Editorial

Gene Therapy in Congestive Heart Failure

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Although research and development of gene therapy have encountered several difficulties in recent years, they have also begun to show potential for the treatment of acquired and inherited human diseases. Gene therapy may have wide applicability for the treatment of numerous cardiovascular diseases, including congestive heart failure (CHF), ischemic heart disease, and cardiomyopathy. CHF remains a leading cause of death and morbidity in industrialized nations, and the number of patients with CHF continues to increase as the population ages. Pharmacological therapies control symptoms, reduce left ventricular (LV) dilation, improve function, and reduce mortality. These therapies control the disease but do not cure it. Currently, however, the growing knowledge of molecular mechanisms involved in myocardial dysfunction has allowed the identification of potential therapeutic targets designed with the aim of curing CHF. Several studies in animal models have provided hope that gene therapy may be one of the future strategies for the treatment of clinical CHF. In these studies, myocardial gene transfer has been used to target at least 3 different biological pathways that play a crucial role in the pathophysiology of CHF: Intracellular calcium signaling, β1-adrenergic receptor (β1-AR) signaling, and antiapoptotic signaling. The activity of sarcoplasmic reticulum Ca2+ ATPase (SERCA2a) is decreased in failing myocardium, and this results in abnormal Ca2+ handling.1 In an animal model of heart failure, Miyamoto et al.2 showed that in vivo overexpression of SERCA2a, achieved by catheter injection of an adenovirus carrying the SERCA2a gene into the aortic root, restored systolic and diastolic function to normal levels. In another study, Muller et al.3 analyzed the chronic overexpression of SERCA2a in both normal and pressure-overloaded hearts (induced by abdominal aortic banding of transgenic rats). Overexpression of SERCA2a resulted in a positive inotropic effect under both conditions. Recently, Iwanaga et al.4 examined the effects of inhibition of phospholamban (PLN), an endogenous inhibitor of SERCA2a, in a rat model of heart failure. They showed that in vivo delivery of the S16EPLN gene (a pseudophosphorylated mutant PLN peptide) using a recombinant adeno-associated virus vector improved LV function and remodeling.

Cardiomyocyte toxicity and CHF can be caused by excessive activation of β1-AR5 and also by alterations of the β1-AR signaling system at different steps along the signal transduction pathway, including the G-protein coupled receptors and adenyl cyclase.6 Therefore, to improve heart function, gene transfer therapies were designed to downregulate the β1-AR,7 inhibit the activity of G protein-coupled receptor kinase-2,8,9 and increase the expression of adenyl cyclase type VI (AC6).

The other promising strategy to promote myocardial survival and function is augmenting cardiomyocyte resistance to apoptosis. Based on the finding by Hirota et al.10 that glycoprotein 130-knockout mice develop dilated cardiomyopathy associated with cardiomyocyte apoptosis, in vitro studies have reported inhibition of LV apoptosis and survival of cardiomyocytes after adenoviral gene transfer of Bcl-211 and Akt.12,13

Gene Delivery Methods

Current strategies for myocardial gene transfer have used viral vectors. Among the different types of viruses available, adenoviruses are vectors with high transfection efficiency for most cell types and easy to produce in high titers, but they cause severe inflammatory reaction and immune responses that limit transgene expression and may also cause myocardial necrosis.2,14 Retroviruses are useful vectors for ex vivo approaches; they are relatively non-immunogenic and efficiently transduce dividing cells in vitro. The requirement that the target cells should be proliferating and the fact that prolonged expression is difficult to attain are the limits of retroviral vectors.15 Adeno-associated viruses and lentiviruses (derived from human immunodeficiency virus, HIV) are emerging as the most promising vectors. They are able to infect both proliferating and non-proliferating cells, have limited inflammatory response, and can induce prolonged, high-level transgene expression. Unfortunately, they also have some disadvantages: The method of production results in a relatively low yield, the total length of the insert in the adeno-associated virus vectors cannot exceed 4.7 kilobases, and lentiviruses contain some residues of the HIV gene.

Other than viral methods of gene-delivery, there are some non-viral options: Plasmid DNA vectors are easy to produce in large scale and require only a small number of proteins. However, they are not very efficient, which can result in prolonged low-level expression in vivo and can stimulate immune responses.16 Liposomes are another non-viral option, composed of a lipid sphere with a fraction of aqueous fluid in the center. The liposome fuses with cell membranes and DNA within the liposome is transferred into the target cell. Despite the progress made in vector development, and despite the recent increased interest in non-viral techniques, the ideal vector has not yet been developed.

Adenylyl Cyclase as the New Therapeutic Target in CHF

Adenylyl cyclase (AC), a very important enzyme in transmembrane signaling, has been tested for gene transfer and has shown success.
in animal models. Investigators have shown that this enzyme plays a key role in the signal transduction pathway that includes β-adrenergic receptor, G-protein, and AC, and therefore in the regulation of cAMP generation.\textsuperscript{17,18} In previous articles,\textsuperscript{17,18} they have established that in murine models of cardiomyopathy, overexpression of the isoform AC\textsubscript{VI} increases cardiac responsiveness to β-adrenergic receptor stimulation (without changes in the amount of the receptor or G-protein), resulting in increased cAMP production and improved cardiac function. In this new study,\textsuperscript{19} published in the present issue of \textit{Circulation}, the authors report a novel therapeutic strategy to treat CHF. They demonstrated that intracoronary delivery of a recombinant adenovirus encoding AC\textsubscript{VI} (1.4 x 10\textsuperscript{12} vp) improves global left ventricular function associated with reduction in LV remodeling in a large animal model of heart failure. After gene transfer, improvements in different parameters of LV function were observed, such as reduction in LV end-diastolic and end-systolic dimensions and wall stress, improved systolic wall thickening, increase in LV +dP/dt (change in ventricular pressure over change in time) and -dP/dt during stimulation with isoproterenol, increase in cAMP generation, and decrease in plasma concentrations of brain natriuretic peptide (a marker of LV dysfunction). A strength of this study\textsuperscript{19} is that the authors have chosen a model of CHF that mimics dilated heart failure: Large animals (pigs) subjected to LV pacing and treated only after they displayed obvious signs of heart failure. Their therapeutic approach is clinically relevant and involves an intracoronary administration of genes that is safe, effective, feasible, and non-surgical. Notably, increasing the expression of AC without altering the number of β-adrenergic receptors or the content of G-protein is a strategy different from others that utilize agents (β-agonists and milrinone) to produce a sustained increase in intracellular levels of cAMP. Thus, this approach, aimed to restore cardiac AC function, has advantages and salutary benefits because it avoids the potential deleterious effects of sustained adrenergic stimulation.

Questions
Some questions regarding this approach remain to be addressed. Is the benefit long lasting—present over months or years—or is the improvement in LV function only transient? Because efficiency of gene transfer in humans is limited, will this therapeutic approach be equally effective in human CHF? Although the authors have previously reported no evidence of cardiotoxicity after long-term exposure to AC\textsubscript{VI} expression in mice with Qq cardiomyopathy,\textsuperscript{20} can we translate this into people with CHF, or will long-term deleterious cardiac effects be associated with increased myocardial AC\textsubscript{VI} content in humans? Will this treatment be beneficial in patients with advanced or end-stage CHF? Three out of 7 treated pigs developed mild interstitial inflammation, which may have been related to the adenovirus. The long-term effect of this inflammation will require future analyses.

Perspectives
Gene therapy raises a number of ethical issues and concerns. Some are common to any new therapy that involves human experimentation, whereas others are related to the specific methods used. The safety of gene delivery by using viral vectors is one of the major concerns because of their potential toxicity, the immune and inflammatory responses that they can induce, and the worry that the viruses may regain their infective ability once present in the patient.\textsuperscript{16} Future pre-clinical and clinical studies will determine the most effective and safe gene therapy for heart failure, with the hope that gene therapy becomes practical. Although advances in the areas of gene discovery and development of novel vectors and devices for delivering therapeutic genes to different tissues in vivo have accelerated the pace of progress of gene therapy, much additional work remains to be done before human gene therapy for cardiac disorders becomes standard. Gene therapy provides hope that the basic molecular defects of CHF can be corrected. Lai and collaborators\textsuperscript{19} should be congratulated for bringing us one step closer to this ultimate goal of a potential cure.

References

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