Emerging Strategies for Chronic Heart Failure: The Role of Gene Therapy

Elena Giulia Milano, MD, Mariantonietta Cicoira, MD, PhD
Department of Medicine, Section of Cardiology, University of Verona, Verona, Italy

Heart failure (HF) is a complex clinical syndrome and a major growing public health problem in Western countries. HF is a leading cause of death and morbidity in modern society, and its incidence continues to increase with the aging population. The complexity of this syndrome and its multifactorial origin constitute problems in the management of patients. Pharmacological treatments aim to interfere with the activation of the neurohormonal and adrenergic systems, which are key pathophysiological mechanisms underlying disease progression. Despite the improvements achieved by current therapies, patients in end stages of the disease still have a poor prognosis. Gene therapy represents a new approach to the treatment of HF, with the ambitious aim of repairing the molecular abnormalities that lead to the disease. Current medical management of clinical HF and novel gene therapies for treatment of HF are presented here.

[Rev Cardiovasc Med. *2012;13(4):e161-e168 doi: 10.3909/ricm0609*]

KEY WORDS

Heart failure • Medical therapy • Gene therapy

Heart failure (HF) is a major and growing public health problem. It is mainly a condition of the elderly; thus, the widely recognized aging of the population, as well as success in prolonging survival after acute coronary events, has contributed to the increasing incidence of HF.[1] HF incidence approaches 10 per 1000 population after age 65 years.[2] HF may result from disorders of the pericardium, myocardium, endocardium, or great vessels, but the majority of patients with HF have symptoms due to an impairment of left ventricular (LV) myocardial function. The multifactorial nature of HF is the result of interaction between genetic and environmental features and is the reason for the

complexity of HF treatment and management. HF can be associated with a wide spectrum of LV functional abnormalities, which range from normal LV size and preserved ejection fraction (EF), to severe dilatation and/or markedly reduced EF, to LV diastolic dysfunction. Coronary artery disease, hypertension, valvular heart diseases, and dilated cardiomyopathy are the causes of HF in a substantial proportion of patients in the Western world.

A new approach to the classification of HF has recently been developed by the American Heart Association (AHA),[3] which emphasizes both the development and progression of the disease. Four stages in the development of HF syndrome have been proposed (Table 1).

LV dysfunction begins with an injury to, or stress on, the myocardium and is generally a progressive process, even in the absence of an identifiable insult to the heart. The principal manifestation of such progression is a change in the geometry and structure of the LV, such that the chamber dilates and/or hypertrophies and becomes more spherical, a process referred to as cardiac remodeling. Although several factors can accelerate the process of LV remodeling, there is substantial evidence that the activation of endogenous neurohormonal systems plays an important role in cardiac remodeling and thereby in the progression of HF.[4] Patients with HF have elevated circulating or tissue levels of norepinephrine, angiotensin II, aldosterone, endothelin, vasopressin (AVP), and proinflammatory cytokines, which can adversely affect the structure and function of the heart. These neurohormonal factors not only increase hemodynamic stress on the ventricle by causing sodium and water retention and peripheral vasoconstriction, but may also directly exert toxic effects on cardiac cells and stimulate myocardial fibrosis, which can further alter the architecture and impair the performance of the failing heart.[3] Neurohormonal activation also has direct deleterious effects on the myocytes and interstitium, because it may alter the performance and phenotype of these cells. The decreased cardiac output results in an unloading of high-pressure baroceptors in the left ventricle, carotid sinus, and aortic arch. This afferent signal to the central nervous system stimulates the release of AVP, which acts as a powerful vasoconstrictor and stimulates the absorption of water in the kidney. The sympathetic stimulation of the kidney leads to the activation of the renin-angiotensin-aldosterone system (RAAS). The RAAS promotes salt and water retention, vasoconstriction, myocyte hypertrophy, and myocardial fibrosis (Figure 1).

Current Treatments

According to the AHA guidelines, the treatment for chronic HF

TABLE 1

Stages of Heart Failure

Stage	Definition	Therapy Goals	Drugs
Stage A	At high risk of HF but without structural heart disease or symptoms of HF	Treat hypertension, lipid disorders, smoking cessation, regular exercise, control metabolic syndrome	ACE inhibitors or ARB only in appropriate patients
Stage B	Structural heart disease but without signs and symptoms of HF	All measures under stage A	ACE inhibitors or ARB in appropriate patients; β-blockers in appropriate patients
Stage C	Structural heart disease with prior or current symptoms of HF	All measures under stage A and B; dietary salt restriction	Diuretics, ACE inhibitors, β-blockers; aldosterone antagonists, ARBs, digitalis, nitrates in selected patients
Stage D	Refractory HF requiring specialized intervention	All measures under stage A, B, and C; appropriate level of care	End-of-life care; extraordinary measures: heart transplant, chronic inotropes, permanent mechanical support

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker; HF, heart failure.
Adapted from the American Heart Association 2005 Guidelines.[3]

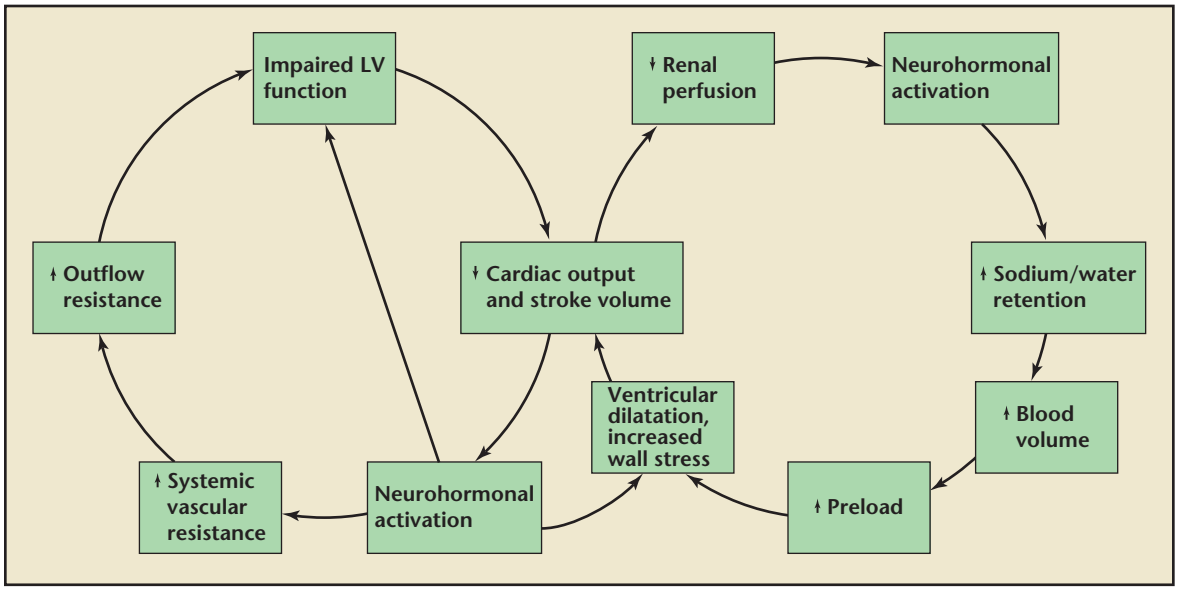

Figure 1. The vicious cycle of heart failure. LV, left ventricular.

differs depending on the New York Heart Association (NYHA) class and the stage of the disease.[3] Once a patient develops structural heart disease and symptoms of HF, management includes several therapeutic strategies.

Inhibitors of the RAAS Pathway

Although many factors are involved in the acceleration of LV remodeling, activation of endogenous neurohormonal mechanisms plays a particularly important role in cardiac remodeling; thus, their modulation might be beneficial in patients with HF. Angiotensin-converting enzyme (ACE) inhibitors were evaluated in more than 7000 patients (mostly with reduced EF) in more than 30 placebo-controlled trials.[5] These studies show that ACE inhibitors improve symptoms, reduce hospitalization, and decrease mortality in patients with HF.[6] Aldosterone antagonists were shown to further reduce the risk of hospitalization and death in patients with NYHA classes II-III and IV HF in two different clinical trials.[7]

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β-Blockers

β-blockers inhibit the adverse effects of activation of the sympathetic nervous system in patients with HF. β-blockers have now been evaluated in more than 20,000 patients with HF who participated in more than 20 published placebo-controlled clinical trials.[8-10] This collective experience indicates that long-term treatment with β-blockers can lessen the symptoms of HF, improve clinical status, enhance the patient's overall sense of well-being, and prolong survival.[11]

Diuretics

Diuretics interfere with sodium and water retention and have been shown to improve symptoms in patients with decompensated clinical HF, but do not affect outcome.[11] They reduce pulmonary congestion, peripheral edema, and body weight and improve exercise tolerance in patients with HF (Stages C and D).

Digitalis

Digitalis glycosides inhibit sodium-potassium adenosine triphosphatase (Na-K ATPase) in cardiac cells to increase contractility, but also in vagal afferent fibers and the kidneys, which helps modulate the neurohormonal imbalance in HF. Placebo-controlled trials showed that treatment with digoxin improves symptoms in HF but has no effect on mortality. However, it has a narrow therapeutic window and should be used with caution.[12]

Cardiac Resynchronization Therapy

Cardiac resynchronization therapy (CRT) is a pacemaker-based approach to the treatment of patients with HF. Its purpose is to provide electromechanical coordination and improved ventricular synchrony in symptomatic patients.[4] When used in association with medical therapy, CRT improves the quality of life and survival.[13]

LV Assist Devices and Cardiac Transplantation

LV assist device implantation and cardiac transplantation represent the last therapeutic choices available in patients with severe end-stage disease.[11] Due to the limited number of donors, cardiac transplantation is available only to few patients, and an LV assistance device is indicated in very selected cases.

Our increasing understanding of the molecular mechanisms involved in the pathogenesis of HF is paving the way to new possibilities for treating this disease.

New Gene Therapies

During the past 30 years, hundreds of pharmacological agents have been developed for the treatment of HF, yet few of them have been tested in patients. Pharmacological intervention with β-adrenergic receptor (β-AR) antagonists, inhibitors of angiotensin II and aldosterone, and diuretics are currently standard treatments for HF. The introduction of these pharmacological interventions substantially increased survival and decreased morbidity in HF; however, mortality and hospitalization rates remain unacceptably high, and consequently, there is an urgent need to discover and develop new, improved therapeutic strategies.[14] Our increasing understanding of the molecular mechanisms involved in the pathogenesis of HF is paving the way to new possibilities for treating this disease.

Cardiovascular gene therapy models were among the earliest in the field, and more recently gene and cell therapies have demonstrated significant potential for the treatment of common cardiovascular problems such as refractory myocardial ischemia and dysfunction.[15] This promising field is gaining attention; preclinical studies in larger animals (pigs and sheep)[16] have recently been carried out and the first in human clinical trial has now reached phase 2.[17]

In principle, gene therapy in HF must aim at correcting key molecular mechanisms in cardiac tissue so as to reduce or reverse the inevitable cardiac deterioration. This requires the introduction of DNA/RNA that targets specific cardiomyocyte processes, which alter HF outcomes, using specific vectors and delivery techniques. The ideal gene therapy strategy would depend on the specific cause of the disease (ischemic, valvular, hypertension, genetic)[14] and the molecular targets would vary depending on the etiology of HF. As an example, gene therapy for HF which is a consequence of ischemic heart disease would ideally involve (1) targeting vascular cells by stabilizing coronary plaques, (2) preventing viable cardiomyocytes from dying (via inhibiting apoptosis and/or necrosis), (3) reducing the risk of arrhythmias by targeting myocardial electrophysiological alterations, and (4) reducing myocardial remodeling by targeting fibroblasts.[14] The genetic intervention to achieve these results could be (1) overexpression of a target molecule, (2) alteration of the target's intracellular shuttling routes, (3) loss of function approaches using dominant negative molecules or using RNA interference, and (4) correction of damaging gene mutations/deletions at the genome or primary messenger RNA level.[14]

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Vectors

Successful gene therapy requires efficient and specific transduction and long-term transgene expression. The ideal vector would have to have some important features, such as high affinity for the tissue target, promoters to maximize transgene expression, and regulatory elements to obtain synchronization between transcription and biochemical stimuli.[18,19] Only viral vectors have been shown to meet these requirements, and of these, adenoviruses (AdVs) and adeno-associated viruses (AAVs) are the most commonly used.

Gene delivery systems can be classified into two main groups: nonviral physicochemical systems and recombinant viral systems. Nonviral vectors can be loosely grouped as plasmid DNA, liposome-DNA complexes (lipoplexes), and polymer-DNA complexes (polyplexes).[15,20] The strengths of nonviral systems include the ease of vector production, reduced limitation of expression cassette size, and relatively minimal biosafety risks. The limitations include low transfection efficiency and transient effect due to intracellular degradation.

The predominant use of viral vector systems in preclinical models of gene therapy reflects the increased gene transfer efficiency achievable with these systems.[15] The strengths of viral systems include relatively high gene transfer efficiency and, when integrating vectors are used, the capacity for long-term transgene expression. The limitations include reduced packaging capacity and biosafety risks.[18] The latter include the generation of replication competent viruses, toxicity due to viral gene products, and insertional mutagenesis when integrating vectors are used.[21] The three most common viral vectors are shown in Table 2.

TABLE 2

Characteristic of Viral Vectors

Vectors	Adenovirus	AAV	Lentivirus
Genome	Double-stranded DNA	Single-stranded DNA	Single-stranded RNA
Insert capacity	7-30 kb	4.8 kb	7-10 kb
Pattern of gene expression	Transient	Long term	Long term
Cell cycle–dependent transduction	No	No	No
Host/vectors interaction	Cytotoxic and immunogenic	Minimally immunogenic	Minimally immunogenic

AAV, adeno-associated viruses.
Adapted from Ly H et al.[15]

The advantages of AdVs are that they have excellent tissue-specific myocardial transduction, large transgene cloning capacity (7-8 kb), and can be easily manipulated.[22] AdV vectors have been an extremely useful research tool.[18] However, in vivo delivery leads to an inflammatory response that causes a transient transgene expression; furthermore, repeated delivery results in a secondary immune response.[23] These side effects can be minimized by using the newer "gutted" AdVs, which lack the immunogenic epitopes. The advantages of AAVs are that they are less immunogenic and have excellent long-term stable transgene expression.[22] There are currently 12 different AAV serotypes, each with different tissue tropisms.[24] AAV type 1 (AAV1) transduces to skeletal and cardiac muscle efficiently. Moreover, some serotypes display tropism toward cardiac tissue. However, they can carry only small amounts of genetic material (4-5 kb), and some are targeted by inherent antibodies in humans, which limits their use.[11]

Delivery Techniques

Once a molecular target and gene delivery system have been chosen, the next step is to deliver the vector to the site of interest within the cardiovascular system. There are several approaches to vector delivery in rodents. Many of these are relevant only to small animal models, whereas others rely on percutaneous techniques that are readily transferable from large animals into clinical practice.[15] The ideal delivery technique would have the following characteristics: safety, minimal invasiveness, and repeatability in order to increase the concentration in target zones.[25]

The success of gene therapy will certainly depend on improvements to the current delivery methods.

Gene delivery has been performed in animal models through the intravenous, intracoronary, and direct intramyocardial routes. An intravenous approach would be ideal but may prove to be impossible in humans, as large blood volume dilutes the effective concentration of viral vectors reaching the myocardium; however, the cardiac muscle tropism of some AAV serotypes, even in large animals, suggests that this approach is feasible.[14] Intracoronary delivery is clinically applicable; however, this approach is also generally inefficient unless certain adjuvant measures are taken to enhance transduction. Direct intramyocardial injection results in good transduction and can be used at the time of cardiovascular surgery,[11] but could be performed only in the relatively few patients selected for surgery. In the first in human trial,[26] percutaneous intracoronary delivery was chosen and accomplished using standard catheters, with infusion occurring over a 10-minute period.

Many questions remain unanswered: what the appropriate levels of gene transcription and translation are, whether restoration of myocardial contractility requires gene transfer to the majority of target cells, and what proportion of cardiac myocytes need to be transfected to obtain global gene delivery.[27] The success of gene therapy will certainly depend on improvements to the current delivery methods.

Targets

The number of molecular targets for each pathophysiology is likely to increase with advances in the knowledge of the molecular basis of cardiovascular disease. Many targets have already been identified (Table 3).

Improving Perfusion. Ischemic heart disease is the most important cause of congestive HF; therefore, angiogenic gene therapies aiming to improve perfusion could be

TABLE 3

Molecular Gene Targets

Mechanism	Molecular Target
Improve perfusion	VEGF
	FGF
Calcium handling	SERCA
	Phospholamban
	Protein phosphatase and inhibitor protein
β-adrenergic receptor pathway	β_2 receptor
	GRK-2
	Adenilate cyclase

FGF, fibroblast growth factor; GRK, G protein-coupled receptor kinase; SERCA, sarcoplasmic reticulum Ca^{2+} adenosine triphosphatase; VEGF, vascular endothelial growth factor.
Adapted from Jameel and Zhang.[11]

extremely beneficial.[11] Several clinical trials of angiogenic therapies have been conducted. The phase II Kuopio angiogenesis trial showed improvement in myocardial perfusion at 6 months after intracoronary administration of Ad-VEGF165 in patients undergoing angioplasty, but no difference in the restenosis rate.[28]

Targeting Proteins Involved in Cardiomyocyte Calcium Handling. The handling of calcium during excitation contraction coupling is abnormal in failing hearts. Several molecular targets in this pathway have been used in gene therapy.[11]

Sarcoplasmic reticulum Ca^{2+}-ATPase. The sarcoplasmic reticulum (SR) Ca^{2+}-ATPase in myocytes is known as SERCA2a and its activity is reduced in HF, resulting in decreased calcium uptake and impaired relaxation.[29] SERCA2 activity is controlled by phospholamban (PLN); dephosphorylated PLN inhibits SERCA2a, whereas phosphorylated PLN reduces this inhibition. SERCA2a gene therapy would aim to increase SERCA2a activity, resulting in quicker calcium uptake and thus improved diastolic relaxation using two approaches: SERCA2a overexpression and phospholamban inhibition. This would also increase contractile reserve because of higher SR calcium concentration.[11] SERCA2a gene therapy in isolated cardiomyocytes from failing human hearts restored their contractile function.[30] The positive results observed in preclinical tests led to the initiation of the first human clinical trial of gene therapy, now in phase II. In the Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease study (CUPID), a randomized double-blind trial, a total of 39 patients with advanced HF received intracoronary AAV1/SR Ca^{2+}-ATPase or placebo. AAV1/SERCA2a demonstrated an acceptable safety profile in a population with advanced HF. In the trial, patients who received an intracoronary administration of high-dose AAV1/SERCA2a showed clinically significant improvements in symptoms, functional capacity and cardiac structure, as well as a significant reduction of clinical events and hospitalization times.[17]

PLN. Through its potent action on SERCA2a activity, PLN plays a critical role in the regulation of SR Ca^{2+} homeostasis, mediating slower cytosolic Ca^{2+} decay in cardiomyocytes, which translates into prolonged diastolic relaxation both in vitro and in vivo. Recombinant gene transfer in human family cardiac myocytes with antisense PLN resulted in enhanced contractile properties, similar to those of cardiac myocytes infected with SERCA.[31] Even with a substantial amount of evidence showing that abolishment of PLN and/or removal of the inhibitory effects of PLN on SERCA2a can be beneficial in experimental HF, it is still unclear what the outcome would be if used in a human HF population.[14]

Protein phosphatase 1 and inhibitor protein-1. The inhibitory action of PLN on SERCA2a is subject to tight secondary control mediated by protein phosphatase (PP)1 dephosphorylation. PP1 activity itself is also subject to rigorous control via the actions of phosphatase inhibitors, inhibitor protein I-1 and I-2. β-AR–mediated PKA activation promotes I-1 to attenuate PP1 activity toward PLN, and this results in sustained PKA-mediated phosphorylation of PLN, which increases SERCA2a activity. Therefore, targeting PP1 and its inhibitory proteins to ultimately increase SERCA2a pump activity may constitute a promising gene therapy strategy in human HF, although there is clearly a need for further studies involving different etiologies of HF and preferably larger experimental animal models.[14]

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Targeting the β-Adrenergic System. Chronic HF is associated with increased sympathetic outflow, which contributes to worsening LV

function. A number of alterations in the β-AR signaling cascade have been described in this context, and these include β-AR down-regulation, up-regulation of β-AR kinase (βARK), and increased inhibitory G-protein α-subunit function. Together, these alterations desensitize β-ARs and diminish signaling through this pathway.[15]

β_2-AR overexpression. Overexpression of β_2-AR in mouse hearts results in improved systolic and diastolic function; however, with extremely high levels, mice develop fibrotic cardiomyopathy and HF after 40 weeks. β_1-AR signaling leads to cell death and apoptosis, but β_2 signaling leads to cell survival and protects against apoptosis. These findings led to the use of β_2-AR gene delivery in animal models of HF. AdV β_2-AR intracoronary delivery in rabbits resulted in enhanced cardiac function. Further studies will determine its long-term effects and future clinical use.[11]

Inhibition of G protein-coupled receptor kinase-2. Desensitization is the process by which kinases dampen the interaction between activated β receptors and their G proteins. Homologous desensitization (agonist dependent) is mediated by G protein-coupled receptor kinases (GRKs). GRK-2 is up-regulated in the failing human heart and is responsible for the desensitization. βARK C terminus (βARKct) is a peptide that has been used to inhibit GRK-2–mediated β-AR desensitization. β*ARKct* gene transfer to isolated failing human cardiomyocytes improved their contractile function.[32]

Adenylate cyclase type 6. β-AR stimulation activates adenylate cyclase (AC) through G protein activation and leads to production of cyclic adenosine monophosphate (cAMP). AC then activates protein kinase to exert its downstream effects. AC5 and AC6 are the predominant cardiac isoforms; of these, cardiac AC6 overexpression leads to increased LV function and increased cAMP levels during β-AR stimulation, whereas basal levels are normal. Intracoronary AdV-AC6 gene delivery in pigs with pacing-induced HF resulted in improved LV contractility. Further preclinical studies are expected before clinical trials.[11]

Conclusions

HF remains a leading cause of death and morbidity in industrialized countries. The etiology of this disease is complex and multifactorial. Activation of endogenous neurohormonal systems plays a major role in the progression of the disease. Research into the molecular and pathophysiological mechanisms underlying HF has led to the development of new drugs and therapies for HF.

Standard medical treatments currently used in the management of these patients target neurohormonal pathways, aiming to modulate their abnormal activation. The therapeutic choices for end stages of the disease are still limited and patients have a poor prognosis because current medications are not able to produce regression of functional and structural damage.

In recent years, the molecular bases of HF have been better elucidated. Gene therapy represents a new approach to the treatment of HF because its targets are inside cardiac tissue. Many molecules inside the cardiomyocyte have been indicated as suitable therapeutic targets, including genes encoding for proteins involved in contractile function and β-adrenergic signaling. SERCA2a, a cardiac Ca^{2+}-ATPase, shows reduced activity in HF and was chosen as the target in a randomized, double-blind, placebo-controlled phase II study in patients with advanced HF (CUPID). The up-regulation of this protein has led to both clinical and functional improvements in patients given high doses of AAV1/

MAIN POINTS

- Heart failure is a major growing public health problem and a leading cause of death and morbidity in Western countries.
- Current treatments interfere with the activation of the neurohormonal and adrenergic systems; however, patients in end stages of the disease still have a poor prognosis.
- Gene therapy in heart failure aims to correct key molecular mechanisms in cardiac tissue through the introduction of DNA/RNA, which targets specific cardiomyocyte processes.
- Many targets have been identified and the success of this novel therapeutic strategy will probably depend on the development of efficient delivery techniques.

SERCA2a. Moreover, the study demonstrated the safety of AAV1/SR Ca^{2+}-ATPase in advanced HF, which would support larger confirmatory trials.

Future research will probably focus on the gene transfer techniques, which are the most important limitation to gene therapy. It will aim to modify vectors in order to further improve both biosafety and gene transfer efficiency. The success of gene therapy depends on the efficiency of DNA/RNA transduction and long-term transgene expression. ■

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