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Adeno-associated virus-mediated gene therapy in cardiovascular disease

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Abstract

Purpose of review—The use of adeno-associated virus (AAV) as an efficient, cardiotropic, and safe vector, coupled with the identification of key molecular targets have placed gene-based therapies within reach of cardiovascular diseases. The purpose of this review is to provide a focused update on the current advances related to AAV-mediated gene therapy in cardiovascular diseases and particularly in heart failure (HF) wherein gene therapy has recently made important progress.

Recent findings—Multiple successful preclinical studies suggest a potential utility of AAV gene therapy for arrhythmias and biological heart pacing, as well as RNA overexpression. Moreover, AAV-mediated overexpression of several molecular targets involved in HF has demonstrated promising results in clinically relevant large animal models. In human, a safe and successful completion of a phase 2 clinical trial targeting the sarcoplasmic reticulum calcium ATPase pump with AAV has been reported. Serial studies are ongoing to further prove the efficacy of AAV-mediated sarcoplasmic reticulum calcium ATPase pump gene transfer in human HF.

Summary—Significant progress in clinical translation of AAV-mediated cardiac gene therapy has been achieved in recent years. This will prompt further clinical trials, and positive results could open a new era for cardiac gene therapy.

Keywords

adeno-associated virus; arrhythmias; gene therapy; heart failure; sarcoplasmic reticulum calcium ATPase pump

INTRODUCTION

Cardiovascular disease is a major cause of morbidity and mortality in the United States [1]. Despite optimal guideline-directed therapy employing a wide range of pharmacological, device, and surgical options, many patients deteriorate over time and develop refractory

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Conflicts of interest

R.J.H. is a cofounder of Celladon, which is developing AAV1.SERCA2a for the treatment of heart failure.

symptoms [2]. Considering the medical, economic, and quality-of-life consequences of these trends, novel treatment strategies for cardiovascular diseases, especially those aimed at reducing hospitalizations, are needed.

Gene therapy was initially applied clinically for inherited monogenic disorders. It is now apparent that gene therapy has a broader potential for acquired diseases such as congestive heart failure (HF). Improvement in our understanding of the molecular mechanisms in cardiovascular diseases has led to the identification of novel targets that are difficult to manipulate pharmacologically, but may be more amenable to gene therapy.

The application of adeno-associated viral (AAV) vectors has accelerated the translation of gene therapy to the cardiac field because AAVs confer prolonged gene expression after a single delivery compared with adenovirus or plasmid-based gene delivery approaches [3]. Furthermore, the lack of human disease caused by AAV makes them an attractive tool for clinical cardiac gene therapy [4]. Indeed, a first-in-human clinical trial targeting HF with virus-mediated overexpression of sarcoplasmic reticulum calcium ATPase (SERCA2a) pump employed AAV serotype 1 [5] has shown to be safe and effective in phase 1 and phase 2 trials [6,7▪].

The purpose of this review is to provide a focused update on the current advances related to AAV-mediated gene therapy in cardiovascular diseases and particularly in the HF field in which gene therapy has recently made important progress.

ADENO-ASSOCIATED VIRUS FOR GENE THERAPY

The appropriate combination of specific vectors and therapeutic genes are key for successful gene therapy. AAV had originally been identified as a contamination in Simian adenovirus preparations [8]. AAV is part of the parvovirus family and is a small, nonpathogenic human virus with a nonenveloped capsid and single-stranded DNA [4]. Of the 13 serotypes known so far, AAV 1, 6, 8, and 9 have shown cardiac tropism [9]. There are several reasons why AAV vectors are increasingly used as gene therapy vectors in the setting of cardiovascular diseases. First, recombinant AAVs used for gene therapy are nonpathogenic in humans and do not integrate in the host genome [4]. Second, AAVs are minimally immunogenic especially when compared with adenovirus [10]. Third, the small size of AAV is an advantage as it allows for their delivery to the myocardium by infusion through the coronary arteries; and finally, AAV vectors are able to induce long-term transgene expression in nondividing cells after a single delivery [11].

One of the main drawbacks of using AAV in gene therapy applications is the limited packaging capacity of ~4.7 kb [4]. To overcome this limitation, trans-splicing based or recombination-based overlapping AAV vector approaches have increased the gene delivery capacity. Two independent AAV vectors each carrying parts of a transgene with appropriate splice signals or with overlapping sequence elements are independently packaged in AAV vectors [12]. More recently, a triple AAV vector coinfection was tested with success in mice to reconstitute the functional, full-length dystrophin coding sequence [13]. Whether these approaches are effective in cardiac gene transfer remains to be evaluated in future studies.

The fact that many AAV serotypes appear to be endemic, results in extensive antiviral immunity in human populations [14]. From a clinical point of view, this issue is very significant because the presence of neutralizing antibodies against the AAV serotype limits the potential clinical candidates for the gene therapy [15▪]. Over the past years, significant effort has been put into the development of AAV variants that circumvent the effect of neutralizing antibodies [14,16]. Recently, a chimeric AAV vector developed by capsid reengineering was shown to effectively transduce the heart for the first time in a clinically relevant large animal model [17▪]. Additionally, plasmapheresis has shown encouraging results in preclinical studies to reduce the effect of antibodies [14,18].

ADENO-ASSOCIATED VIRUS CARDIAC DELIVERY METHODS

The vector delivery method is another important factor for successful cardiac gene transfer. Although intravenous injection is effective to transduce the heart in mice, more advanced animals require cardiac-specific delivery even with the cardiotropic AAV vectors. In the first-in-human clinical trial of gene therapy in the setting of HF, antegrade intracoronary injection was the choice for AAV delivery because of its relatively safe, simple, and minimally invasive nature [5]. Several different approaches are proposed to increase the transduction efficacy and cardiac specificity using large animals. Retrograde delivery into the coronary sinus was tested in pigs using AAV-6 with successful transduction to the heart [19]. There is also a surgical recirculation delivery method, which was recently applied to deliver AAV-9 SERCA2a in a sheep model that resulted in positive improvement in the left ventricular function [20▪]. This approach may reduce the extracardiac gene expression, and at the same time circumvent the antibody inhibition by AAV. Although this approach is more invasive than catheter-based delivery, it may be first applied to patients who are scheduled for bypass surgery or valve replacement surgery to establish its clinical utility. The same group also reported a novel method to deliver AAV by liquid jet injection [21]. Although safety and efficacy of these delivery methods need to be further established in future studies, such attempts for novel delivery approaches will certainly advance the cardiac gene therapy field. The optimal clinically translatable technique for global cardiomyocyte delivery has yet to be developed and the ideal delivery method is likely to vary depending on the target gene.

MOLECULAR TARGETS

AAV-mediated overexpression of several molecular targets has demonstrated promising results in cardiovascular disease.

Sarcoplasmic reticulum calcium ATPase pump

Abnormal calcium cycling is a common characteristic in patients with advanced HF regardless of cause. Both the amplitude and decay rate of the intracellular calcium transient are blunted in cells and tissues from failing hearts [22]. SERCA2a plays a pivotal role in calcium handling and numerous studies have shown that SERCA2a expression is decreased in HF, whereas restoring the SERCA2a expression level improves cardiac function [23]. Supported by extensive preclinical studies focusing on efficacy and safety of SERCA2a

gene therapy using an AAV vector, this approach is currently being investigated in humans. The results of clinical trials are discussed later in this review [6,7▪].

Abnormal calcium cycling plays a key role in the pathophysiology of vascular remodeling and SERCA2a is downregulated. Recently, another beneficial effect of AAV-1 mediated SERCA2a gene therapy on the vasculature was focused and investigated. In a pig model of mitral regurgitation, AAV-1 mediated SERCA2a gene transfer increased coronary flow through enhanced endothelial nitric oxide synthase expression in endothelial cells [24]. Furthermore, aerosol delivery of AAV-1 SERCA2a in a mouse model of monocrotaline-induced pulmonary arterial hypertension decreased pulmonary arterial remodeling and suppressed right ventricular remodeling [25▪]. Further study in more advanced species is awaited to enable translation of this promising approach into clinics.

Small ubiquitin-like modifier 1

AAV-based experiments have proven the important role of proteins associated with SERCA2a. A recent study has brought to light the interaction between SERCA2a and the small ubiquitin-related modifier 1 (SUMO-1) through a posttranscriptional modification process called SUMOylation [26]. The amount of myocardial SUMO-1 is decreased in failing hearts, and its knockdown results in severe HF in mice [27]. SUMO-1 was shown to preserve SERCA2a function and stability, and the overexpression of SUMO1 in a rodent model of HF had favorable effects on myocardial function [26]. AAV-9 gene transfer of SUMO-1 prevented the heart from undergoing hypertrophy after transverse aortic constriction and prevented the development of left ventricular dysfunction in mice. Furthermore, SUMO-1 expression protects SERCA2a from oxidative stress [28]. Toward clinical translation, a recent study evaluated the effects of SUMO-1 gene transfer using AAV-1 in a swine model of ischemic HF. Compared with control animals, SUMO-1 gene transfer and its combination with SERCA2a improved cardiac function and halted left ventricular remodeling [29▪]. These results support the critical role of SUMO-1 in SERCA2a regulation and underline the therapeutic potential of SUMO-1 for HF patients.

S100A1

The calcium sensor protein S100A1 has emerged as an attractive target for HF gene therapy because of its characteristic molecular profile [30]. The S100A1 protein regulates a network in cardiomyocytes that controls sarcoplasmic reticulum calcium cycling and mitochondrial function through interaction with the ryanodine receptor, SERCA2a, and mitochondrial F1-ATPase activity, causing antihypertrophic, positive inotrope, and antiarrhythmic effects and reducing energy depletion in HF [31]. Recently, large animal studies in a swine model of HF showed the feasibility, therapeutic efficacy, and safety of AAV-6-mediated and AAV-9-mediated S100A1 gene therapy [32,33▪]. Clinical trials with AAV9.S100A1 are being planned in the coming few years.

Protein phosphatase 1 inhibitor-1

The decrease in activity of SERCA2a observed in HF is at least partially attributable to enhanced phospholamban (PLN) inhibition. It has been found that protein phosphatase 1 activity serves to regulate the phosphorylation pattern of PLN [34]. Expression of protein

phosphatase 1 is elevated in HF, limiting the deactivation of PLN, and this is associated with decreased expression of inhibitor-1 (Fig. 1) [34,35]. Overexpression of the constitutively active, truncated form of inhibitor-1(I-1c) in the heart results in significant enhancement of PLN phosphorylation that results in increased cardiac function and abrogation of the negative effects in small animal aortic constriction models [36]. Recent studies report improved cardiac function after I-1c gene transfer in clinically relevant models of postmyocardial infarction in pigs using AAV-9 and a chimeric vector of AAV-2 and AAV-8 [17▪, 37▪]. This novel chimeric vector was able to de-target the liver, which is an intriguing feature for more cardiac-specific gene delivery [17▪]. Clinical trials with the chimeric vector expressing I-1c are planned to start in 2015 in patients with severe heart failure.

As inhibitor-1 is an upstream regulator of SERCA2a, this molecule also seems to have influence on vascular remodeling. Phenotypic switching of vascular smooth muscle cells from a contractile/quiescent to a proliferative/synthetic phenotype plays a key role in vascular proliferative syndromes such as atherosclerosis [38]. Recently, a synergistic role of I-1 and SERCA2a in the acquisition of the vascular smooth muscle cells contractile phenotype was reported [39▪]. Moreover, local I-1c gene transfer in a rat model of carotid injury decreased neointimal formation by preserving vascular smooth muscle cell contractile marker expression, and therefore preventing a vascular smooth muscle cell phenotypic switch [39▪]. Hence, in addition to the potential application in HF, I-1c gene transfer appears to be a promising strategy for preventing vascular proliferative disorders.

Inhibition of G protein-coupled receptor kinase-2 by overexpression of βARKct

β-adrenergic receptor system dysregulation is another molecular characteristic of HF [40]. In the course of HF, the hyperactivity of the sympathetic nervous system becomes detrimental over time, causing uncoupling and downregulation of β-adrenergic receptors [41]. G protein-coupled receptor kinase-2 (GRK2), which is markedly upregulated in failing human myocardium plays a key role in the downregulation of β-adrenergic receptors [40]. Targeted inhibition of GRK2 is possible using a peptide inhibitor known as βARKct. When βARKct is expressed in transgenic mouse models or through adenovirus administration, GRK2 activity is blocked, leading to improved cardiac function [42]. Recently, a large animal preclinical model of HF showed that myocardial AAV-6 delivery of βARKct reversed ventricular dysfunction, remodeled the heart, and lowered sympathetic outflow of catecholamines, indicating that βARKct administration and GRK2 inhibition are promising therapeutic targets in HF [19].

RNA

Several studies have attempted to overexpress RNAs using AAV. Successful transduction of RNAs enabled overexpression of therapeutic microRNAs (miRNAs), as well as gene silencing by RNA interference using short-hairpin RNA or small interfering RNA [43,44]. miRNAs are small noncoding RNA that modulate gene expression [45]. Accumulating evidence suggests a critical involvement of miRNAs in the development of cardiovascular disease. Recently, two promising miRNAs have demonstrated therapeutic efficacy in reducing pathological cardiac remodeling using AAV-mediated gene transfer. miR-1 overexpression with AAV-9 resulted in cardiac hypertrophic regression and halted

functional deterioration in a rat model of transverse aortic constriction [46]. Similarly, miR-378 overexpression was associated with prevention of cardiac hypertrophy and reduced fibrosis in a mouse model of HF [47]. Whether miRNA-based approaches are effective in clinically relevant models currently remains unknown, and future studies are expected to test these promising approaches in larger animals. The use of AAV to increase the level of miRNAs also represents a valuable investigational tool to explore the role of miRNAs in cardiovascular disease pathophysiology. For example, this technology helped to describe the involvement of miR-25 in HF and to identify it as a potentially new therapeutic target [48▪].

Other targets

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is an inherited arrhythmogenic disorder characterized by sudden cardiac death. Drug therapy is still insufficient to provide full protection against cardiac arrest. The disease is related to abnormal calcium storage and release from sarcoplasmic reticulum within the cardiomyocytes, with causative mutations in two key proteins: the ryanodine receptor and the calcium buffering and ryanodine receptor regulatory protein calsequestrin (CASQ2) [49]. In a CPVT knock-in mouse model carrying the CASQ2(R33Q/R33Q)(R33Q) mutation, Denegri *et al.* [49] recently showed that AAV-9.CASQ2 gene therapy prevents and reverts severe manifestations of CPVT.

Electronic cardiac pacing provides effective treatment for atrial-ventricular block and/or sinus node dysfunction. However, the possibility of replacing wires and power supplies with a biological pacemaker using gene therapy is very attractive [50]. A recent proof-of-concept study demonstrates the feasibility of a gene therapy somatic cell reprogramming strategy for creating a biological pacemaker in a large animal preclinical model of complete heart block [51▪]. The gene of the embryonic transcription factor T-box 18 (TBX18) using an adenoviral vector delivery, conferred a sinus-node-like phenotype on adult porcine ventricular myocytes, generating ventricular pacemaker activity that was responsive to autonomic regulation during daily activity [51▪]. An earlier study described the feasibility of another gene therapy approach leading to the coexpression of skeletal muscle sodium channel 1 with HCN AQ5 channel 2 in another large animal model [52]. Before a translation to human clinical studies, ongoing efforts focus on safe delivery of long-term biological pacemaker function based on AAV-mediated gene transfer [50].

CLINICAL TRIALS

A Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease phase 2 trial using AAV-1 to deliver SERCA2a gene therapy by coronary anterograde infusion to patients with chronic HF has been successfully completed. Clinical event rates were significantly lower 3 years after gene transfer in patients receiving high-dose AAV-1.SERCA2a compared with those receiving 0.9% saline [6,7▪]. In addition, this study has shown the long-term persistence of the SERCA2a gene in the myocardium up to 31months after gene transfer [7▪]. Further clinical studies are currently underway including a large international study in 250 patients, evaluating whether high-dose AAV-1.SERCA2a (1×10^{13} viral genomes) versus placebo, randomized 1 : 1, is an effective therapy to reduce cardiovascular events in advanced HF [15▪]. In Europe, two additional double-blind

randomized placebo-controlled studies are currently recruiting participants in which the safety and feasibility of AAV-1.SERCA2a therapy is being tested in 24 HF patients that have received a left ventricular assist device (LVAD) for an accepted clinical indication (SERCA-LVADstudy; NCT00534703). Moreover, the impact of AAV-1.SERCA2a therapy on left ventricular remodeling is specifically studied as a primary end point using multimodality cardiac imaging in 44 patients with severe HF (Agent-HF study; NCT01966887).

CONCLUSION

Vector modification to enhance and control gene transduction, and improvement in delivery methods, together with identification of novel therapeutic targets will continue to drive the cardiac gene therapy field forward. Increasing numbers of cardiac gene therapy studies using AAV are being conducted in clinically relevant large animal models. This will pave the way to more trials in clinical gene therapy that will ultimately benefit patients with cardiovascular diseases.

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KEY POINTS

- AAV vectors are safe and confer long-term expression.
- A large clinical trial testing the efficacy of AAV-1. SERCA2a gene therapy is underway.
- Several molecular HF targets such as SUMO-1, S100A1, and βARKct have been successfully targeted in relevant large animal models and are likely to be clinically evaluated in the near future.
- In addition to HF, preclinical studies suggest the potential utility of AAV gene therapy for arrhythmias and biological heart pacing.

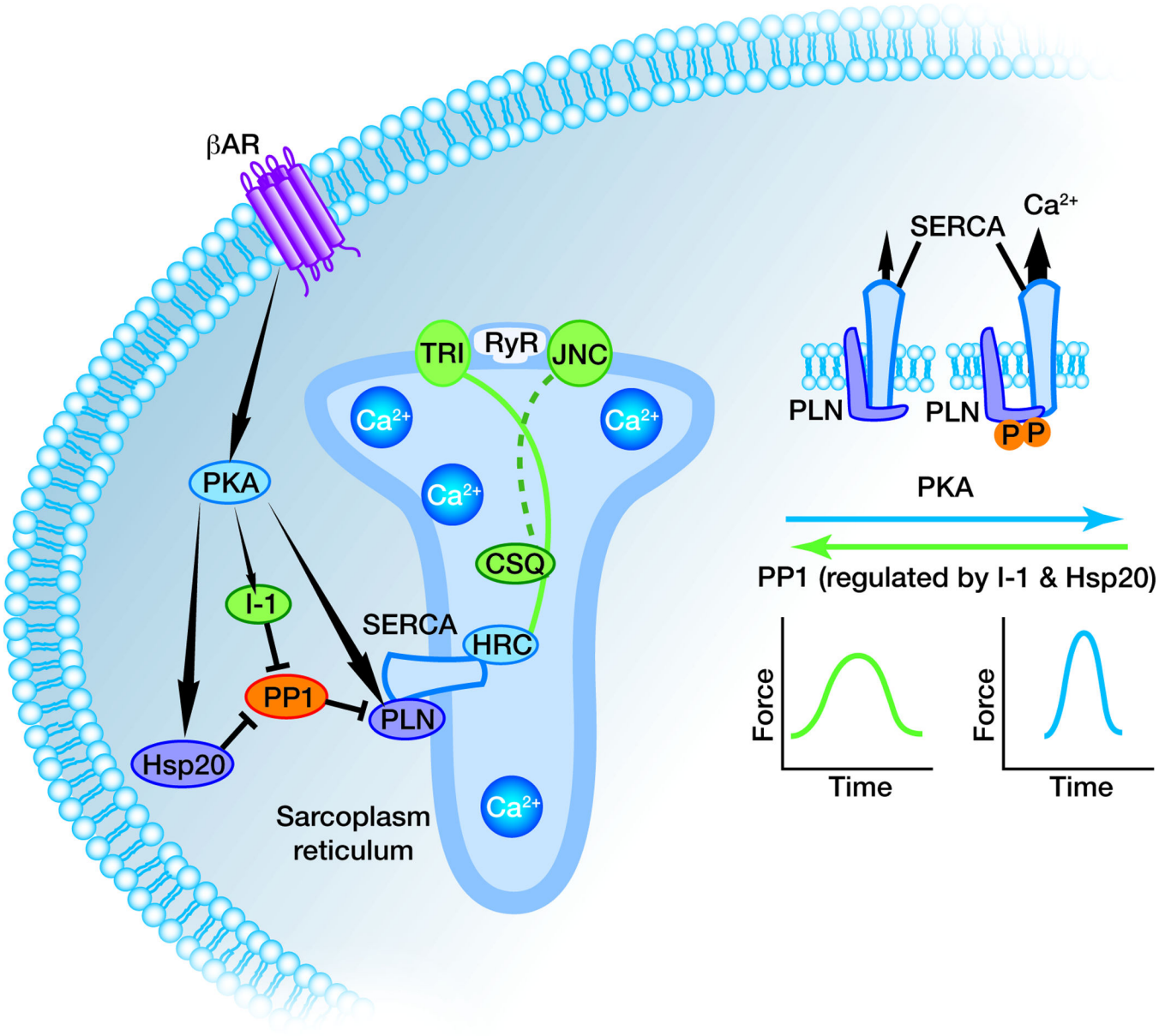

FIGURE 1.

Schematic representation of the sarcoplasmic reticulum calcium ATPase (SERCA2a) regulation by protein AQ6 phosphatase 1 inhibitor-1, SERCA2a activity is downregulated by phospholamban. The phosphorylation of phospholamban (PLN) reduces its inhibitor effect on sarcoplasmic reticulum calcium ATPase (SERCA2a) pump. During β-adrenergic stimulation PLN is phosphorylated by protein kinase A (PKA). PLN dephosphorylation is due to protein phosphatase 1 (PP1). Inhibition of PP1 by inhibitor-1 (I-1) leads to enhance the phosphorylation of PLN that results in increased SERCA2a activity. PP1 is also regulated by heat shock protein 20 (Hsp) 20. The sarcoplasmic reticulum calcium (Ca^{2+})

release complex included ryanodine receptor (RyR), triadin (TRI), junctin (JNC) and calsequestrin (CSQ). Reproduced with permission from [35].