Gene Therapy for Heart Failure

Jang-Whan Bae, M.D. and Myeong-Chan Cho, M.D.
Department of Internal Medicine, Chungbuk National University College of Medicine, Medical Research Institute, Chungbuk National University, Cheongju, Korea

ABSTRACT

Heart failure is an important public medical problem in the developed countries. Although there are numerous and fascinating medicines given by physicians for the treatment of heart failure, the general 5 year survival rate for advanced heart failure is only about 50%. There are still many theoretical and practical problems to overcome before the application of gene therapy to cardiovascular disease becomes a reality in the future. With our increased knowledge on the development of gene delivery, vector systems, molecular basis of cardiac dysfunction, and the pathogenesis of heart failure at the cell level, gene therapy is emerging as a new therapeutic option for heart failure. (Korean Circulation J 2005;35:345–352)

KEY WORDS: Heart failure; Gene therapy; Vector.

Introduction

Cardiovascular gene therapy is a novel approach for the treatment of cardiovascular disease by replacing defective or missing gene products including cellular proteins, receptors and inhibitors, and this approach shifts the therapeutic focus toward correcting the pathophysiology at the cellular and subcellular level. Although gene transfer technology has progressed, many problems still exist with regard to the delivery systems, efficiency of gene transfer and control of expression, and all of these problems must be properly addressed before gene therapy can be applied safely and effectively in the clinical setting.1) After a decade of pre-clinical and early phase I clinical investigations, gene therapy has now emerged as a genuine therapeutic option for the two most common cardiovascular diseases in adults—ischemic heart disease and congestive heart failure.

Heart failure is a progressive disease that displays various stages of evolution and cardiac dysfunction. Genetically engineered mice and some of the larger animal models of this disease have led to the discovery of molecular targets in the myocardium of failing heart. Although studies have yet to be initiated on human subjects, the candidate genetic targets of gene therapy for heart failure are the intracellular calcium regulation and homeostasis,2) β-adrenergic receptor signaling,3) antiapoptotic signaling,4) and myocardial angiogenesis.5) In the animal models of heart failure and in the ex vivo studies, these gene therapies relieved the heart failure symptoms and improved the heart function.

The purpose of this review is to offer a glimpse into the future of gene therapy for the potential treatment of heart failure.

Potential Gene Therapy Strategies for Heart Failure

Calcium regulation and homeostasis

Cardiomyocyte depolarization induces an influx of Ca²⁺ from the extracellular milieu via the voltage-dependent L-type Ca²⁺ channels, and this in turn activates the ryanodine receptor (RyR) to release the intracellular stores of Ca²⁺ from the sarcoplasmic reticulum (SR). The combination of Ca²⁺ influx and release raises the free intracellular Ca²⁺ concentration, allowing Ca²⁺ to bind to the myofilament protein troponin C that then switches on the contractile machinery.7–11) For relaxation to occur, the intracellular Ca²⁺ concentration must decline to allow the Ca²⁺ to dissociate from troponin. This requires Ca²⁺ transport out of the cytosol via four pathways: SR Ca²⁺ ATPase (SERCA), sarcolemmal Na⁺:Ca²⁺ exchanger (NCX), sarcolemmal Ca²⁺ ATPase or mitochondrial Ca²⁺ uniport (Fig. 1).

SERCA2a (the predominant isoform in cardiomyocytes) activity is regulated by phospholamban (PLB).
Unphosphorylated PLB is an endogenous inhibitor of SERCA2a activity, and the phosphorylation of PLB by protein kinase A and/or Ca\(^{2+}\)/calmodulin-dependent protein kinase causes the disinhibition of PLB's negative effects on SERCA2a (Fig. 2). In the failing heart, several alterations in this overall cardiac Ca\(^{2+}\) homeostasis have been observed. Of particular interest is the SERCA2a activity, which is decreased in the failing cardiomyocytes in addition to the decreases of SERCA2a mRNA and protein. Moreover, although the level of PLB expression is unchanged, its phosphorylation state can be altered. This causes an important change in the ratio of PLB to SERCA2a, with its relative increase in the failing heart and the inhibitory effect of PLB being even greater. The primary focus of molecular therapy for heart failure has been to enhance the activity of SERCA2a in the SR in order to restore the intracellular Ca\(^{2+}\) regulation in the failing heart. Therefore, as potential gene therapy approaches, research efforts have concentrated on either overexpressing the SERCA2a or decreasing (via inhibition) the PLB.

The adenoviral-mediated overexpression of SERCA2a in the isolated cardiomyocytes from failing human hearts has recently resulted in enhanced pump activity, improved contraction and a return of the relaxation velocity to the levels observed in non-failing cardiomyocytes. Based on the favorable results of in vitro research, several in vivo studies have been performed in heart failure animal models. The adenoviral-mediated gene transfer of SERCA2a into failing rat hearts improved the LV...
systolic function and pressure, the rate of isovolumetric relaxation, the phosphocreatine/ATP ratio (as a marker of cardiac metabolism), and the survival rate at 4 weeks after the gene transfer. \textsuperscript{14,15} PLB is an alternative gene therapy target for regulating calcium in heart failure, and inhibiting PLB activity is the main approach method that’s been used for PLB based gene therapy.\textsuperscript{16,17} In vitro studies on isolated cardiomyocytes from patients with end-stage heart failure have revealed an improved contractile function after the adenoviral-mediated transfer of PLB antisense oligonucleotide.\textsuperscript{17} In addition, the overexpression of a pseudophosphorylated mutant of PLB in cardiomyopathic hamsters by using an adenovirus-associated virus prevented the progressive impairment of the systolic and diastolic function that was observed in the untreated animals.\textsuperscript{18} More recently, Ziolo et al.\textsuperscript{19} have shown that PLB inhibition by using a dominant negative mutant PLB adenovirus enhanced SERCA2a function and this restored Ca\textsuperscript{2+} transients and positive force frequency response in cardiomyocytes obtained from an arrhythmogenic rabbit heart failure model.

In addition to reduced the SR Ca\textsuperscript{2+} uptake, the increased Ca\textsuperscript{2+} leakage through the RyRs is a significant component of the altered excitation-contraction coupling that is seen in heart failure. A leak of Ca\textsuperscript{2+} from the SR decreases the SR Ca\textsuperscript{2+} content and this decreases the release of systolic Ca\textsuperscript{2+}, and it may be a trigger for arrhythmias. Moreover, the altered cytosolic Ca\textsuperscript{2+} concentration that occurs by the increased Ca\textsuperscript{2+} leakage may contribute to the altered gene expression and the myocardial remodeling. FK-506 binding proteins (FKBPs) are a good candidate to stabilize the RyR. In this regard, Prestle et al. have demonstrated that Ca\textsuperscript{2+} leakage through the RyRs was reduced in adenoviral mediated FKBP12.6 overexpressed cardiomyocytes.\textsuperscript{20} Yano et al.\textsuperscript{21} have recently reported that preventing Ca\textsuperscript{2+} leakage through the RyRs improved the ventricular function and it prevented heart failure in a dog model, when using the agent JTV519 that restores the FKBP12.6-mediated stabilization of the RyR.

The β-adrenergic receptor system

The β-adrenergic receptor (βAR) signaling pathways are important for the regulation of cardiac contractility in response to sympathetic activation in both the normal physiologic state and the pathologic condition. Downregulation of βARs and the diminished contractile response to the adrenergic agonists are characteristic of chronic heart failure. An enhanced βAR desensitization can occur due, in part, to the impaired function of the remaining receptors (a 50% loss of βAR density) and also because of the increased mRNA, protein and activity of βAR kinase-1 (βARK1) in the failing heart (Fig. 2). The βARKct, a 194 amino acid peptide consisting of the carboxyl terminal of βARK1, is able to inhibit the endogenous βARK1 by competing for a G\textsubscript{βγ}, binding and so it prevents the excessive desensitization of βARs and the other G-protein coupled receptors (GPCRs).

A series of transgenic mice with cardiac targeted overexpression of several components of the β-adrenergic signaling pathway have recently been created, including the β\textsubscript{2}-AR, βARK1, an inhibitor of βARK1 (βARKct) and the G-protein-coupled receptor kinase-5. The overexpression of β\textsubscript{2}-ARs in the heart can lead to a marked enhancement of the myocardial contractility and relaxation.\textsuperscript{22} Mice that were overexpressing βARK1 demonstrated attenuation of the isoproterenol stimulated increase in the in vivo contractility, whereas the opposite phenotype a significant enhancement of the resting cardiac function was observed in the βARKct transgenic mice. These experiments document the importance of βAR system as determinants of the in vivo cardiac function.\textsuperscript{23} In this regard, the preliminary data has shown that the adenovirus-mediated intracoronary gene transfer of β\textsubscript{2}-AR can significantly potentiate the β-adrenergic signaling in adult rabbit ventricular myocytes. In a large animal model, transfer of adenovirus mediated human β\textsubscript{2}-AR gene delivery resulted in increased βAR density and the enhanced β-agonist stimulated +dP/dt\textsubscript{max} and reduced LVEDP.\textsuperscript{24} Mechanistically, the recent studies have shown that β\textsubscript{2}-ARs can evoke apoptotic signaling in myocytes, while β\textsubscript{2}-ARs do not and they may actually prevent the pathological β\textsubscript{1}-AR signaling.

Besides the overexpression of β\textsubscript{2}-ARs, the specific inhibition of βARK1 can prevent the phosphorylation and desensitization of not only βARs in the heart, which are known to be functionally uncoupled, but also potentially other GPCRs as well that are also targets for βARK1-mediated desensitization. Importantly, adenoviral-mediated gene transfer of βARKct in both transgenic mouse models and larger animals of heart failure resulted in increased cardiac function with augmented responses to βAR stimulation. Cross-breeding βARKct transgenic mice with several murine heart failure models (MLP knockout mice or calsequestrin transgenic mice) has resulted in functional rescue being seen in the offspring, including prevention of LV remodeling, enhanced cardiac function and improved survival.\textsuperscript{25} A reduced βAR density and transient impaired LV function has been observed following cardiopulmonary bypass and cardioplegic arrest. Adenoviral βARKct delivery to the heart has also been shown to be effective for the transient models of cardiac dysfunction, including cardioplegic arrest.\textsuperscript{26}

Another alternative therapeutic approach is to increase the expression of adenylyl cyclase type VI (AC\textsubscript{VI}). Lai et al.\textsuperscript{27} have demonstrated that intracoronary delivery
of a recombinant adenovirus ACvI improves the global LV function associated with a reduction in LV remodeling in a pig model of heart failure.

Resisting apoptosis
Cardiomyocytes undergo apoptosis in response to a myriad of stimuli including hypoxia, reoxygenation, acidosis, oxidative stress, serum deprivation, β-adrenergic agonists, TNF-α and anthracycline. Heart failure involves low, but abnormal rates of cardiomyocyte apoptosis that persist for months (0.08% to 0.25% in the failing human heart compared with 0.001% to 0.002% in the normal human heart). Cardiomyocyte apoptosis has been observed in failing human heart specimens, and a gene therapy that resists apoptosis or promotes cardiomyocyte survival has been proposed as a potential therapeutic modality for heart failure. The conceptual support for this idea is derived from mice that have undergone ventricular-restricted knockout of gp130 because these mice develop rapid-onset, dilated cardiomyopathy.26)

Although adenovirus-mediated Bcl-2, activated PI3-kinase, and Akt and p35 gene deliveries into ventricular cardiomyocytes have significantly reduced apoptosis that was induced by p53, hypoxia or doxorubicin respectively.5) Insulin growth factor 1 (IGF-1) has been shown to block apoptosis in many settings, and transgenic mice over-expressing IGF-1 have less myocyte apoptosis and an improved cardiac function in an infarction model.27)

Myocardial angiogenesis
The preliminary experimental data support the idea that therapeutic angiogenesis may have the potential to elicit functional improvement in individuals with heart failure.28) The previous studies have established that interstitial fibrosis, decreased capillary density and the subsequent impaired myocardial blood flow reserve are characteristic findings in patients with dilated cardiomyopathy. Impaired myocardial angiogenesis and ischemic cardiomyopathy were demonstrated in mice lacking the vascular endothelial growth factor (VEGF) isoform VEGFβ165. Knock out of the VEGF-1 gene in ventricular cardiomyocytes leads to increased embryonic lethality and severe cardiac defects in the surviving mice.29) These results imply that the coronary microvasculature is responsible for both the myocardial ischemia and the LV dysfunction. Angiographic observations made in those individuals who received phVEGFβ165 gene transfer have likewise suggested that the increased vascularity responsible for the improvements in myocardial perfusion and anginal symptoms occurs in the microvascular coronary circulation.30)

Methods of Gene Transfer into the Myocardium

Direct myocardial injection
Direct injection of the genetic materials into a beating heart can be performed under visual control during open-chest surgery, but the invasive nature of this technique and the local expression limits its widespread application. Endocardial gene transfer directly into myocardium using a needle-equipped catheter will allow for multiple and repeated injections. However, the low efficiency of gene transfer and the transient nature of the gene expression are still considerable hurdles that need to be overcome before these techniques can be applied as treatment modalities. Gene expression is limited to the small region of myocardium that skirts the needle track; therefore, this method has a disadvantage for attempting to treat globally involved myocardial dysfunction. Direct myocardial damage, minor myocardial inflammation and tissue edema are inevitable consequences of this technique. In addition to these mechanical injuries, the arrhythmogenic possibility around scar tissue should not be underestimated. Because of these problems, this method may be difficult to apply to human gene therapy.

Pericardial injection
Gene delivery to the myocardium via the pericardial space was effective in small animal models. Generally, 25-30% of the injected materials were recovered in the myocardium, but about a 40% delivery rate can be achieved by performing the injection with a mixture of hyaluronidase and collagenase.31) This method could be applicable to clinical study under the small sized thoracotomy after the safety of this procedure has been confirmed in large animals.

Retroperfusion
Cannulation of a coronary sinus (or the subselective vein) with the concurrent occlusion of outflow from the coronary sinus distributes the transgene in a retrograde fashion from the coronary venous circulation through the capillary bed to the cardiomyocytes and interstitium in potentially all the regions of myocardium.

Intraventricular delivery
The most global and efficient gene delivery method to the myocardium in small animals is an intraventricular injection via the apical myocardium to the aortic root with clamping aorta and/or main pulmonary artery during 10 to 40 seconds.14) In patients with heart failure, this method permits the efficient global gene delivery to the entire myocardium in some limited cases undergoing
cardiopulmonary bypass for some other indications e.g. coronary bypass or valve surgery.

**Intracoronary catheter delivery**

A good gene delivery method in the clinical field should be safe, easy to apply and efficient to myocardial gene transfer. Intracoronary catheter delivery may fulfill these requirements and this method is frequently applied to myocardial stem cell therapy. Because coronary selection is easy and safe even for severe global dysfunction in heart failure, compared to thoracotomy and cardiopulmonary bypass, the intracoronary catheter delivery method will be the most promising method in the clinical field.

**Indirect gene transfer**

A potential method for gene transfer into the myocardium is the implantation of genetically engineered cells. If the damaged myocardium could be replaced with functional and coupled cells, it would be possible to graft healthy or genetically engineered cells back into the diseased heart. Suitable candidate cells include skeletal myoblasts, fetal cardiomyocytes, cardiomyocytes obtained from endomyocardial biopsy and stem cells such as endothelial progenitor cells and mesenchymal stem cells. This cell-based gene therapy may be of use in replacing the diseased myocardium: for example, in ischemic heart disease following myocardial infarction.

Animal studies have established the feasibility, and in some cases the potential advantages, of indirect gene transfer through the administration of stem cells that have been transfected ex vivo before the in vivo delivery (Fig. 3). Endothelial progenitor cells gathered from the peripheral circulation, for example, have been expanded ex vivo and then administered to animals with limb or myocardial ischemia to enhance neovascularization successfully.

**Vectors**

Various viral or non-viral vector systems are available. However, no vector system currently exists that can fulfill the various conditions such as the easy to prepare vectors on a large scale, the transfection efficiency, durable gene expression and the noninflammatory and nonimmunogenic in vivo application (Table 1).

**Adenoviral vector**

The adenoviral vector is the most commonly used
vector system in animal studies because of its high efficiency of infecting non-dividing cells like the cardiomyocyte. But the adenoviral vector has some limitations for applying it to chronic heart failure therapy. The gene expression duration of the adenoviral vector is only 3–4 weeks. Furthermore, the 1st generation of adenoviral vector induces neutralizing antibodies, and these antibodies diminish the gene expression efficacy in repeated treatments. Some studies have reported that the adenoviral vector provoked inflammatory responses and, in a dose related manner, cardiomyocyte apoptosis and necrosis. However, the new generation adenoviral vectors are able to support longer gene expression and they have reduced immunogenicity. A potential use of this vector in the future is still under investigation.

Adeno-associated viral (AAV) vectors

AAV vectors effectively infect both dividing and non-dividing cells, and they integrate in the genome of the host cell and support durable transgenic expression for about 30 weeks. From these points, the AAV vector is a very attractive system for long term heart failure treatment. However, AAV vectors also have several important disadvantages. It’s very difficult to generate a purified high titer of these viruses and their inserting capacity is very limited to less than 4.7 kilobases. The manner in which these vectors integrate into the host genome may disturb the normal host genetic activity and the fate of the cell.

Retroviral and lentiviral vectors

Retroviral vectors are useful for in vitro studies. These vectors are relatively non-immunogenic and they efficiently transfec to dividing cells in vitro. The long term genetic expression can be supported, but their transfection efficacy to non-dividing cells is very low. Therefore, their application for heart failure treatment is very limited. A recent study has suggested that using lentivirus may achieve efficient expression in non-replicating cells or beating cells like cardiomyocytes.

Non-viral vector systems

Plasmid DNA vectors are easy to produce in large scale and the required only a small number of proteins, but their in vivo transfection and gene expression rates are is very low. Low frequency ultrasound local radiation after plasmid DNA injection elevates the cell membrane’s permeability to an enhance uptake of plasmid DNA, and this may augment gene expression. Other than these ordinary vector systems, liposomes also can be used for chronic heart failure gene therapy.

Conclusion

Although there has been a great deal of meticulous evaluation of the gene therapy for heart failure in the preclinical studies during the last decade, there are some important questions remaining for applying this new optional treatment to human heart failure. Is the LV dysfunction improvement going to be long lasting after the gene therapy? Is the transfection efficacy and gene expression rate comparable between an animal heart failure model and human heart failure? Can we prevent or reduce the inflammatory reaction and the immunogenic responses that may aggravate LV fibrosis and systolic dysfunction after the gene therapy?

Even though the gene therapy for heart failure has various technical difficulties and questions to be solved, this option is a very attractive alternative for the treatment of advanced heart failure. We have already targeted some genes for delivery, and additional target should be pursued. Effective and safe vector systems and delivery methods are also very important. The new generation adenovirus vector system can be a recommendable method.
from the point of view of its high transfection rate to non-dividing cells, its low immunogenicity and its longer duration of gene expression. The noninvasive intracoronal catheter delivery method will certainly be more popular than the other invasive ones. Genetically modified stem cell therapy also is recommendable as a more effective and durable therapeutic option. More pivotal gene discoveries, novel vectors, advanced delivery system development and further preclinical trials remain to be done before attempting gene therapy for human heart failure.

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