Development of Therapeutics for Heart Failure

Small Animal Models of Heart Failure
Development of Novel Therapies, Past and Present

Richard D. Patten, MD; Monica R. Hall-Porter, PhD

The study of heart failure requires viable animal models whereby chronic changes in myocardial structure and function can evolve and the progression of heart failure and left ventricular (LV) dysfunction can be quantified. During the past 40 years, basic and translational scientists have used small animal models to explore the pathophysiology of heart failure and to develop novel therapies that might slow the progression of this prevalent and fatal disease. The purpose of this review is to describe commonly used heart failure models in rodents and to cite examples of how these models have been used to evaluate novel therapies for the treatment of heart failure.

Heart Failure Models in Rats
Heart failure models were originally developed in rats because of numerous potential advantages inherent in a small animal model (see Table 1). Housing and maintenance costs for rats are much lower than for large animals, thus reducing costs and increasing the number of animals included in a given study to improve the statistical power. Moreover, more recent technological advances in echocardiography, MRI, and micromanometer conductance catheters have greatly streamlined the assessment of cardiac function in rodents, removing a significant barrier to their use in heart failure research. The development of suitable expertise to perform open-chest surgical procedures and invasive hemodynamic assessments in rats is far easier compared with that required for mice. Additionally, investigators are able to perform a greater number of postmortem histological or molecular biological analyses given the approximately 10-fold greater myocardial mass of rats compared with mice. For these reasons, the rat models detailed below have been the most widely and successfully used heart failure models in basic and translational research.

Rat Models of Myocardial Injury
Myocardial infarctions (MIs) in rats were originally induced by the sequential administration of subcutaneous isoproterenol causing diffuse myocardial necrosis. Subsequent investigators used an electrocautery technique applied to the epicardial surface to induce small, focal infarctions. Soon thereafter, Pfeffer et al developed the rat coronary ligation model that became perhaps the most widely used heart failure and MI model in the decades to follow. The Pfeffer group first applied this model to explore the relationship between infarct size and LV chamber dilatation and function (Figure 1). They observed a proportional increase in LV volume as a function of infarct size that was a highly original and novel finding, at which time, the notion that vasodilators may “unload” and therefore benefit failing hearts was gaining momentum. The angiotensin-converting enzyme inhibitor, captopril, was the first used by Marc and Janice Pfeffer and others who observed that angiotensin-converting enzyme inhibitor therapy reduced LV chamber dilation, improved LV systolic function, and increased survival in rats with moderate or large MIs.6,7 This ground-breaking work in the rat MI model led to clinical trials testing the utility of the angiotensin-converting enzyme inhibitor, captopril, in post-MI patients with reduced LV function. Following a small pilot study,7 the large, multicenter Survival and Ventricular Enlargement trial was conducted in which captopril or placebo was administered 3 to 16 days following MI in patients with reduced LV function. Captopril decreased all-cause mortality by 19% with a 22% reduction in heart failure hospitalizations after a mean follow-up period of 42 months; the reductions in mortality and morbidity were associated with less LV dilation or remodeling during the first year of therapy.9 This is perhaps one of the most elegant demonstrations of the utility of small-animal models to explore new and potentially important therapies for heart failure.

The rat MI model was also essential in establishing the beneficial effects of angiotensin II type 1 receptor antagonists on LV structure and function following MI.10,11 However, the case for endothelin (ET) receptor antagonists illustrates that results in this model are not necessarily mirrored in clinical heart failure trials. Sakai et al first reported that treatment with the combined ET receptor type A and B receptor antagonist, BQ123 (bosentan), improved survival and reduced LV chamber dilation 12 weeks following MI in rats. Subsequent clinical trials using either the ET receptor type A antagonist, darusentan, or the dual ET receptor antagonist, bosentan, demonstrated no added benefit in patients with heart failure and reduced LV ejection fraction. In fact, a propensity toward worsening heart failure was noted early in life when bosentan therapy was used in children with congenital heart disease.
the treatment of patients with heart failure. Interestingly, because of reported reductions in pulmonary arterial pressures in rat models of pulmonary hypertension, such observations ultimately led to the successful application of the nonselective ET receptor antagonist, bosentan, for the treatment of pulmonary hypertension.

More recently, Koch and colleagues used the rat MI model to examine the utility of adeno-associated viral-mediated delivery of a gene construct encoding S100A1, a calcium regulatory protein that facilitates calcium transport across sarcoplasmic reticulum by optimizing the activity of the ryanodine receptor and sarcoplasmic reticulum calcium ATPase pump (Sera2A). Diminished expression of S100A1 in failing hearts is therefore likely a contributing factor to calcium handling abnormalities in failing cardiac myocytes. The S100A1 viral vector administered to rats post-MI restored the expression of S100A1 to normal levels, improved LV function, and reduced LV chamber dilation. It is clear from these examples that the rat MI model has been critical to the development of both well-established heart failure therapies and novel molecular approaches that may hold promise in the future.

Rat Pressure Overload Models

Ascending Aortic Banding

There are numerous surgical methods to induce pressure overload in rats, but the ascending aortic banding is one of the more widely used surgical models, in which a stricture is placed around the ascending aorta of weanling (3- to 4-week-old) rats. As these rats grow, hypertension develops gradually, during which aortic outflow is increasingly impeded. Lorell and colleagues developed this model and showed that 8 weeks postbanding, rats exhibit maintenance of LV chamber size with clear evidence of LV hypertrophy, consistent with “compensated hypertrophy” though Doppler echocardiography at this stage shows evidence of increased left atrial pressure. By 18 weeks, overt signs of heart failure become evident (ie, tachypnea, edema, pleural effusions, and ascites) that are associated with LV dilation and systolic dysfunction. The advantages and disadvantages in this model are similar to those noted for the rat MI model (Table 1) with the added benefit that the stimulus for heart failure (pressure overload) is gradual in onset as is the progression from compensated hypertrophy to decompensated heart failure, therefore making this model potentially more clinically relevant to heart failure progression in humans. Hajjar and colleagues have used this model extensively to explore the utility of adenoexemplar gene therapy to restore levels of the calcium handling protein, Sera2A, whose expression is diminished in failing hearts. After optimizing the technique of gene delivery, they achieved adequate myocardial expression of the Sera2A construct accompanied by clear improvements in LV systolic function, remodeling, and survival. Following preclinical studies in larger animal models, this novel gene therapy approach has now entered the clinical arena as a phase 1 clinical trial.

Genetic Models of Hypertension and Heart Failure in Rats

In addition to inducing pressure overload by surgical means, several genetic models of hypertension and heart failure in rats have been used extensively. In one such model, the Dahl salt-sensitive rat, hypertension and heart failure develop gradually in rats placed on a high-salt diet. LV hypertrophy without chamber dilatation develops within 4 to 6 weeks of a high-salt diet followed by a decompensated phase of heart failure and LV dilation at approximately 15 to 20 weeks. The spontaneously hypertensive heart failure-prone rat developed by McCune et al is another commonly used model. Heyen et al demonstrated that progressive LV hypertrophy with normal ejection fraction develops between 4 and 9 months of age; by 12 months, LV dilation and decreased LV systolic function occurs in tandem with a marked rise in circulating cytokine levels including tumor necrosis factor (TNF)-α and interleukin-6. These genetic models of pressure overload and heart failure offer several advantages. The gradual onset of hypertension with aging has more direct clinical relevance, with both models being characterized by time-dependent, progressive LV hypertrophy in the early stages followed by the development of heart failure and LV dysfunction in later stages. Moreover, no surgical expertise is required, though a notable drawback is the high cost associated with maintaining colonies for an extended period of time (6 to 12 months) to allow emergence of the heart failure phenotype.

Heart Failure Models in Mice

Mouse heart failure models offer many of the same advantages as the rat models discussed previously (also see Table 1). The greatest benefit in using mouse models is the availability of a great number of relevant transgenic and knockout (KO) strains. The advent of cell type-specific, inducible KO or transgenic strategies has made the mouse an invaluable tool to study the pathogenesis of heart failure and to identify novel therapeutic targets. Mouse models have the added benefit of lower housing costs compared with rats. In
addition, cardiac physiological assessments have been facilitated greatly by newer technologies such as ultrahigh resolution ultrasound and micromanometer conductance technology for pressure volume loop analyses. However, such tools are extremely expensive and pose significant technical challenges to laboratories without an established expertise. Whereas the rat models listed above have been used extensively to explore the potential for novel pharmacological or molecular agents for the treatment of heart failure, mouse models are best used as “proof of principle” to identify important gene or protein targets that pave the way for the development of new molecular or pharmacological therapies.

Mouse Models of Myocardial Injury

The development of transgenic and KO methodology led to a virtual explosion in new approaches to study disease pathogenesis at which time the development of surgically induced mouse models of heart failure became imperative. A mouse model of coronary ligation to induce a MI was first described 3 decades ago. More recently, Michael et al. were the first to establish a mouse model of ischemia-reperfusion injury, during which they identified that mouse left coronary anatomy is highly variable (Figure 2A). Models of permanent coronary ligation in mice were followed in our laboratory (Figure 2B) and others in an

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effort to gain further insight into pathophysiology of post-MI cardiac remodeling.32,33

Mouse models of heart failure can be used to identify potentially important and novel targets for pharmacological or molecular therapy. The recent and evolving story of the serine-threonine kinase, calmodulin kinase II (CaMKII), in LV remodeling and heart failure exemplifies this well. CaMKII activity and expression are increased in the failing heart.34 CaMKII targets multiple calcium regulatory proteins within cardiac myocytes including the ryanodine receptor and phospholamban, an important regulator of Serca2A. Heller-Brown and colleagues35 overexpressed the cytoplasmic isoform of CaMKII in the hearts of transgenic mice and observed the development of progressive LV hypertrophy, LV dilation, and heart failure, supporting that CaMKII contributes to heart failure progression. Anderson and colleagues36 then developed transgenic mice that expressed a cardiomyocyte-specific CaMKII inhibitory peptide, which in response to left coronary ligation, exhibited less LV hypertrophy, dilation, and systolic dysfunction (Figure 3). Moreover, a novel pharmacological inhibitor of CaMKII (KN93) attenuated LV dilation and improved LV systolic function post-MI in wild-type mice,37 therefore, supporting that CaMKII is a worthy therapeutic target for drug development. These studies also exemplify how mouse models can unveil the physiological relevance of a given protein using complementary overexpression and molecular and pharmacological strategies.

Mouse Pressure Overload Model of Transverse Aortic Constriction

Although numerous pressure overload models have been developed in mice, the transverse aortic constriction (TAC) model is perhaps the most widely used model; it was first described by Rockman et al.37,38 The greatest advantage of this model is the ability to quantify the pressure gradient across the aortic stricture that allows stratification of LV hypertrophy (Figure 4A). The sudden onset of hypertension achieved with TAC causes an approximately 50% increase in LV mass within 2 weeks, making this model an excellent choice to examine the utility of pharmacological or molecular interventions that may limit hypertrophy. In our experience, performing TAC in mice requires greater surgical expertise than that required for coronary ligation. An important drawback of the TAC model is the variability in the LV remodeling responses among different mouse strains: C57BL6 mice develop rapid LV dilation39 after TAC that may not occur with other strains (unpublished observations and reference 40). Moreover, the acute onset of severe hypertension characteristic of this model lacks direct clinical relevance. Nonetheless, the literature is replete with examples of how this model can be used to identify and modify novel therapeutic targets in heart failure. For example, the protein kinase C (PKC) family of serine-threonine kinases has gained increasing attention41 given that PKC/βII42 is a fundamental regulator of calcium handling and cardiac contractility.42 Molkentin and colleagues43 explored the relevance of PKCα in LV hypertrophy by applying the TAC model to PKCα KO mice that exhibited less LV chamber dilatation and improved systolic function compared with wild types. Jeong et al44 then applied the TAC model to transgenic mice with cardiomyocyte-specific overexpression of an endogenous PKC inhibitor, PKC-interacting cousin of thioredoxin, which developed a phenotype similar to the PKCα KO mice, exhibiting less LV chamber dilation and systolic dysfunction.
Kass and colleagues recently explored the effects of the widely prescribed phosphodiesterase 5 inhibitor, sildenafil, on the development of LV hypertrophy and remodeling using the mouse TAC model. Sildenafil reduced LV dilation and hypertrophy supporting that phosphodiesterase 5 inhibition attenuates pressure overload-induced myocardial hypertrophy. These data are particularly intriguing given the increasing interest in sildenafil as a potential therapy for heart failure because of its favorable effects on endothelial function and functional capacity in patients with heart failure and systolic dysfunction. Whether sildenafil improves LV remodeling and reduces mortality in a large heart failure population has yet to be studied. These studies exemplify, however, the tremendous utility of the TAC model in identifying important therapeutic targets and exploring the effects of molecular or pharmacological inhibitors.

Genetic Models of Dilated Cardiomyopathy in Mice
Numerous KO or transgenic mice have been developed that have implicated a given protein in the pathogenesis of heart failure and dilated cardiomyopathy (reviewed in reference 47). For example, Arber et al developed a model of dilated cardiomyopathy in which homozygous deletion of the gene encoding muscle lim protein caused myofiber disarray, LV hypertrophy, dilation, systolic dysfunction, and heart failure. Muscle lim protein is an actin-based cytoskeletal protein that...
positively regulates myogenic differentiation. The authors hypothesized that its disruption may uncouple mechanical load from the induction of muscle-specific genes that maintain striated muscle function. Indeed, muscle lim protein KO mice develop a cardiac phenotype resembling dilated cardiomyopathy characterized by the development of LV dysfunction, heart failure, and death. Muscle lim protein KO mice have since served as a genetic model of heart failure that is used by many laboratories to explore molecular therapies that might “rescue” the heart failure and dilated cardiomyopathic phenotype. The TNF-α-overexpressing mice represent another model of dilated cardiomyopathy. TNF-α is a circulating cytokine that has long been suspected to contribute to heart failure progression. Accordingly, mice with cardiomyocyte-specific overexpression of TNF-α develop LV hypertrophy, dilatation, and profound systolic dysfunction associated with heart failure and premature death. Treatment of these mice with a soluble chimeric protein comprised of the TNF-α receptor bound to an IgG fragment neutralizes circulating TNF-α and improves indices of cardiac function in these mice. These preliminary experimental observations led to a multicenter clinical trial exploring the efficacy of the soluble TNF receptor chimera in heart failure. Surprisingly, the soluble TNF receptor chimera offered no morbidity or mortality benefits to patients with heart failure and LV systolic dysfunction, illustrating that positive results in preclinical rodent studies do not necessarily translate to clinical benefits when applied to heterogeneous heart failure populations.

Caution must be entertained with respect to analyzing the results from any model of dilated cardiomyopathy caused by the cardiomyocyte-specific overexpression of a given protein. Supernumerical overexpression of any protein in cardiomyocytes may drastically alter their biology in a manner not related to the specific function of a protein. This notion was illuminated by Izumo and colleagues, in which transgenic mice that express the biologically inert, green fluorescent protein in a cardiomyocyte-specific manner developed significant LV hypertrophy, dilation and systolic dysfunction, in a manner directly related to the level of green fluorescent protein expression. This study demonstrates that nonspecific effects on LV structure and function may result from vast overexpression of even biologically inert proteins.

Summary

The use of small-animal models to study complex cardiovascular pathophysiology has proven to be invaluable during the past 4 decades. As a direct result of basic and translational studies in murine models, some of which have been highlighted above, our understanding of pathophysiology of heart failure and its treatment has advanced considerably. Rat models have been used primarily to assess the efficacy of specific pharmacological or molecular therapies. The ability to manipulate the mouse genome has facilitated a particularly elegant approach to identify novel therapeutic targets, offering a “proof of principle” approach to explore the mechanisms underlying heart failure and its progression. Moving forward, these small animal models of heart failure will continue to be critical tools in the identification of new therapeutic targets and evaluation of specific therapies for heart failure.

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