition is relieved by phosphorylation (P) of PLN by protein kinase A (PKA) and Ca\(^{2+}\)-calmodulin-dependent protein kinase (CaMKII; not shown). Dephosphorylation of PLN by protein phosphatase 1 (PP1) restores inhibition. PP1 inhibitor-1 (I-1) opposes the actions of PP1, thus favoring increased SERCA2a activity. Activation of \(\beta\)-adrenergic (\(\beta\)-Ad) receptors leads to an increased activity of adenylyl cyclase (AC), e.g. AC6, and cAMP formation, which in turn activates PKA. \(\beta\)-Ad receptors are desensitized by the serine/threonine kinase \(\beta\)-adrenergic receptor kinase, also referred to as \(\beta\)ARK or G-protein-coupled receptor kinase 2 (GRK2). Besides PLN phosphorylation, PKA enhances contraction by phosphorylating and activating LTC, RyR2 and I-1. The Ca\(^{2+}\) binding chaperone protein S100A1 enhances RyR2 and SERCA2a activity, favoring enhanced calcium turnover during the contractile cycle. Heart failure is associated with reduced expression of SERCA2a and SUMO1, and increased levels of PP1 and GRK2, thereby reducing heart contraction by depleting SR Ca\(^{2+}\) stores and adrenergic stimulation. AAV-mediated gene delivery strategies to increase SERCA2a activity or expression, directly or indirectly by increasing (green star) SUMO1, S100A1, AC6, and I-1 activity/expression, or by decreasing (red star) PP1, PLN, or GRK2 activity/expression have proven beneficial in preclinical models of heart failure. The approach of directly increasing SERCA2a has proven beneficial in early clinical trials. See text for additional details.
Table 2. Summary of preclinical trials targeting calcium handling protein by viral gene therapy that showed benefit for treating heart failure

| Targets                                      | Role                                | Vector/Animals                          | Objective         | Status/Comments          |
|----------------------------------------------|-------------------------------------|-----------------------------------------|-------------------|--------------------------|
| Adenylyl cyclase 6 AC6                       | Indirect increase in SERCA2a activity | Ad-mouse [31,32]/pig [33,34]            | Upregulation      | Ad5.h-AC6 Pl/I clinical trial |
| G protein-coupled receptor kinase (GRK2)     | Desensitize β-adrenergic receptor    | Ad-rabbit [47,48]                       | Inhibition         | Very promising           |
| Phospholamban (PLN)                          | Inhibits SERCA2a activity            | AAV6-sheep [49,50]/rat [51]              | Downregulation    | Promising (Optimization required) |
| Protein phosphatase 1 (PP1)                  | Indirect inhibition of SERCA2a activity via PLB dephosphorylation | AAV-\text{Hamster} [52] | Downregulation/Inhibition | Promising (more studies required) |
| S100 calcium-binding protein AI (S100A1)     | Enhances SERCA2a/RyR2 and SR Ca^{2+} cycling proteins activity | Ad-rat [39] AAV6-rats [40] AAV9-pigs [41] | Upregulation   | Very promising           |
| Small ubiquitin-related modifier 1 (SUMO1)   | Promotes SERCA2a protein stability and activity | AAV9-mouse [26] | Upregulation   | Promising (more studies required) |
| SR Ca^{2+}-ATPase (SERCA2a)                 | Ca^{2+} storage during diastole      | AAV1-rat [20]/pig [21] AAV2-sheep [22,59] AAV6-sheep [60] | Upregulation | AAV1- SERCA2a PlI clinical trial |

Ad, adenovirus; AAV, adeno-associated virus; Pl, Phase I; PlI, Phase II.

SUMO1
Sumoylation is posttranslational modification of SERCA2a that was recently identified as highly relevant to development and potential treatment of heart failure [37]. SERCA2a is sumoylated by SUMO1 on two specific lysine residues in the heart and levels of SERCA2a sumoylation are reduced in the failing human heart and animal models of the failing heart, as are levels of SUMO1 and SERCA2a. Restoration of SUMO1 during transverse aortic constriction-induced heart failure in the mouse by recombinant AAV9 improved cardiac function. Evidence was provided that sumoylation increases both SERCA2a ATPase activity and protein stability. Although SUMO1 seems like a promising target for treating heart failure, its broad-based action in cardiac myocytes may prove an insurmountable limitation [38].

S100A1
This is a Ca^{2+} binding protein that is highly expressed in cardiac myocytes, where it localizes at the sarcoplasmic reticulum, mitochondria, and myofilaments [36]. S100A1 interacts with SERCA2a, phospholamban, and RyR2. Exact details are not defined, but S100A1 seems to function as a Ca^{2+}-sensitive chaperone that enhances the activity of other proteins. End-stage failing human hearts have reduced levels of S100A1 protein, and a number of gene therapy studies have shown the utility of increasing expression of S100A1 to improve cardiac function in heart failure [39-41].

A cautionary note
Results of clinical trials are needed before the utility of gene therapy to treat heart failure can be adequately assessed. A negative outcome in the absence of any serious adverse events may slow, but not deter, further research into this approach. For one thing, there is a certain attractiveness to the simplicity of the idea of treating heart failure by restoring cardiac muscle performance. Of course that begs the question of whether it makes sense to "flog" an already "sick" cardiac muscle cell to make it work harder. Gene therapy to do so may turn out simply to be a way of buying additional time for true myocardial regeneration to take place using cardiac or induced-pluripotent stem cells. The reader is referred to several recent review articles dealing with stem cell therapy in heart failure [42-45].

Summary and future perspectives
Heart failure is a major health issue with a high rate of crippling morbidity and mortality. In the USA, the total annual cost of heart failure is estimated to be greater than 30 billion dollars and is expected to more than double over the next decade, due to an aging population [46]. Thus, there is a substantial need for new strategies to prevent the progression of heart failure. Until now, heart failure was seen as an incurable disease. For the first time, results of recent clinical trials using a viral-delivery gene therapy approach have offered the promise of an effective therapy for reversing systolic heart failure. These trials are the outgrowth of the methodical approach of basic research in identifying viable targets to enhance cardiac contractility. Time will tell whether a single target approach is sufficient to restore heart function and prevent deterioration or whether multiple gene targets are needed along with stem cell therapy to eventually replace the injured myocardium. Using a viral-delivery gene therapy approach to treat heart failure by enhancing contractility is not yet a reality, but substantial progress in that direction has been made in the last few years.
Abbreviations
AAV, adeno-associated virus; AC, adenyl cyclase; GRK2, G-protein-coupled receptor kinase 2; IV, intravenous; PKA, protein kinase A; PP1, protein phosphatase 1; RyR2, ryanodine receptor 2; SERCA2a, sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase pump.

Disclosures
The authors declare that they have no disclosures.

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