

Heart failure and mouse models

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Heart failure is a common, complex condition with a poor prognosis and increasing incidence. The syndrome of heart failure comprises changes in electrophysiology, contraction and energy metabolism. This complexity, and the interaction of the clinical syndrome with very frequently concurrent medical conditions such as diabetes, means that animal modelling of heart failure is difficult. The current animal models of heart failure in common use do not address several important clinical problems. There have been major recent advances in the understanding of cardiac biology in the healthy and failing myocardium, but these are, as yet, unmatched by advances in therapeutics. Arguably, the development of new animal models of heart failure, or at least adaptation of existing models, will be necessary to fully translate scientific advances in this area into new drugs. This review outlines the mouse models of heart failure in common usage today, and discusses how adaptations in these models may allow easier translation of animal experimentation into the clinical arena.

Heart failure: the medical problem

Heart failure is an increasingly common diagnosis, with a dismal prognosis that is worse even than many types of cancer (Ho et al., 1993). There are few therapeutic options. The estimated cost of heart failure is currently between 1-2% of the total healthcare spend in developed economies and is expected to rise (McMurray and Stewart, 2000).

Clinical presentation may be insidious or acute, with decreased exercise tolerance and shortness of breath. Cardiac arrhythmias may accompany heart failure, leading to high rates of sudden death. Current treatment includes simultaneous administration of angiotensin-converting enzyme (ACE) inhibitors (acting principally by vasodilatation), β blockers (slowing the heart rate) and spironolactone (vasodilatation and diuresis). This combination reduces death resulting from heart failure, but these drugs do not 'cure' the condition. There are no truly 'disease modifying' drugs for heart failure.

Many conditions eventually lead to heart failure (Table 1), several of which are associated with each other, such as hypertension, obesity and diabetes, as exemplified in

the case study. Our understanding of the heart failure disease phenotype is not yet sufficient to appreciate whether aspects of the final heart failure syndrome differ with the causative aetiologies. Preliminary evidence, however, demonstrates that different gene expression patterns (Huang et al., 2005) and prognoses (Felker et al., 2000) are associated with heart failure resulting from differing causes.

Heart failure comprises changes in cardiac contractility, electrical conduction and energy metabolism, leading to an inability of the heart to meet circulatory demands (Jessup and Brozena, 2003; Stanley et al., 2005). This activates neurohormonal compensatory mechanisms, such as vasoconstriction, which are thought to be helpful in maintaining general organ perfusion in the short term, but maladaptive with respect to long-term cardiac function. In this way, the physiological and gene expression changes observed in heart failure are not constantly 'adaptive' or 'maladaptive' (and are, thus, not easily amenable to pharmacological modulation). This has introduced therapeutic confusion over the years. For example, use of β -adrenoceptor antagonists was dis-

couraged in heart failure for many years on the basis of studies showing increased mortality when given to acutely unwell patients (Braunwald and Chidsey, 1965). Subsequently, β blockers have been found to be beneficial when used in relatively low doses in stable heart failure patients (CIBIS, 1999).

An increasingly commonly recognised variant of heart failure is 'diastolic' or 'systolic function preserved' heart failure, characterised by resistance to ventricular filling rather than defective contraction (Dodek et al., 1972; Zile et al., 2004). As the commonest causes of diastolic heart failure are ischaemia, obesity, hypertension, diabetes and ageing (Owan and Redfield, 2005), the incidence of this condition is expected to increase with time. Fundamentally, the mechanisms underlying this variant of heart failure are unknown.

There have been major recent advances in the clinical assessment of heart failure patients – the aim being earlier diagnosis and risk stratification (i.e. the identification of high-risk patients). Clinical scoring based on a patient's assessment of their own exercise capacity and basic clinical observations has proved of use in identifying high-risk patients and guiding therapy (Goldman et al., 1981). More recently, imaging technology has been used to improve diagnosis and prognostication in heart failure. Echocardiography has been the historical 'gold standard' for non-invasive evaluation of the failing heart, and is safe and relatively cheap. The recent development of tissue Doppler measurement to evaluate myocardial strain has improved sensitivity with respect to early detection of abnormalities. Further adaptations, such as ultrasonographic tracking of acoustic markers ('speckle tracking') and intravascular ultrasound contrast (Flu et al., 2009), have shown promise at their relatively early stage of development. Cardiac magnetic resonance imaging (cMRI) is increasingly used in heart failure and, as well as being the most accurate method to determine left ventricular mass (an important clinical measurement in certain types of car-

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Case study

A 72-year-old man was admitted to hospital complaining of a progressive three-day history of shortness of breath at rest, exacerbated by lying flat. He had suffered a myocardial infarction four years earlier, which was treated by primary angioplasty. His past medical history was significant for hypertension, hypercholesterolaemia, and type II diabetes, which was diagnosed at the time of his infarct. Following the angioplasty, he was prescribed a β blocker, an ACE inhibitor, a statin, anti-platelet agents (all of which have been shown to reduce mortality following myocardial infarction) and oral anti-diabetic medication. He had remained well in the intervening four years. On examination, he was found short of breath at rest with crackles audible in both lung fields, consistent with left ventricular failure. Echocardiography was performed, revealing a significantly depressed left ventricular ejection fraction and normal valvular function. He was treated with intravenous diuretics to good symptomatic effect, and had the doses of his other cardiac medications increased. Over the next six months, he had two further admissions with left ventricular failure, both of which were treated with diuretics.

diomyopathy and heart failure), can also determine the cause of heart failure by quantifying microvascular perfusion, myocardial iron and fibrosis (Karamitsos et al., 2009). Diagnostic imaging of the myocardium using radio- or nanoparticle-labelled probes ('molecular imaging') is an area of active development (Saraste et al., 2009) and is potentially one of the quickest ways to translate advances in basic science into the clinical arena. Many of these tech-

niques are still experimental in that they have not yet been proven to predict clinical events.

The gene expression changes that lead to heart failure are not definitively known. Many changes in gene expression have been described in biopsies taken from human heart failure patients. However, biopsies are necessarily small and usually taken blindly through the endocardial route via a vascular catheter. The logistical, ethical and

legal difficulties in obtaining high-quality biopsies from relevant patient groups have been addressed by a working group of the American Heart Association and European Society of Cardiology (Cooper et al., 2007). In the absence of widely available biopsy material, clinical trials and medical practice have relied on the use of biomarkers that are essentially unvalidated. A comparison of the gene expression changes in animal models and human failing hearts is vital for validating the findings from animal models of heart failure, and eventually for assaying biomarkers used in humans.

Unanswered clinical questions in heart failure

It is largely unknown whether the individual changes in gene expression and physiology that are observed in heart failure patients are adaptive or maladaptive, and how this changes with the evolution of the disease. Further questions include whether there are novel biomarkers (Lainscak and Anker, 2009), and which imaging modalities are optimal that will facilitate clinical decision-making in heart failure patients.

Two obvious deficiencies are hindering the development of new heart failure therapies: (1) high-resolution, longitudinal phenotyping of heart failure patients (i.e. at several stages in the evolution of the condition) has not yet been carried out; and (2) the development of new animal models that more closely mimic the medical treatment of heart failure, and the common causes of heart failure (such as obesity and hypertension) that interact with treatment, would facilitate convergence between clinical and animal modelling fields. Given the huge recent advances in our understanding of murine biology, mouse models would arguably be of the most use to answer these questions.

Recent insights into cardiac biology

The last two decades have seen rapid advances in our understanding of the genetic and physiological processes involved in the development and maintenance of a healthy mammalian heart, and how perturbations may result in disease; for example, the discovery of autologous cardiac repair through pluripotent cells, which has been extensively reviewed elsewhere (Chien et al., 2008; Hansson et al., 2009; Nakano et al., 2008). Development of this technology towards the clinic requires reli-

Table 1. Commonest documented causes of heart failure

Cause of heart failure	
No single identifiable cause found in 50% of cases	
Ischaemia	
Pressure overload	Hypertension Aortic valve stenosis Aortic coarctation
Volume overload	R-L (right-to-left) shunting Mitral valve regurgitation
Infection	Viral cardiomyopathy/myocarditis HIV
Infiltration	Sarcoid Amyloid
Peripartum cardiomyopathy	
Connective tissue disease	
Toxins	Alcohol Cocaine Anthracyclines Other drugs (corticosteroids, lithium)
Diabetes	
Endocrine	Thyroid disease Corticosteroid excess Growth hormone excess Pheochromocytoma
Inherited cardiomyopathy	

Table adapted from Felker et al., 2000.

able animal models, which arguably do not exist yet.

Strikingly, the failing adult heart resembles the foetal heart in many ways. Several gene expression changes that have been reported in failing hearts are consistent with 're-expression of the foetal gene expression program.' The expression of numerous transcription factors that are associated with heart development (Rajabi et al., 2007), gene activation programmes via histone deacetylases (HDACs) (Molkentin et al., 1998), and foetal-type microRNA (miRNA) expression profiles (Rooij et al., 2006; Thum et al., 2007) have been reported in failing hearts. Furthermore, 'foetal' contractile protein isoforms and ion channels have been documented in human failing hearts. An important question is whether this gene expression shift contributes to the heart failure phenotype, or whether it is a protective response. Recently, it has been postulated that foetal isoforms of the contractile protein myosin are beneficial in the failing heart as they reduce oxygen consumption, albeit at the expense of reduced contractile function (Krenz and Robbins, 2004; Lowes et al., 1997; Rajabi et al., 2007; Tardiff, 2006). It is perhaps a paradox that reducing cardiac contraction in a condition defined by an insufficiency in cardiac output should be beneficial. However, there is supporting evidence from human adult therapeutics for this hypothesis. When administered appropriately to heart failure patients, β blockers afford a clear survival advantage and are negatively inotropic, but reduce cardiac ATP consumption (CIBIS, 1999; Marian, 2006). It is now becoming clear that understanding the alterations in energy generation that are seen in heart failure may be pivotal in advancing our knowledge of heart failure. The failing heart shifts energy generation from predominantly lipid oxidation, seen in the healthy adult heart, to glycolysis, which predominates in the foetal heart. This is another aspect in which failing hearts seem to resemble foetal hearts, and a key area of research has been the possible mechanisms controlling this switch.

There are a number of biochemical 'stress pathways' that are activated in the failing heart in response to a number of stimuli, and that may lead to changes such as metabolic substrate switching (Pu and Izumo, 2001). It is now thought that activation of these signalling axes, although initially

adaptive with respect to survival, eventually results in cardiac dysfunction when activated chronically (Olson and Schneider, 2003). In addition, pharmacological inhibitors of HDACs, part of the 'stress pathways', are being investigated as potentially novel treatments for heart failure (Kee et al., 2006). It has been pointed out that stress pathways are also activated in the foetal heart, adapting cardiac function to a low-oxygen environment (Rajabi et al., 2007).

Transgenic mouse experiments are now investigating whether these changes in gene expression drive the heart failure phenotype (and, thus, whether they are therapeutic targets), or whether they are protective in the context of heart failure. Arguably, more effective models of heart failure would be of great use in this area.

Animal models of heart failure

The aim of animal modelling of heart failure is to simplify an extremely complex syndrome into manageable research questions. A key decision is the choice of animal system – often this is a trade off between convenience/cost and physiological applicability. Animal models of heart failure, as opposed to isolated organs or cells, do enable analysis of the physiological effects of cardiac dysfunction, which are of great importance in the overall heart failure phenotype.

The relatively complete annotation and simple manipulation of the mouse genome have allowed significant mechanistic insights into human disease. Mouse models are indispensable tools in many areas of medical research and the mouse is, most often, the animal system of choice when disease modelling.

Although mice are relatively inexpensive and convenient, there are significant differences between mouse and human hearts. Mouse hearts are obviously very small, and beat very quickly [400-600 beats per minute (bpm)] compared with humans (60-90 bpm), leading to important differences in calcium handling and ion currents between mice and human hearts. For example, heterozygous deletion of the calcium channel SERCA2a in mice leads to a contractile dysfunction, whereas in humans there is no apparent phenotype (Mayosi et al., 2006). There are also important differences in the predominant myosin isoforms expressed in adult human and

Clinical terms

ACE inhibitors – drugs that block angiotensin-converting enzyme (ACE) to inhibit the conversion of angiotensin I to angiotensin II. Less angiotensin II reduces blood pressure, increases parasympathetic activity in the heart to control cardiac arrhythmia, and inhibits cardiac muscle wasting (cachexia) in heart failure patients

β blocker/ β -adrenoceptor antagonists – propranolol-based compounds that inhibit sympathetic neuron (epinephrine)-induced cardiac stimulation, which are used to control cardiac arrhythmias and elevated blood pressure

Cardiac contractility – the intrinsic ability of a heart muscle fibre to contract at a certain length, it is a measure of muscle performance

Cardiac output – the blood volume pumped out from the heart

Cardiac MRI – cardiac imaging modality providing direct visualisation of the heart chambers and myocardium without the risk of potentially harmful ionising radiation

Coronary CT scan – cardiac imaging modality that is used primarily to evaluate coronary atherosclerotic disease

Echocardiography – sonogram or ultrasound of the heart, it allows real-time visualisation of blood dynamics and wall motion in the heart

Heart failure – inability of the heart to pump a sufficient blood supply to meet tissue consumption needs

Primary angioplasty – dilation of a narrowed or stenosed coronary artery, with the goal of increasing blood flow through the artery

mouse hearts (Swynghedauw, 1986). Having said this, the common features of murine and human hearts have allowed for many important observations regarding embryology, physiology, cell signalling, energetics and stem cell function.

Surgical models of heart failure

The most commonly used group of mouse models of heart failure uses the response to a surgical intervention, such as banding an aorta or clipping a coronary artery, to model the multisystem effects of heart failure (Table 2) (Mayosi et al., 2006). These surgical models of heart failure have the advantage of very closely replicating specific disease situations of myocardial infarction (coronary artery ligation) and heart failure owing to hypertension or aortic valve stenosis (aortic banding). However, heart failure occurs suddenly post-surgery in the context of a relatively young heart, whereas in humans, the onset may be insidious over several years in the context of comorbid-

ties and age-related changes. All surgical models are relatively expensive and technically demanding with high rates of intraoperative mortality, which reduces reproducibility. Interventions such as long-term pacing of the heart at high rates are also used commonly in larger animals, such as dogs and rabbits, but this is not (yet) technically feasible in mice.

Transverse aortic banding seeks to model heart failure resulting from aortic stenosis. A ligature or clip is placed across the ascending or descending aorta, inducing abnormally increased intracardiac pressure ('pressure overload'). Typically, intraoperative mortality is high and operator-dependent, with heart failure developing within weeks of the procedure in the surviving mice. This procedure is assumed to model heart failure resulting from aortic stenosis in humans, although the time scale of the development of heart failure differs significantly. How much relevance this model has to other mechanisms of heart failure is unknown.

Surgical clipping of a coronary artery, typically the left anterior descending artery, models heart failure following myocardial infarction. In the postoperative days and weeks, a myocardial scar and dilated cardiomyopathy develop. This clearly models the effect of a completed human myocardial infarction, but has the experimental drawbacks of relatively high perioperative mortality, relatively poor reproducibility, and the technical challenge of such a delicate procedure.

Surgical models of heart failure/hypertrophy have afforded many insights into human disease. For example, there is direct

evidence from animal experimentation that the nature of the stimulus for cardiac hypertrophy (which often precedes heart failure and is associated with activation of the same 'stress pathways') determines the type of hypertrophic response, and potentially the clinical outcome (for example exercise training versus intermittent transverse aortic banding) (Perrino et al., 2006; Tardiff, 2006).

Surgical models of heart failure model specific cardiac conditions that represent a portion of the heart failure cases that are encountered clinically. The major disease burden of heart failure in the future is expected to come from patients with the complex phenotype cluster of hypertension/hyperlipidaemia/obesity/diabetes. It is not obvious how closely the heart failure resulting from, for example, hypertension and diabetes resembles the current animal models. There are increasing numbers of spontaneously occurring or engineered mouse strains that exhibit hypertension, diabetes or obesity (Russell and Proctor, 2006). Combination of these models may provide 'polygenic' models of heart failure, perhaps in conjunction with existing surgical techniques.

Toxin-induced heart failure

Administration of a single toxin or drug is a theoretically attractive method of modelling heart failure. For example, ethanol (Berk et al., 1975) and the cytotoxic drug doxorubicin (Delgado et al., 2004) are known cardiotoxins in humans and have been used in mice to induce heart failure syndromes. Elevated levels of the non-protein amino acid homocysteine, suggested to

be a risk factor for the development of heart failure in humans (Herrmann et al., 2006), have been induced in mice by heterozygous deletion of cystathionine- β -synthase (Vacek et al., 2009), again modelling a specific cause of heart failure. Isoproterenol infusion in rodents models the cardiotoxic effects of chronic activation of the sympathetic system (Balazs and Herman, 1976; Oudit et al., 2003). Technically, these models are simple and reproducible, but the question of general applicability of these experiments to all forms of heart failure arises.

Genetic models of heart failure

One approach has been to individually investigate the changes in gene expression associated with heart failure by targeted mutagenesis/transgenesis or pharmacology. This has proved very fruitful, but any such reductive approach to heart failure is liable to charges of oversimplification. Over 5000 cardiac transgenic/knockout studies have now been published (http://circres.ahajournals.org/cgi/collection/animal_models_of_human_disease). Although not in themselves disease models, they have proven indispensable for investigating individual signalling pathways, or to investigate the role of individual genes. This approach has been criticised on several grounds – most importantly, that the study of individual gene manipulations has little relevance out of context (Cook et al., 2009).

In their simplest forms, genetic alterations can be induced by overexpression under the control of heart-specific promoters, or deletion by targeted deletion of

Table 2. Advantages and limitations of some commonly used animal models of heart failure

Model	Advantages	Limitations
Surgical		
Aortic banding	Model of human pressure overload	High mortality; technically demanding; relevance to other conditions?
Coronary ligation	Directly applicable to human disease	
Cryoinjury	Technically simple	Relevance to human disease?
Toxic		
Ethanol	Non-invasive; technically simple; reproducible	High mortality; non-cardiac effects; not generally applicable?
Doxorubicin		
Homocysteine		
Genetic		
Transgenic	Directly reproduces gene expression changes seen in disease	Non-specific effects of overexpression
Targeted		Developmental effects of null allele
Inducible null/transgenic	Control of time course of induction/deletion	Control of level of transgene often impossible

a locus or crucial part of it (Yutzey and Robbins, 2007). Temporospatial control of genetic manipulations is now possible using tet-inducible transgenic systems (Gulick and Robbins, 2005; Sanbe et al., 2003), and inducible deletion using hormone-fused Cre recombinase constructs (Sohal et al., 2001).

Relating the expression of a transgene to endogenous levels (and human expression data) is absolutely crucial – this applies as much to deletion experiments as transgenic overexpression ones. Very high levels of even ‘inert’ proteins such as Cre recombinase or green fluorescent protein (GFP) can lead to dose-related phenotypes (Buerger et al., 2006). Therefore, misexpression or deletion of a single gene has a high chance of leading to artefactual phenotypes. Constitutive (i.e. non-inducible) misexpression runs the risk of generating artefacts owing to developmental effect. Additionally, mosaicism of expression of the transgene, or of deletion, is invariably present and rarely assessed.

The targeting strategy is also crucial; Yutzey and Robbins use the example of myogenic regulatory factor 4 (MRF4) to show that three different ‘null’ mutants have three different phenotypes resulting from differences in target loci and targeting vectors (Baker et al., 2000; Stull et al., 2002; Yutzey and Robbins, 2007).

It is difficult to see how signalling pathways could be dissected, and the effects of specific disease alleles tested, without the use of genetically modified mice. These technologies have allowed the investigation of many cardiac signalling pathways, and very many individual gene products, with respect to their potential role in heart failure and their potential as therapeutic targets.

Models of heart failure in rabbits, dogs, sheep and larger animals have had varying degrees of success (Halapas et al., 2008). These all have their own advantages and disadvantages, and several are arguably more physiologically applicable to humans, but they are uniformly more expensive than mice and with fewer molecular biology resources. It has been estimated that, for example, the housing costs of experimental rabbits are 20 times those of mice, and that heart failure phenotypes may take years to develop in a rabbit, as opposed to weeks/months in a mouse (Marian, 2005; Marian, 2006).

Conclusion Opportunities for making mouse models of heart failure more clinically applicable

The end points used in mouse studies should, where possible, be clinically valid (for example, by using the same imaging modalities when studying cardiac morphology in mice and heart failure patients) (Allen et al., 2009). It is worth pointing out that many of the clinical end points that are important to doctors, patients and health-care systems alike, such as quality of life, exercise tolerance and hospital admission, are unlikely to be modelled adequately in any animal system. The use of surrogate markers (measured end points in clinical trials to substitute for clinical events such as death) in heart failure clinical trials is complex and controversial (Gheorghade et al., 2003). As the field advances, it will be important to establish which of the indices measured in mice most truly model human disease. In some areas, such as mouse cMRI, there are still technological limits, but echocardiography in mice has now reached the point where human echocardiographers can easily interpret mouse data. Currently, the limiting factor is the size of the mouse heart – it is not clear whether this will remain the case. Molecular imaging of mouse hearts is developing in parallel with human technology.

Validation of the effect of drug treatment in mouse models against human heart failure is vital. For example, experimental interventions in surgical models should be made on a background of standard medical therapy (β blockers and ACE inhibitors) in the same way as in patients in clinical trials.

The effect of comorbidities on the development and treatment of heart failure is of importance, because human heart failure most often occurs as a cluster of related medical conditions whose contribution to the development of the phenotype and response to treatment is unknown. In future, mammalian systems other than mouse may be needed to model complex metabolic interactions such as obesity and dyslipidaemias.

The number of heart failure patients is increasing owing to the lack of necessary therapeutic tools to treat them. A dialogue between clinicians and scientists is necessary to develop animal models of heart failure that accurately replicate a complicated clinical syndrome, and to allow exploration

Clinical and basic research opportunities

- Investigate whether heart failure from different causes benefits from different treatments
- Research to understand the complex elements that contribute to heart failure. Central to this, is how changes in gene expression and energy dynamics induced by the disease influence its progression
- Define reliable biomarkers to diagnose and phenotype heart failure
- Establishment of relevant and informative indices that correlate well between animal models and human patients with heart failure

of novel disease targets. Relatively simple modifications in the experimental design of animal models of heart failure have the potential to allow increased understanding of the complexity of the clinical heart failure syndrome. This may lead to clinical advances using currently available technologies. Future animal models of heart failure will hopefully give mechanistic insights that lead to novel classes of therapies.

COMPETING INTERESTS

The authors declare no competing financial interests.

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