

CardiologyRounds™

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Rescuing the Failing Heart by Targeted Gene Transfer

BY ROGER J. HAJJAR, M.D.

Congestive heart failure (CHF) represents an enormous clinical problem demanding effective therapeutic approaches. Despite advances in its treatment, including novel pharmacologic management, myocardial revascularization, mechanical assistance, and transplantation, CHF remains a leading cause of death in the United States and Europe.^{1,2} Although new treatments for CHF have had a significant impact on mortality and the course of the disease, they do not reverse or cure the underlying pathological state of the heart. To this end, clinical research has demonstrated that gene transfer is a potential therapeutic option to restore diseased cardiomyocytes and rescue the failing heart. This issue of *Cardiology Rounds* will focus on recent developments in this field and underscores some potential targets for gene transfer relative to the failing heart.

The cells and microcirculation that make up the failing heart contribute to contractile dysfunction as shown in Figure 1. The contributions of the microcirculation and fibroblasts to the phenotype of the failing heart are beyond the scope of this review.^{3,4} In the failing heart, there are many types of cardiomyocytes:

- cardiomyocytes that have undergone either apoptosis or necrosis
- diseased cardiomyocytes that are characterized by contractile dysfunction
- nondiseased cardiomyocytes that are exposed to neurohormonal stimulation and are at risk of becoming dysfunctional or undergoing necrosis and apoptosis.⁵

At our laboratory, we have focused on using gene transfer to restore diseased cardiomyocytes in order to improve contractile function and survival in failing cardiomyocytes.⁵ The targets for gene transfer are membrane channels, intracellular transporters involved in calcium homeostasis, and other intracellular pathways involved in cell survival.

The myopathic heart has a number of abnormalities that have been characterized at the cellular level, including changes at the level of the sarcolemma, sarcoplasmic reticulum, myofilaments, and mitochondria, all of which contribute to depressed contractile function and reserve. Identifying the mechanisms associated with these changes is frequently confounded by simultaneous alterations in multiple signaling pathways in the complex milieu of the failing or myopathic heart. For this reason, gene transfer has the potential to alter our understanding of the different mechanisms involved in heart failure in two distinct, yet related, ways.

First, the ability to genetically reprogram the heart in relevant *in vitro* and *in vivo* models of cardiovascular disease allows us to test the role of the specific restored molecular pathways in disease pathogenesis. In this way, mechanistic hypotheses can be tested and potential targets for therapeutic intervention can be identified.

Secondly, gene transfer allows us to rapidly translate the latest developments in molecular and cell biology into clinically relevant models. Once validated, a potential target can be approached with the full spectrum of therapeutic options, including traditional pharmaceuticals,



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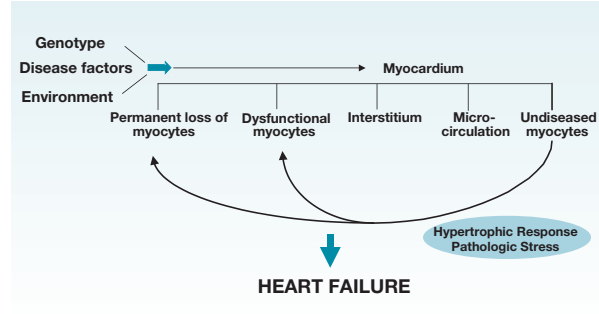
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Figure 1: Cardiomyocytes, fibroblasts and microcirculation that make up the failing heart contribute to the contractile dysfunction observed in systolic heart failure. Within the failing heart, there are many types of cardiomyocytes (cardiomyocytes that have undergone either apoptosis or necrosis, diseased cardiomyocytes characterized by contractile dysfunction, and nondiseased cardiomyocytes that are exposed to neurohormonal stimulation and are at risk of becoming dysfunctional or undergoing necrosis and apoptosis).



targeted synthesis of small molecule agonists or antagonists, biological agents (cells, antibodies, genetic material), or gene-based therapy. Undoubtedly, lessons gleaned from gene transfer experiments about local modulation of cardiac genetic programs will better guide attempts to transform early investigations into established therapy.

Vectors for cardiac gene delivery

Vectors available for gene transfer have recently improved significantly.^{6,7} A growing number of vectors are available for experimental and clinical gene transfer experiments (Table 1). Many of these systems are not applicable to cardiac gene transfer, which requires *in vivo* gene transfer (as opposed to *ex vivo* transduction of cells) to cells that are generally not replicating. For this reason, this discussion will focus on the systems that are most relevant to cardiac gene transfer.

Plasmid DNA: Plasmid DNA is often referred to as “naked DNA” to indicate the absence of a more elaborate packaging system. Over the past decade, multiple investi-

gators have demonstrated the ability of the heart to take up and express genes directly injected as plasmids. A major advantage of this approach is that it avoids many of the biosafety concerns associated with viral vectors. However, in general, the *level* of transgene expression and the *efficiency* of gene transfer (per cent of target cells expressing the transgene) are substantially lower with unmodified plasmid DNA than with more elaborate chemical or biological packaging systems. Whether the expression level and efficiency are adequate to achieve the experimental or clinical goals will depend critically on the particular application. For example, expression of secreted angiogenesis factors after muscle injection of plasmid DNA, despite relatively low levels of focal transgene expression, has demonstrated significant biological effects in animal models⁸⁻¹⁰ and appears promising clinically. However, efforts to more directly target cardiac dysfunction have generally focused on gene transfer approaches that achieve more effective transgene expression, such as the viral vectors considered below.

Adenoviruses: Recombinant adenoviral vectors offer several significant advantages for cardiac gene transfer. The viruses can be prepared at extremely high titer, infect non-replicating cells, and confer high-efficiency and high-level transduction of cardiomyocytes *in vivo* after direction injection or perfusion approaches.⁵ The major disadvantages to adenoviral gene transfer have been its transience and the immune response it evokes. In animal models, adenoviral gene transfer to adult myocardium *in vivo* has generally been found to mediate high-level expression for approximately one week,^{5,11-13} although the duration transgene expression may improve in systems utilizing cardiac-specific promoters, possibly because of reduced expression in antigen-presenting cells. Other improvements in the molecular engineering of adenoviral vectors can be expected to produce more sustained transgene expression.

Adeno-associated viruses: Recombinant adeno-associated viruses (rAAV) are derived from non-pathogenic parvoviruses. They evoke essentially no cellular immune response and produce transgene expression lasting months

Table 1: Vector systems for gene transfer

	Duration of expression	Advantages	Disadvantages
Naked DNA	4-7 days	No viral proteins	Inefficient entry Lack of stability
Adeno-associated virus	Onset of expression at 4 weeks; lifelong expression	Long-term expression No immune response Integration at specific site	Requires adenovirus to grow Limited insert size Complex to prepare
Lentivirus (pseudo-typed virus)	Lifelong	Long-term expression High efficiency	Complex preparation Insertion site
Herpesvirus/Amplicon	10-20 days	Large transgenes	Complex construction
DNA liposomes	4-10 days	More efficient entry	Limited persistence
Adenoviral Polylysine DNA conjugates	7-14 days	More efficient entry	Complex to construct Immunogenic

in most systems.¹⁴⁻¹⁶ They appear promising for sustained cardiac gene transfer.^{7,16,17} In comparison with adenoviral gene transfer, cardiac injection of rAAV produces less initial, but more sustained transgene expression.^{7,16,17} rAAV vectors appear particularly promising for sustained expression of secreted gene products. Whether the expression level and efficiency produced by rAAV will also be sufficient to modulate overall cardiac function, as has been achieved with adenoviral vectors,¹⁸ remain to be seen. Although systems for initial generation and purification of rAAV have improved, large-scale production for clinical applications remains challenging.

Lentiviruses: Lentiviruses are derived from a family of retroviruses that include human immunodeficiency virus and feline immunodeficiency virus. These viruses have enveloped capsids and a plus-stranded RNA genome. Like all retroviruses, the RNA genome is converted to DNA by reverse transcriptase after infection and is then firmly integrated into the host genome. However, unlike retroviruses that only infect dividing cells, lentiviruses can infect both dividing and nondividing cells. Lentiviruses have a specific tropism that restricts their targets; however, by pseudotyping the viral envelope with vesicular stomatitis virus, lentiviruses have a much broader range.¹⁹

Herpesvirus/amplicon: Herpes simplex 1 has a number of important characteristics that make it an important gene delivery vector *in vivo*. There are two types of HSV-1 based vectors: those produced by inserting the exogenous genes into a backbone virus genome, and HSV amplicon virions that are produced by inserting the exogenous gene into an amplicon plasmid that is subsequently replicated and then packaged into virion particles. HSV-1 can infect a wide variety of cells (both dividing and nondividing), but has an obvious strong tropism towards nerve cells. It has a very large genome size and can accommodate very large transgenes (>35 kb). In cardiovascular gene transfer, ryanodine receptors and titin (both are very large proteins) have been encoded in herpes viruses.²⁰

Concerns and limitations

The biologic advantages and limitations of the viral vectors are described above and in Table 1. These vectors have also been used clinically with variable success. Through these early clinical experiences, the occurrence of a number of complications is of concern. The most widely publicized case was that of Jesse Gelsinger. Gelsinger was a 17-year-old patient with partial ornithine transcarbamylase (OTC) deficiency, an X-linked defect of the urea cycle in which nitrogen metabolism is affected, leading to a spectrum of neurological symptoms including seizures and mental retardation.²¹ Therapy for the condition relies on alternative substrate administration, but mortality rates with the disease are high. Following administration of an adenovirus carrying OTC, Gelsinger developed acute respiratory distress syndrome (ARDS) and died 2 days later of multiple organ failure due to anoxia. Measurements of inflammatory

cytokines suggested that the vector had caused the systemic inflammatory response syndrome. This case illustrates that the immune response mounted against the viral vectors not only limits the efficiency of such vectors, but can elicit catastrophic reactions from the body.

Cardiac gene delivery

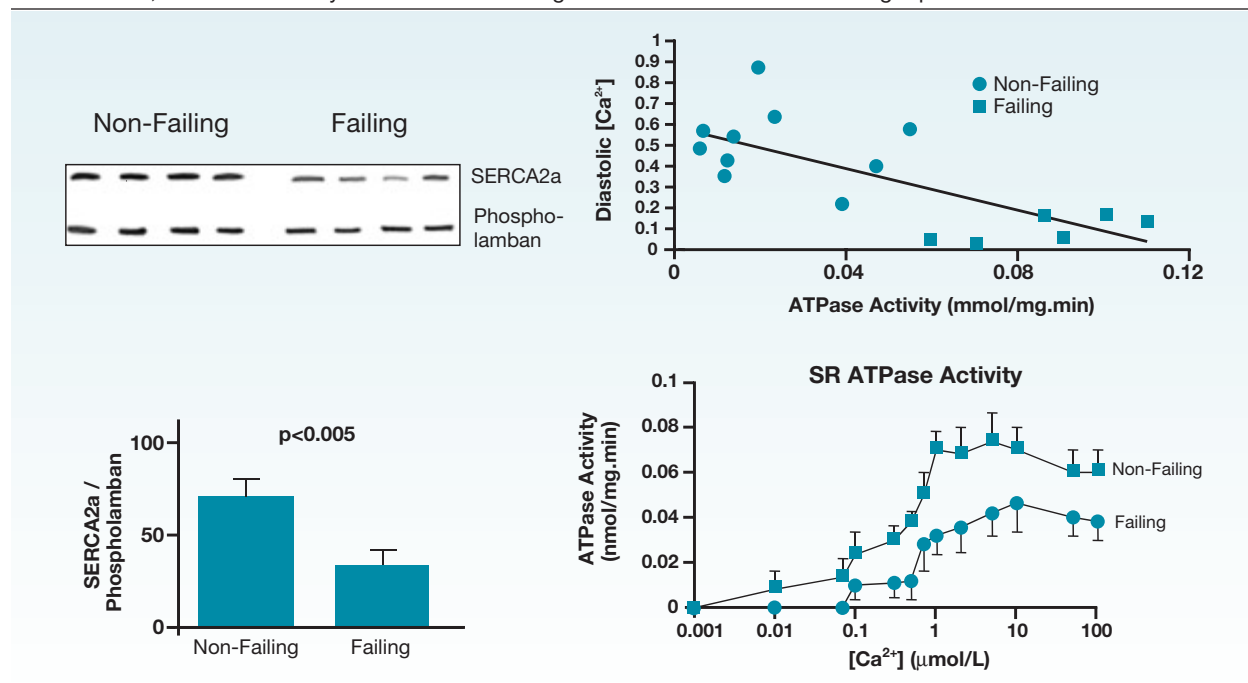
A number of mechanical approaches have been attempted to transduce myocardium using adenoviral vectors. Some of the techniques used have been intracoronary catheter delivery, direct injection of the virus into the myocardium, intraventricular delivery with retroinfusion of the coronary vein, and injection of adenovirus into the pericardial sac.^{12,22-25} Most of these approaches lead to focal transduction of the myocardium, with viral expression in only specified regional areas of the heart. This may not be adequate, as effective therapy in heart failure requires global transduction of the heart.

More recently, a new technique for cardiac gene delivery was developed in rodents that involves injecting adenovirus into the aortic root just above the aortic valve, while the aorta and pulmonary artery are transiently cross-clamped.²⁵ This technique achieved homogeneous transduction of the myocardium and has also been shown to produce transgene-specific physiological effects on ventricular function *in vivo*. More recently, the cross-clamping technique was extended to hamsters where cooling the animals allowed prolonged cross-clamping (~5 minutes) and better efficiency of gene transfer.²⁶ Further animal studies have established that *in vivo* adenoviral gene transfer cannot only achieve transgene expression in the myocardium, but can also modulate intrinsic functional properties of the intact heart. These studies have also been extended to larger animals and have involved retrograde perfusion through the coronary sinus and cardiopulmonary bypass with viral perfusion to the heart. These latter techniques may be more amenable to translation into humans.

Calcium handling in heart failure

The myopathic heart exhibits abnormalities in both the systolic and diastolic phase. Changes in diastolic function often appear earlier than in systolic dysfunction. In fact, compensated hypertrophy phenotypically demonstrates impaired relaxation parameters in the presence of normal or increased systolic function.²⁷ Abnormalities in calcium handling were noted more than 15 years ago when calcium transients, (recorded with the calcium indicator aequorin) from trabeculae in myopathic human hearts removed at the time of cardiac transplantation, revealed a significantly prolonged calcium transient with an elevated end-diastolic intracellular calcium.²⁸ These defects were subsequently found in single isolated cardiomyocytes loaded with the fluorescent indicator Fura-2 from myopathic hearts.²⁹ The calcium transients were characterized as having elevated diastolic calcium levels, a decreased systolic Ca²⁺ and a prolonged relaxation phase. Studies in both muscle strips

Figure 2: Failing hearts are characterized by reduced SERCA2a protein levels as shown in the upper left panel. Relative to phospholamban, SERCA2a levels are substantially decreased as shown in the bottom left panel. The decrease in SERCA2a activity is inversely related to diastolic calcium as shown in the upper right panel. Throughout a large range of calcium concentration, SERCA2a activity is decreased in failing hearts as shown in the lower right panel.



and isolated cardiomyocytes demonstrated that the systolic calcium concentration was decreased in the failing state, while diastolic calcium concentration was elevated.²⁹ These differences were more accentuated at higher stimulation rates.

The slow relaxation and abnormal force-frequency relationship observed in isolated muscles, as well as in isolated myocytes from failing hearts, indicate a deficiency in calcium reuptake by the sarcoplasmic reticulum (SR). Calcium transport into the SR occurs via the SR calcium ATPase calcium pump (SERCA2a). Failing hearts have been characterized by defects in SR function. Specifically, “relaxation abnormalities” correlated with deficient SR Ca²⁺ uptake have been associated with a decreased expression level of SR Ca²⁺-ATPase and reduction in SR Ca²⁺-ATPase activity.³⁰⁻³⁴ A number of investigators have shown that the levels of SERCA2a message and protein to be consistently decreased in heart failure³⁰⁻³⁶ in relation to phospholamban. Associated with a decrease in mRNA, there is a decrease in SR Ca²⁺-ATPase activity and SR Ca²⁺ uptake from SR vesicles and membranes isolated from failing human hearts as shown in Figure 2. Indeed, in experiments where the SR vesicles were isolated from human hearts, vesicles from failing human hearts had decreased rates of Ca²⁺ uptake when compared to normal hearts. Furthermore, the SR Ca²⁺ ATPase activity is inversely related to diastolic calcium (Figure 2).³⁰⁻³⁶ In failing hearts, diastolic calcium is elevated and ATPase activity is low relative to normal hearts.

Calcium is removed from the cytosol by the sarcolemmal Na⁺/Ca²⁺ exchanger, which has high capacity, but low affinity and is the major calcium extrusion mechanism of

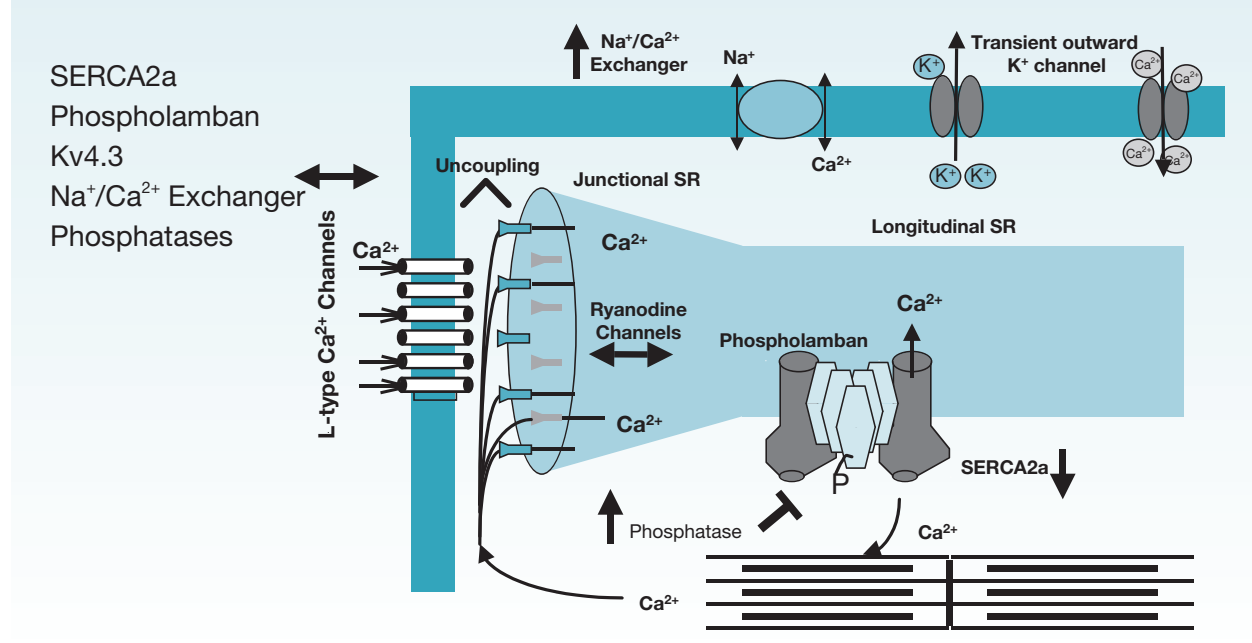
the cardiac myocyte.³⁷ This system returns calcium concentrations to diastolic levels (~100-300 nM) and may therefore, contribute significantly to myocardial relaxation. Most studies indicate that mRNA and protein levels of the Na⁺/Ca²⁺ exchanger are increased in human myopathic hearts, but not in all preparations.³⁷⁻⁴¹ Likewise, studies find the functional capacity of the Na⁺/Ca²⁺ exchanger to transport calcium is increased. The consequence of increased activity of the Na⁺/Ca²⁺ exchanger in failing hearts may be to compensate for the reduction in SR Ca²⁺-ATPase activity. Increased activity of the Na⁺/Ca²⁺ exchanger should aid in myocardial relaxation, albeit at the cost of reduced calcium release from the SR during systole. This would be particularly evident at higher rates of stimulation and thus lead to a blunted frequency response as is commonly seen in myopathic human myocardium.

The Na⁺/Ca²⁺ exchanger can operate to bring calcium into the cell or extrude calcium out from the cell.³⁷⁻⁴¹ There is an increase in sensitivity to compounds that produce positive inotropic effects through raised intracellular Na⁺, either by inhibiting the Na/K-ATPase or by opening Na⁺ channels, in muscle strips from failing human hearts. It has been suggested that the relaxation abnormalities produced by the loss of SERCA2a activity could be, at least partially, compensated for by an increase in the activity of the Na⁺/Ca²⁺ exchanger.³⁷⁻⁴³

Gene transfer of calcium handling proteins

Gene transfer provides a unique opportunity to manipulate the expression of essential proteins and alter the expression of specific downstream signaling pathways

Figure 3: Excitation-contraction coupling in heart failure and molecular targets amenable to gene therapy. Excitation of the cardiac cell induces the entry of a small amount of calcium, which in turn, causes the release of a larger amount of calcium from the sarcoplasmic reticulum, which in turn, activates the myofilaments and eventually produces force. During relaxation, most of the calcium is taken up by SERCA2a back into the SR and a smaller amount is extruded outside the cell by the $\text{Na}^+/\text{Ca}^{2+}$ exchanger. SERCA2a is under the endogenous control of phospholamban, a protein that when unphosphorylated, inhibits SERCA2a, and when phosphorylated, the inhibition is removed. The action potential duration is also determined by the transient outward current, which is dependent on the expression of the potassium channel (Kv4.3). In heart failure, there is a down-regulation of SERCA2a, an increase in $\text{Na}^+/\text{Ca}^{2+}$ exchanger, and a decrease in Kv4.3. These become targets for molecular repair.



implicated in the pathogenesis of heart failure. Contractile dysfunction of the myocardium results from abnormalities in many subcellular mechanisms. Specifically, three major areas of calcium handling have been targeted:

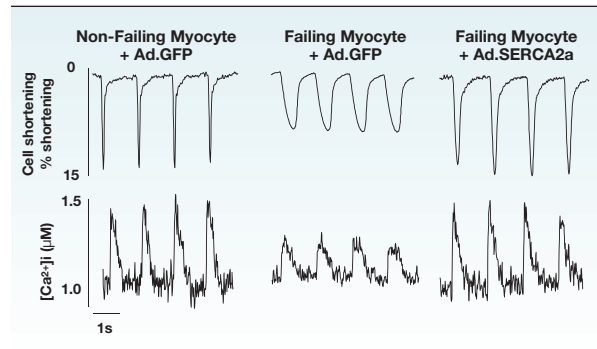
- the calcium handling proteins involved in excitation-contraction coupling
- potassium channels and their role in arrhythmia genesis
- abnormalities in neurohormonal receptors, specifically, the beta-adrenoceptor signaling pathways (Figure 3).

Adenoviral gene transfer has been instrumental in elucidating the molecular basis of these abnormalities and has also shown that many of the changes seen in excitation-contraction coupling can be halted and even reversed in failing myocardium. Transfer of SERCA2a, for example, has been shown to lead to an increase in SR Ca^{2+} -ATPase activity, an increase in the amount of Ca^{2+} released, a faster relaxation phase, and a decrease in diastolic calcium. Conversely, using gene transfer to increase phospholamban relative to SERCA2a in isolated myocytes, simulates abnormalities in calcium handling seen in failing ventricular myocardium.^{11-13,44} These include prolongation of the relaxation phase of the calcium transient, a decrease in Ca^{2+} release, and an increase in resting Ca. Furthermore, over-expressing SERCA2a can largely rescue the phenotype created by increasing the phospholamban/SERCA2a ratio.^{11-13,44} More recently, restoration of SERCA2a in

failing human cardiomyocytes was shown to restore the contractile function of these failing human cells to normal as shown in Figure 4.^{11-13,44} This study validated the premise that targeting SERCA2a by gene transfer may offer a new therapeutic modality in patients with heart failure.

At our lab, we recently investigated whether increasing SERCA2a expression can improve ventricular function in a rat model of pressure-overload hypertrophy and failure. After 19-23 weeks of banding, during the transition from compensated hypertrophy to heart failure, over-expression of SERCA2a restored both SERCA2a expression and ATPase activity to nonfailing levels. Furthermore, rats infected with Ad.SERCA2a had significant improvement in left ventricle systolic pressure (LVSP), $+\text{dP}/\text{dt}$, $-\text{dP}/\text{dt}$, and rate of isovolumic relaxation, enabling them to normalize to levels that were comparable with sham-operated rats.^{11-13,44,45} Load-independent parameters of contractility were also shown to be improved. More recently, transfer of SERCA2a in a rat model of heart failure was shown to effectively decrease mortality.^{11-13,44-49} In fact, survival was increased significantly in animals receiving gene transfer compared to those who did not (63% vs 9%) as shown in Figure 5. Furthermore, cardiac metabolism measured by the ratio of creatine phosphate to ATP was also restored to normal. The restoration of the energetics was surprising because enhanced contractility is associated with increased energy demand. The fact that gene transfer of SERCA2a

Figure 4: Effect of adenoviral gene transfer of SERCA2a (Ad.SERCA2a) in failing cardiac myocytes loaded with the fluorescent calcium indicator, Fura-2. In failing cardiac myocytes (infected with the adenovirus carrying the reporter gene green fluorescent protein [GFP]), contraction amplitude is decreased and prolonged compared to non-failing cardiac myocytes. Gene transfer of SERCA2a in the failing cardiac myocytes restores contraction to normal levels. (Modified from Reference #44)

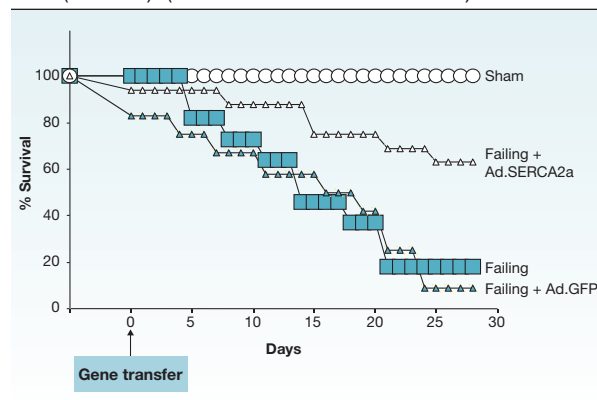


was not associated with a compromise in energetics means that a beneficial remodeling occurred within the cardiac cell restoring the metabolic machinery. The decrease in diastolic calcium seen with restoration of SERCA 2a levels to normal has also been postulated to reduce pro-apoptotic and pro-hypertrophic signaling (as sustained elevations of intracellular calcium lead to activation of serine-threonine phosphatases, including calcineurin, inducing hypertrophy and cell death).

β-adrenergic signaling

The β-adrenergic signaling pathway provides an important target for intervention in heart failure. β-adrenergic signaling defects, including down-regulation of myocardial β-adrenergic receptors, β-AR uncoupling, and an up-regulation of the β-AR kinase (βARK1), are central features in human and animal heart failure. In isolated

Figure 5: Survival curves in failing rats compared with failing rats who have received SERCA2a gene transfer. Note the improved survival compared to failing rats who either had no gene transfer or received the reporter gene GFP (Ad.GFP). (Modified from Reference #11)



ventricular myocytes from a model of heart failure in the rabbit, adenoviral gene transfer of the human β₂-AR or an inhibitor of βARK1 led to the restoration of β-AR signaling and an increase in cytosolic cAMP levels. More recently, overexpression of an inhibitor of βARK1 by gene transfer of a truncated and dysfunctional βARK1 (βARKct) rescued function in a model of left ventricular dysfunction in the rabbit. These studies, along with the finding that overexpression of βARKct prevents the development of cardiomyopathy in a murine model of heart failure, emphasize the importance of β-adrenergic signaling defects in the pathogenesis of heart failure and raises the possibility that targeting this system may restore function in failing cardiomyocytes.⁵⁰⁻⁵² However, stimulation of the β-adrenergic system induces an increase in intracellular cAMP which, when sustained, can be cardiotoxic and arrhythmogenic. In fact, overexpression of the β₁ receptor induces fibrosis, severe left ventricular dysfunction, and arrhythmias.⁵³ It is possible that this mechanism may underlie the clinical observation that inotropic interventions that increase cellular cAMP increase mortality in chronic heart failure. In fact, a recent study found that in mice overexpressing β₂-adrenergic receptors, development of heart failure was exacerbated when these mice were subjected to aortic stenosis. Moreover, the transgenic mice had more severe left ventricular dysfunction and a higher incidence of premature deaths. Interestingly, intracoronary injection of a recombinant adenovirus encoding adenylyl cyclase provided enduring increases in cardiac function in normal pigs, even though adenylyl cyclase modulates cAMP.⁵⁴ Nevertheless, the critical role of the β-adrenergic pathway suggests further investigation of this pathway as a target for intervention, despite the cautionary clinical and experimental experience of direct β-agonism. As shown in Figure 6, a summary of the effects of overexpressing SERCA2a by gene transfer on cAMP, intracellular calcium, arrhythmias, survival, and energetics, while at the same time enhancing contractility, point towards an overall beneficial effect of this mode of inotropy over conventional inotropic agents.

Future directions

Ongoing advances in vector technology, cardiac gene delivery, and most importantly, our understanding of heart failure pathogenesis, encourage consideration of gene therapy for heart failure at this time. Strategies that enhance sarcoplasmic calcium transport are supported by substantial evidence in both cardiomyocytes derived from patients with heart failure and in animal models. Initial studies evaluating other novel targets appear promising, but have not been as fully evaluated. In ongoing efforts to target cardiac dysfunction, gene transfer provides an important tool to improve our understanding of the relative contribution of specific pathways. Through such experiments, molecular targets can be validated for therapeutic intervention, whether pharmacological or genetic.

Figure 6: Differences in conventional treatment for heart failure versus targeting specifically SERCA2a on survival, cAMP, calcium handling, energetics, and arrhythmogenic potential.

	Conventional inotropic agents	Targeting SERCA2a
cAMP	↑	↔
Intracellular Ca ²⁺	↑Dias. ↑Sys.	↓Dias. ↑Sys.
Arrhythmogenicity	↑	↓
Survival	↓	↑
Energetics/Metabolism	↓	↑

However, translating these basic investigations into clinical gene therapy for heart failure remains a formidable challenge. Further development of concepts established in rodent models will be required in large animal models with clinical grade vectors and delivery systems to evaluate both efficacy and safety of these approaches. Nevertheless, practical advances and our growing understanding of the molecular pathogenesis of heart failure provide reason for cautious optimism.

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