

Gene Therapy for Heart Failure: An Investigational Treatment That is Coming of Age

Donna Mancini and Mary Jane Farr

Department of Medicine, Division of Cardiology, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Over the past 2 decades, despite advances in the management of heart failure with the addition of highly effective drugs and devices, the number of heart failure patients continues to increase. Heart failure is the end result of a variety of disease states including coronary artery disease, hypertension, alcohol abuse, and infections. Irrespective of the etiology of heart failure, many changes in the genetic expression of contractile proteins occur in the cardiac myocyte that may create effective targets for molecular therapies.¹ Whether gene therapy can improve cardiac function by normalizing gene expression of contractile proteins is an avenue of active clinical research in the 21st century. In this brief review, the early state of gene therapy for heart failure will be described.

Gene Therapy

Gene therapy is the insertion of a gene into a human cell to treat a disease. This can be a hereditary disease with a defined genetic defect such as cystic fibrosis, muscular dystrophy, or hemophilia. Some cardiomyopathies are genetically based, such as hypertrophic cardiomyopathies. However, gene therapy can also be used to normalize the expression of down-regulated genes in a disease state. In heart failure, at the cellular level the cardiac myocyte responds to a physiological stress, i.e volume or pressure overload, with activation of what has been called a “fetal gene program.”² This hypertrophic response is associated with a decline in the gene

expression for several critical proteins, including decreased expression of alpha myosin heavy chain, beta myosin heavy chain, sarcoplasmic reticulum, calcium ATPase (SERCA2a), and phospholamban. Several heart failure pharmacologic therapies and devices, such as neurohormonal blockade, mechanical unloading with left ventricular assist devices³ and cardiac resynchronization, result in molecular “remodeling” of these genetic changes with a return of gene expression towards normal levels.² These molecular remodeling effects correspond to improvement in left ventricular function and are potential targets for gene therapy.

Administration of Gene Therapy

Gene therapy requires the transfer of the therapeutic gene into the cells of the patient. The usual delivery system involves vectors which frequently are viral in origin.¹ The usual viral vectors include retroviruses, adenoviruses, adeno-associated viruses. Adenovirus vectors were first used in the late 1990, but significant concerns arose regarding the pathogenesis of the viral vector following the death of a young recipient in an early clinical trial. Moreover, some viruses such as adenoviruses generate an inflammatory response that decreases the long term effectiveness of this therapy and precludes recurrent administration due to the secondary immune response. Adeno-associated viruses are small single stranded DNA viruses derived from the parvo virus family.⁴ Recombinant adeno-associated viruses contain only the therapeutic gene and cannot integrate into the human genome. The therapeutic gene is free in the nucleus of the cell. It does not replicate if the cell undergoes division. Adeno-associated viruses are non-pathogenic and have minimal immunogenicity, but are difficult to produce and generally deliver only small quantities of DNA. However, the non-pathogenicity of this vector has made it an attractive option in current clinical trials. Additionally, some adeno-associated viruses exhibit a selective tropism for cardiac tissue. The only human heart failure trial investigating gene therapy currently uses an adeno-associated virus as the gene delivery mechanism.⁴

Disclosure: Prof. Mancini is a consultant for the Celladon Corporation. Her role is the interpretation of cardiopulmonary tests for the phase I/II Cupid trial.

Correspondence: Dr D. Mancini,
Division of Cardiology, Columbia University College
of Physicians & Surgeons,
622 West 168th Street, PH1273, New York, NY 10032, USA
E-mail: dmm31@columbia.edu

Application of Gene Therapy in Heart Failure

Several potential gene therapy targets in heart failure patients have been identified from experiments using transgenic mouse models. These targets include: SERCA2a, phospholamban, G protein-coupled kinase 2 receptor, protein phosphatase 1, adenylate cyclase, among others.¹

Calcium cycling mediated by SERCA2a is a critical control mechanism in regard to cardiac contraction. During the action potential, calcium enters the cell through L type calcium channels as inward calcium current. Calcium entry triggers calcium release from the sarcoplasmic reticulum into the cytosol by activation of the ryanodine receptor. The increase in cytoplasmic free calcium contraction allows calcium to bind to troponin C, triggering contraction. Relaxation depends on the release and decline of cytosolic calcium. This requires calcium transport out of the cytosol by multiple pathways including SERCA2a, sodium-calcium exchange pump, mitochondrial transport, and ryanodine receptors. SERCA2a removes up to 90% of intracellular calcium in rodents and 70% in humans and large animals. Abnormal cellular calcium handling is observed in the failing heart. Increased sodium calcium exchange mechanism, reduction in SERCA2a activity, decreased phospholamban/SERCA2a ratios and increased phosphorylation of ryanodine receptor producing "leakiness" all result in decreased sarcoplasmic reticulum calcium content and a prolonged calcium transient. Any one of these proteins could be a molecular target for the treatment of heart failure.^{1,4-6}

So far, SERCA2a^{7,8} and the G protein-coupled kinase 2 receptor (GRK2 or BARK1)⁹ have been studied in animal models using gene transfer with adeno-associated viral vectors.

Limiting beta receptor desensitization by GRK2 inhibition may be a beneficial molecular strategy in heart failure. In a rat heart failure model, direct intramyocardial injection of beta adrenergic receptor kinase resulted in improved cardiac contractility and reversal of left ventricular remodeling.⁹ Studies using this approach in larger animal models are planned and not yet reported.

SERCA2a gene therapy has been studied in small and large animal models and is now undergoing evaluation in human subjects. Dr Roger Hajjar has pioneered the use of SERCA2a therapy in heart failure.⁶ An initial proof of concept study published in 1999⁵ demonstrated that failing human cardiomyocytes showed normalization of contractility after SERCA2 gene transfer. SERCA2a was overexpressed in human ventricular myocytes from 10 patients with end stage congestive heart failure (CHF). Overexpression of SERCA2a resulted

in an increase in SERCA2 protein¹⁰ expression and pump activity with a greater contraction velocity and enhanced relaxation. Hajjar's group subsequently went on to demonstrate the efficacy of this therapy in small and large animal models of heart failure. In a rat model of heart failure caused by ascending aortic constriction, increasing SERCA2a expression by gene transfer improved ventricular function.^{7,8} Moreover, in the rats who received gene therapy, overexpression of SERCA2a gene expression prolonged survival, normalized LV volumes and improved cardiac metabolism compared to sham treated control rats.¹¹

Similar results were observed using a large animal model. In swine, heart failure from volume overload was induced by severing the chordar tendininae of the mitral valve apparatus.¹² After 2 months of severe mitral regurgitation, 16 pigs underwent intracoronary delivery of the recombinant adeno-associated virus type 1 vector carrying SERCA2a and 6 pigs received saline. The treated animals displayed improved left ventricular (LV) contraction with significantly higher LVdp/dt and a decrease in ventricular volume compared to the control animals. Moreover, the treated animals had restoration of SERCA2a gene expression to normal levels. The kinetics of expression of the adeno-associated virus serotype 1 (AAV1) gene was dose dependent with significant rise in gene expression by 4 weeks after administration. Long-term expression of the gene was shown. These animal studies provided the basis to proceed with the first human gene trial in heart failure subjects.

The Calcium Upregulation by Percutaneous Administration of Gene Therapy in Cardiac Disease (CUPID) is a phase I/II study investigating the safety and biologic activity of SERCA2a replacement therapy in human heart failure.^{13,14} The study population consisted of patients with class III/IV CHF left ventricular ejection fractions <30%, and maximal oxygen consumption <16 mL/kg/min. All had implantable defibrillators and had been on a chronic stable outpatient oral heart failure regimen for at least 30 days. Prior to enrollment in the trial, patients were screened for neutralizing antibodies to the viral capsid protein. Approximately 60% of patients had neutralizing antibody titers of >1:2. Patients were eligible for study if neutralizing antibody titers against AAV1 viral capsid protein was ≤1:2. Given the large percentage of patients with neutralizing antibodies, if this therapy is ultimately found to be effective, neutralizing antibodies will be a limiting factor. Additionally, if the patient had coronary artery disease, coronary angiograms were reviewed to determine if the anatomy precluded adequate delivery of the gene product. Patients with prior coronary artery bypass grafting, left main or

ostial right stenoses were excluded. Other major exclusion criteria included treatment in the last 30 days with intravenous inotropic therapy; restrictive, infiltrative or obstructive cardiomyopathies; myocardial infarction within the past 6 months, or severe intrinsic liver or renal disease.

The safety study consisted of intra-coronary administration of MYDICAR (AAV1/SERCA2a; Targeted Genetics Corporation, Seattle Wash) in ascending doses. The intracoronary infusion was accomplished with standard catheters connected to a MEDRAD Mark V ProVis angiographic system (Indianola PA). Four dose levels in groups of 3 patients separated by time intervals were included in the study to determine safety and adequate dose level. A single antegrade infusion of AAV1/SERCA2a was administered over a 10-minute period starting at 1.4×10^{11} , 6×10^{11} or 3×10^{12} doses.

Enzyme Linked ImmunoSPOT (ELISPOT) assays were used to assess for potential cellular response to the AAV1 capsid proteins. Efficacy assessments included 2D- and 3D-echocardiograms, New York Heart Association Functional Class, Minnesota Living with Heart Failure Questionnaire, 6-minute walk tests, cardiopulmonary exercise testing, biomarkers (BNP), and clinical outcomes (all cause death, heart failure hospitalization, need for intravenous medications). Safety assessments included complete blood counts, cardiac enzymes, ELISPOT, serum chemistries, electrocardiograms, and interrogation of the implantable defibrillator.

The results for the first 9 heart failure patients enrolled in the study were recently reported. Safety data as well as 6- to 12-month follow-up data in regard to quality of life, functional assessments and echocardiograms were described. Seven men and 2 women, all with New York Association Class III CHF were studied. Age averaged 52 (7) years, left ventricular ejection fraction was 22% (5%), peak VO_2 was 14.2 (2.5) mL/kg/min. All patients tolerated the intracoronary infusions without significant adverse events. There was no evidence of increase in ventricular ectopy, no systemic effects and no myocarditis. Only 1 patient had transient elevation in the ELISPOT assay when the patient had a coexistent upper respiratory viral syndrome. Eight of the 9 patients were alive at the time of the report. One patient died suddenly more than 3 months post SERCA2a. This patient had a prior history of sudden death syndrome; his death was attributed to natural progression of his severe heart failure. One patient was transplanted at month 8 and another deteriorated and required a mechanical assist device. The 2 patients who went on transplant and device therapy both had neutralizing antibody levels of 1:2. In the 7 patients without neutralizing antibodies, 5 demonstrated symptomatic improvement, 4 had

functional improvement, and 5 had improved LV remodeling. On the basis of these initial findings, 34 patients have been enrolled in a phase 2 trial. Two of 3 patients receive active therapy. Enrollment is complete and data collection is ongoing, with results available in 2010.

Conclusions

Gene therapy appears to be well tolerated and safe in a small study of heart failure patients. The results of the Phase II placebo controlled trial using SERCA2a gene therapy will be of great interest and the first of what may be many future gene trials.

REFERENCES

- Vinge L, Raake P, Koch W. Gene therapy in Heart Failure. *Circ Res.* 2008;102:1458-70.
- Coppola T. Molecular remodeling in Human Heart Failure. *J Am Coll Card.* 2008;51:137-8.
- Burkhoff D, Klotz S, Mancini D. LVAD-induced reverse remodeling: basic and clinical implications for myocardial recovery. *J Card Fail.* 2006;12:227-39.
- Zsebo K, Jaski B. Serca 2a Gene Transfer for Heart Failure. *Touch Briefings 2009.* US Cardiology. London: Touch Group; 2009. p. 46-9.
- Minamisawa S, Hoshijima M, Chu G, Ward CA, Frank K, Gu Y, Martone ME, et al. Chronic phospholamban sarcoplasmic reticulum calcium ATPase interaction is the critical calcium cycling defect in dilated cardiomyopathy. *Cell.* 1999;99:313-22.
- Meyer M, Schillinger W, Pieske B, Holubarsch C, Heilmann C, Posival H, et al. Alterations of sarcoplasmic reticulum proteins in failing human dilated cardiomyopathy. *Circulation.* 1995;92:778-84.
- Hajjar R, Kang J, Gwathmey J, Rosenzweig A. Physiological effects of adenoviral gene transfer of sarcoplasmic reticulum calcium ATPase in isolated rat myocytes. *Circulation.* 1997;95:423-9.
- Miyamoto MI del Monte F, Schmidt U, DiSalvo TS, Kang ZB, Matsui T, et al. Adenoviral gene transfer of SERCA2a improves left-ventricular function in aortic banded rats in transition to heart failure. *Proc Natl Acad Sci U S A.* 2000;97:973-8.
- Rengo G, Lympopoulos A, Zincarelli C, Donniacuo M, Soltys S, Rabinowitz J, Koch W. Myocardial adeno-associated virus serotype 6-BARKct gene therapy improves cardiac function and normalizes the neurohormonal axis in chronic heart failure. *Circulation.* 2009;119:89-98.
- del Monte D, Harding S, Schmidt U, Matsui T, Kang ZB, Dec GW, et al. Restoration of contractile function in isolated cardiomyocytes from failing human hearts by gene transfer of SERCA2a. *Circulation.* 1999;100:2308-11.
- del Monte F, Williams E, Lebeche D, Schmidt U, Rosenzweig A, Gwathmey K, et al. Improvement in patient survival and cardiac metabolism after gene transfer of sarcoplasmic reticulum Ca ATPase in a rat model of heart failure. *Circulation.* 2001;104:1424-9.
- Kawasi Y, Ly H, Pruner F, Lebeche D, Shi Y, Jim H, et al. Reversal of cardiac dysfunction after long term expression of Serca 2a by gene transfer in a pre-clinical model of heart failure. *J Am Coll Cardiol.* 2008;51:1112-9.

13. Haijar R, Zsebo K, Deckelbaum L, Thompson C, Rudy J, Yaroshinsky A, et al. Design of a phase 1 / 2 trial of intracoronary administration of AAV1/Serca 2a in patients with heart failure. *J Cardiac Fail.* 2008; 14:355-67.
14. Jaski B, Jessup M, Mancini D, Coppola T, Pauly D, Greenberg B, Borow, et al. Calcium upregulation by percutaneous administration of gene therapy in cardiac disease (Cupid Trial) a first in human phase 1 / 2 Clinical Trial. *J Cardiac Failure.* 2009;15:171-81.