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INVITED REVIEW

Small animal models of heart failure

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Abstract Heart disease is a major cause of death worldwide with increasing prevalence, which urges the development of new therapeutic strategies. Over the last few decades, numerous small animal models have been generated to mimic various pathomechanisms contributing to heart failure (HF). Despite some limitations, these animal models have greatly advanced our understanding of the pathogenesis of the different aetiologies of HF and paved the way to understanding the underlying mechanisms and development of successful treatments. These models utilize surgical techniques, genetic modifications, and pharmacological approaches. The present review discusses the strengths and limitations of commonly used small animal HF models, which continue to provide crucial insight and facilitate the development of new treatment strategies for patients with HF.

Keywords Animal models • Rodents • Heart failure • HFpEF • HFrEF

1. Introduction

Heart failure (HF) is the leading cause of death worldwide. The mortality rate of HF is high, with about 50% of patients dying within 5 years after the initial diagnosis, which exceeds most types of cancer (www.who.int). Furthermore, the prevalence of HF in industrialized nations is increasing, which results in an enormous economic burden. The increase is attributable, at least in part, to the improved treatment following acute myocardial infarction (MI), which has decreased the mortality rate, but not morbidity, and is based on the number of surviving patients. Additional factors comprise an increased prevalence of comorbidities, which predispose and accelerate the development of HF. Therefore, there is an urgent need to modify these risk factors and to develop new therapeutic strategies for HF patients.

Based on left ventricular (LV) ejection fraction (LVEF), HF can be categorized as *h*eart *f*ailure with *p*reserved ejection *f*raction (HFpEF; LVEF ≥50%), *h*eart *f*ailure with *m*id-range ejection *f*raction (HFmrEF; LVEF 40–49%), or *h*eart *f*ailure with *r*educed ejection *f*raction (HFrEF; LVEF < 40%).[1] About 50% of HF patients are afflicted with HFpEF and exhibit HF symptoms, which include exercise intolerance, congestion, and oedema that are associated with cardiac hypertrophy, increased fibrosis, and decreased capillary content. Common risk factors for the development of HFpEF include arterial hypertension, obesity, diabetes mellitus, atrial fibrillation, and renal dysfunction (*Figure 1*). This implies that impaired cardiac compliance and contractile dysfunction found in HFpEF can be triggered by associated comorbidities. Importantly, the postulation that diastolic dysfunction is equivalent to HFpEF is an oversimplification and only partially correct. This emanates from the observation that diastolic dysfunction has also been detected in normal subjects without clinical HFpEF symptoms.[2] In contrast, HFrEF is typically associated with loss of cardiomyocytes, which can be a consequence of myocardial damage of different aetiologies (*Figure 1*) and may increase wall stress, as reflected by higher levels of natriuretic peptides compared to HFpEF.[2]

Small animal models, including mice, rats, and guinea pigs,[3] continue to improve our understanding of the various aspects and aetiologies of HF and help to develop novel treatment strategies. Mice and rats are the most commonly used animal models and share a high degree of homology to the human genome, with ∼30 000 protein-coding genes each. Major advantages of rodent models include relatively short breeding cycles and low housing costs. Numerous small animal models have been generated as tools to decipher HF aetiologies and develop new HF treatment strategies. These models typically utilize genetic modifications, pharmacological and surgical approaches, which can also be combined. The pathogenesis of HFpEF and HFrEF is multifactorial. Thus, it is often impossible to discern the underlying mechanisms, which can be overlapping and interconnected. This provides a challenge to investigate co-existing risk factors for HF development in a single model organism,[4,5] especially in models of diabetic cardiomyopathy and HFpEF,[6] necessitating the thoughtful selection of the best animal model for a given hypothesis. However, small animal HF models enable the study of specific risk factors without the confounding effect of comorbidities. Over the last few decades, numerous small animal models have greatly advanced our

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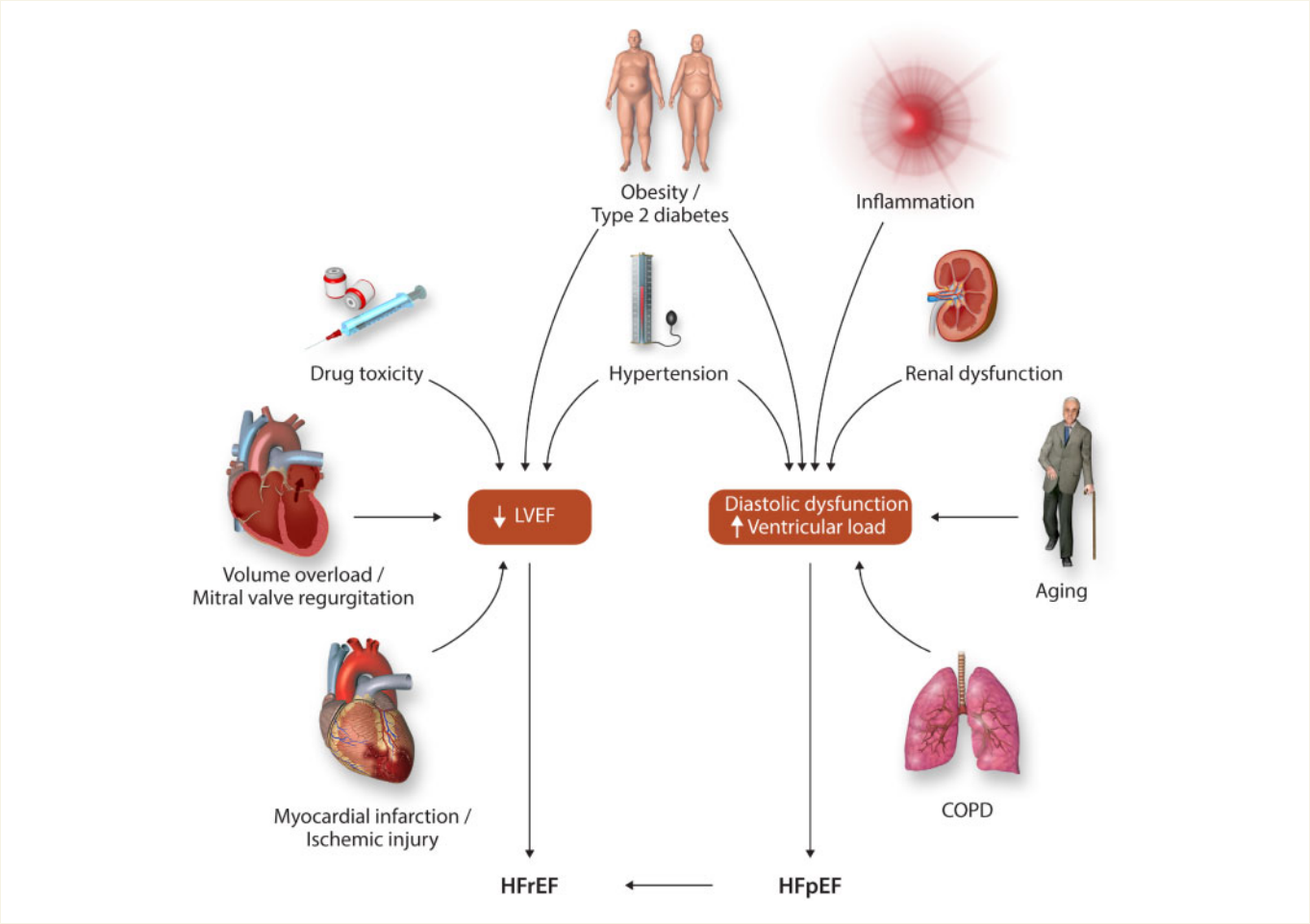

Figure 1 Schematic depicting selected risk factors for the development of heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). Note that some risk factors increase the risk of both HFrEF and HFpEF, and HFpEF may precede the later onset of HFrEF. COPD, chronic obstructive pulmonary disease; LVEF, left ventricular ejection fraction.

understanding of the pathogenesis of HFrEF and HFpEF, many of which will be highlighted in this article and are summarized in *Table 1*.

2. Small animal models of HFrEF

The following sections discuss rodent models, which typically provoke HFrEF (*Figure 2*). It is important to note that some of these models induce HFpEF, which precedes the later onset of systolic dysfunction and HFrEF.

2.1 Surgical models

2.1.1 LV pressure overload

Chronic LV pressure overload causes HF in mice[7–16] and rats,[7,17,18] which is accomplished by various surgical approaches to mimic the adaptations associated with hypertension and aortic valve stenosis in patients. Transverse aortic constriction (TAC) in mice was first described by Rockman *et al.*[15] and has been subsequently used as a method for LV pressure overload by numerous laboratories. TAC increases LV afterload, which results in concentric cardiac hypertrophy and, ultimately, HFrEF. Several surgical techniques for TAC have been developed, including minimally invasive approaches by a small incision in the proximal sternum[7,9,10,14] and placement of surgical clips or sutures to impede blood flow across the aortic arch. Recently, a novel method using o-rings with fixed inner diameters has been described, which are placed around the ascending aorta in mice.[83] Measurement of the peak flow velocity difference of the right relative to the left carotid artery enables the quantification of the pressure gradient post-surgery.[7] Important parameters for the hypertrophic response and progression of HF include sex, weight, age, and the genetic background of the species used. Mice with the C57BL/6J genetic background develop HF more rapidly post-TAC compared to the 129S1/SvImJ strain[11] and have similar gene expression patterns of human dilated cardiomyopathy compared to 129S1/SvImJ mice.[85] The identified pathways contributing to accelerated HF in C57BL/6J mice include periostin, angiotensin, and IGF1 signalling. Different adaptations for the response to pressure overload have also been reported for the different C57BL/6 substrains, i.e. C57BL/6NCrl (maintained by the Charles River Laboratories), C57BL/6NTac (maintained by the Taconic Laboratories), and C57BL/6J (maintained by the Jackson Laboratory).[84,86] C57BL/6J mice have a mutation in the *nicotinamide nucleotide transhydrogenase (Nnt)* gene, which regenerates NADPH from NADH. This mutation protects C57BL/6J mice from oxidative stress and HF post-TAC compared to the inbred C57BL/6N strain.[84]

Table 1 Characteristics of selected small animal models of heart failure

Model	HF stimulus	Advantage	Limitation	Species	Selected references
Surgical					
LV pressure overload	TAC	Reliable model to induce cardiac hypertrophy and HF.	The acute increase in afterload does not reflect the gradual progression of arterial hypertension and aortic valve stenosis in patients.	Mouse Rat	7–16 7,17,18
	Ascending aortic constriction	Gradual onset of pressure overload, which mimics the gradual progression of arterial hypertension in patients.	Limited relevance to human disease as pressure overload is induced in young animals, whereas arterial hypertension is primarily observed in elderly patients.	Rat	19,20
Temporary LV pressure overload	TAC + removal of the stenosis	Reliable model of cardiac hypertrophy followed by removal of stressor to study reverse cardiac remodelling.	Two surgeries required. Technically challenging technique to remove suture or clip.	Mouse	21–25
MI	LAD ligation	Reliable model to induce tissue damage and HF.	Model does not reflect the clinical setting with reperfusion of the occluded vessel during coronary angiography performed after an acute MI.	Mouse Rat	26–33 34–40
Ischaemia/reperfusion injury	Temporary LAD ligation	Close to clinical scenario with reperfusion of the occluded vessel during coronary angiography performed after an acute MI.	Surgery is more time consuming and more complex than placement of permanent LAD ligation.	Mouse Rat	41–44 44,45
MI (neonatal)	LAD ligation	Identification and characterization of pathways involved in cardiac regeneration.	Limited relevance to human disease. MI and coronary artery disease primarily occur in elderly patients.	Mouse	46
Pressure overload + MI	TAC + LAD ligation	Model mimics the relevant co-morbidities of arterial hypertension and ischaemic heart disease. Gradual and predictable progression of HF.	The acute increase in afterload does not reflect the gradual progression of arterial hypertension in patients.	Mouse	47
	Ascending aortic constriction + LAD ligation	Same as for mouse model of TAC + LAD ligation.	Same as for mouse model of TAC + LAD ligation.	Rat	48
	Abdominal aortic constriction + LAD ligation	Same as for mouse model of TAC + LAD ligation.	Same as for mouse model of TAC + LAD ligation.	Rat	49
Pulmonary hypertension	Pulmonary artery constriction	Model mimics characteristics of RV HF, including increased liver weight and peripheral oedema.	The acute increase in afterload does not reflect the gradual progression of pulmonary hypertension in patients.	Mouse Rat	50–52 53
Volume overload	Aorto-caval fistula (shunt)	Model of chronic volume overload as observed in patients with mitral valve regurgitation.	The acute increase in volume overload does not reflect the gradual progression of mitral valve regurgitation in patients.	Mouse	12
		Reproducible model of volume overload-induced HF.	Shunt creates an artificial mix of arterial with venous blood.	Rat	54,55

Continued

Table 1 *Continued*

Model	HF stimulus	Advantage	Limitation	Species	Selected references
Drug induced					
Toxic cardiomyopathy	Doxorubicin	Potent stimulus to induce dilated cardiomyopathy.	Systemic toxic effects, especially on bone marrow cells, and gastrointestinal system.	Mouse Rat	56–59 60
	Isoproterenol	Potent stimulus to induce cardiac hypertrophy. Drug is easy to administer (i.p. injection or osmotic mini pump).	Chronic activation of adrenergic signalling is only one contributing factor to the development of HF in patients.	Mouse Rat	8,61 62
	Monocrotaline	Model of predominantly RV hypertrophy and RV failure.	Toxicity on other organ systems, i.e. pulmonary and kidney injury.	Rat	63,64
	Homocysteine	Potential clinical relevance; hyperhomocysteinaemia is a risk factor for HF.	Hyperhomocysteinaemia represents only one aspect in the development of HF in humans, which is conversely discussed. Non-specific side effects and toxicity on other organ systems, especially vasculature.	Rat	65–67
	Ethanol		Non-specific side effects and toxicity on other organ systems, especially liver.	Rat	68
Hypertension					
Angiotensin II infusion	Chronic stimulation of angiotensin II Type 1 receptor (AT1R) signalling	Reliable model of cardiac hypertrophy. Technically easy surgery to implant osmotic minipumps.	Unspecific side effects on organ systems, especially kidney.	Mouse Rat	69 70
Dahl salt-sensitive rat	Inbred strain of Sprague-Dawley rats, which are susceptible to hypertension following a high-salt diet	Induction of hypertension and HF by high-salt diet feeding without additional surgery. Slow progression of hypertension and HF development as observed in patients.	High housing costs based on the slow progression of hypertension and HF.	Rat	71,72
Spontaneously hypertensive rat (SHR)	Inbred strain of Wistar-Kyoto rats with hypertension	Slow progression of hypertension and HF development as observed in patients.	High housing costs based on the slow progression of hypertension and HF.	Rat	73
T1D					
Akita (*Ins2*$^{Akita+/-}$)	Pancreatic β-cell failure based on mutation in the *Insulin2* gene	Robust model for T1D.	High housing costs based on the time-dependent progression of the phenotype.	Mouse	74–76
High-dose STZ	Pancreatic β-cell toxin	Robust model for T1D.	Does not capture the autoimmune contribution to the development of T1D in patients.	Mouse/rat	6

Continued

Table 1 *Continued*

Model	HF stimulus	Advantage	Limitation	Species	Selected references
Metabolic syndrome/ T2D					
ob/ob	Hyperphagia based on leptin deficiency	Robust phenotype of obesity and T2D.	High housing costs based on the time-dependent progression of the phenotype. Potentially confounding effects of altered leptin-mediated signalling.	Mouse	77,78
db/db	Hyperphagia based on leptin resistance	Robust phenotype of obesity and T2D.	High housing costs based on the time-dependent progression of the phenotype. Potentially confounding effects of altered leptin-mediated signalling.	Mouse	79,80
ZF/ZDF rats	Hyperphagia based on leptin resistance	Model of metabolic syndrome with increased levels of circulating lipids and cholesterol.	High housing costs based on the time-dependent progression of the phenotype. Potentially confounding effects of altered leptin-mediated signalling.	Rat	81,82
High-caloric diet (± low-dose STZ)	High caloric intake (± pancreatic β-cell toxin)	Additional low-dose STZ treatment mimics β-cell failure and late stage T2D.	High housing costs based on the time-dependent progression of the phenotype. Additional low-dose STZ treatment mimics mixture of T1D and T2D.	Rat/mouse	6

Note that housing costs for mice are typically lower than for rats. Another advantage of mouse models is the availability of numerous transgenic strains available. General advantages of rat models are that surgical techniques are easier to perform than in mice.

HF, heart failure; i.p. intraperitoneal; LAD, left anterior descending artery; LV, left ventricular; MI, myocardial infarction; RV, right ventricular; STZ, streptozotocin; T1D, Type 1 diabetes; T2D, Type 2 diabetes; TAC, transverse aortic constriction; ZDF, Zucker diabetic fatty; ZF, Zucker fatty.

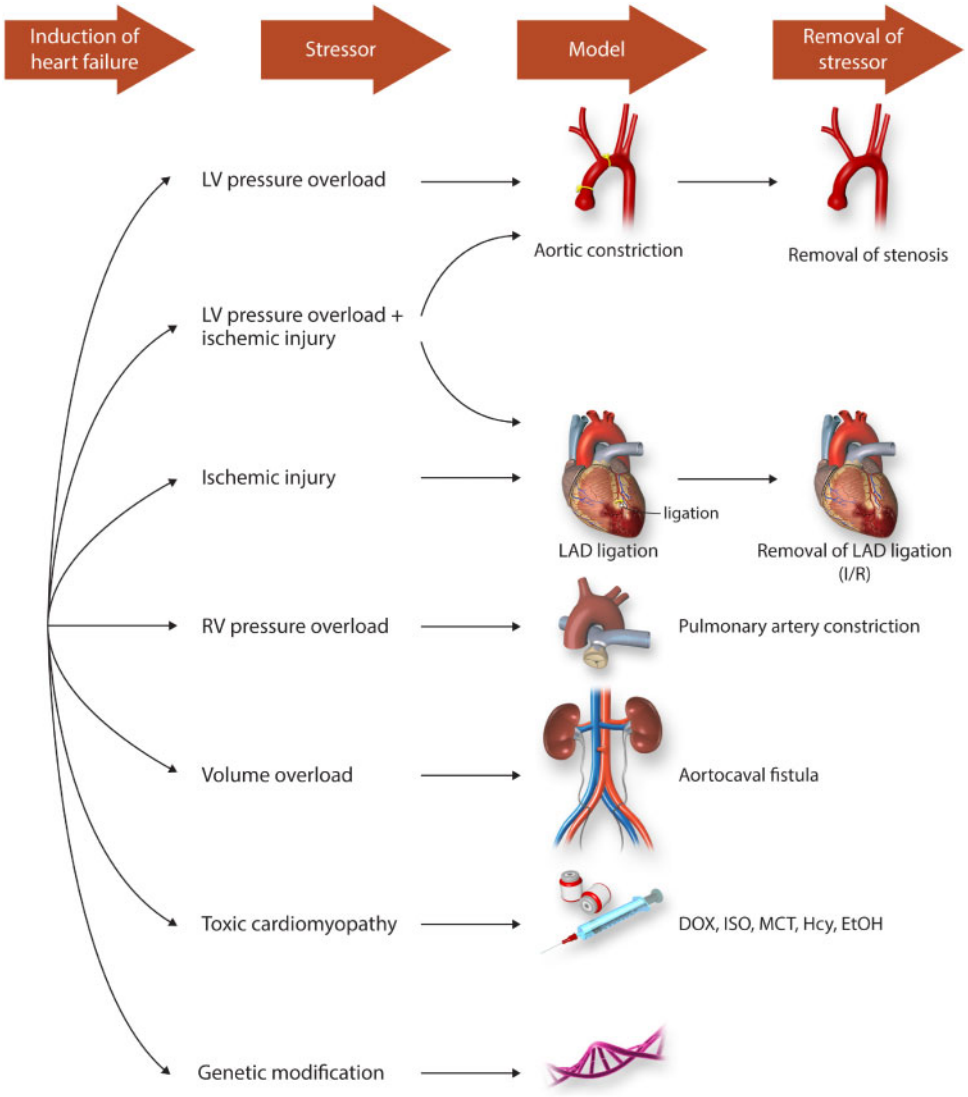

Figure 2 Schematic depicting selected stressors to induce heart failure with reduced ejection fraction (HFrEF) in small animal models. Note that these models may also induce heart failure with preserved ejection fraction (HFpEF), which precedes the later onset of HFrEF. Additional animal models with temporary exposure to drugs and temporary genetic gain-of-function or loss-of-function modifications have been developed. DOX, doxorubicin; EtOH, ethanol; Hcy, homocysteine; I/R, ischaemia/reperfusion injury; ISO, isoproterenol; LAD, left anterior descending artery; LV, left ventriclular; MCT, monocrotaline; RV, right ventriclular.

One important limitation of TAC is the immediate onset of pressure overload, which is in contrast to the slow progression of hypertension and aortic valve stenosis in patients. To overcome this potential drawback, constriction of the ascending aorta has been performed in 3- to 4-week-old rats. In this model, LV hypertrophy is observed by 6 weeks and overt HF by 18 weeks post-surgery.[19,20] Aortic constriction in rats has also been performed around the abdominal aorta both in the infrarenal and suprarenal position, the latter of which induces renal hypoperfusion, hypertension, and LV hypertrophy. Abdominal aortic constriction typically contributes to a slower progression of the HF phenotype.[87] Recently, additional models have been developed that facilitate the study of reverse cardiac remodelling. The models described use different surgical approaches to remove the TAC stenosis and subsequently decrease cardiac workload.[21–25]

2.1.2 Ischaemic injury

Coronary artery ligation is a commonly used, small animal HF model[88] that was initially established by Pfeffer *et al.*[34] in rats and has been subsequently used by numerous groups.[35–38] The Pfeffer group performed groundbreaking studies and demonstrated that infarct size, post-MI LV chamber dilatation and LV function are correlated. They subsequently showed that treatment with the angiotensin-converting enzyme (ACE) inhibitor captopril improves contractile function and survival following MI in rats.[39,40] The impact of ACE inhibition was subsequently tested in large clinical trials in patients post-MI, which improved contractile function and survival.[89] These studies established pharmacological ACE inhibition for patients with MI, which is now a commonly used, standard treatment. Coronary artery ligation has also been performed in mouse models.[26–33] Ligation of the left anterior descending (LAD) artery

typically results in HF 4 weeks post-surgery and strongly depends on the genetic background of the mice used.[32] One potential limitation of these animal models with permanent coronary artery occlusion is the differences in their observed phenotypes relative to those observed in patients; atherosclerosis of the coronary arteries in patients results in ischaemic heart disease that slowly progresses and coronary artery blood flow can eventually be re-established during coronary angiography performed after an acute MI. To overcome this limitation, ischaemia/reperfusion (I/R) models have been established, which facilitate the investigation of molecular mechanisms and tissue damage following temporary LAD occlusion.[41–45,90] The I/R model typically exhibits less tissue damage compared to permanent LAD occlusion. Importantly, ischaemic injury is typically induced in young rodent models. This is in contrast to the patient population, in which elderly and multimorbid patients exhibit the greatest risk of coronary artery disease and acute MI.

MI has also been induced in neonatal mice to identify and characterize pathways that are involved in cardiac regeneration. Ischaemic injury in neonatal mice is provoked by LAD ligation and complete recovery is observed by 3 weeks of age. The regenerative potential decreases as the mice age and the abundance of proliferating cardiomyocytes diminishes.[46] Similar to TAC, the adaptations post-MI have been compared across the most commonly used mouse strains and are dependent on genetic background. While infarct rupture was most frequently observed in 129S6 mice, cardiac dilatation was most prominent in Swiss mice.[32] Therefore, the genetic background should be an important consideration in study designs.

2.1.3 Combined LV pressure overload/ischaemic injury

To explore the coexistence of clinically relevant morbidities of arterial hypertension and coronary artery disease present in patients, recent surgical HF models combine the techniques of TAC surgery and LAD ligation. This combined surgical approach was first described in rats[48] and has since been modified for mouse models.[47] Various models have been published with different locations for the placement of the aortic stenosis, all of which exhibit adverse LV remodelling and rapid HF progression.[49] Recently, a mouse model with combined MI and temporary TAC was developed,[91] which has enabled the elucidation of the impact of mechanical unloading following ischaemic injury.

2.1.4 Right ventricular pressure overload

Similarly to TAC surgery, which increases LV afterload, pulmonary artery banding increases right ventricular (RV) afterload, and mimics pulmonary hypertension in mice[50–52] and rats.[53] Pulmonary artery banding results in RV hypertrophy and pathological remodelling.[50] As reported for TAC, the severity of the pulmonary artery stenosis correlates with the progression of contractile dysfunction and mortality.[51] Notably, the acute increase in RV afterload does not reflect the gradual progression of pulmonary hypertension in patients.

2.1.5 Volume overload

Chronic volume overload in small animal models reproduces the pathologies observed in patients with mitral valve regurgitation, which typically increase diastolic wall stress and cause eccentric cardiac hypertrophy.[12] Cardiac volume overload is accomplished in rodents by creating a surgical aorto-caval shunt and has been reported for rats[54,55] and mice.[12] Volume overload in rats initially decreases LV function. The subsequent compensatory hypertrophy normalizes contractile function at one month post-surgery,[54] with the time course of HF development strongly depending on the shunt volume and being less predictable compared to TAC models. The shunt creates an artificial mix of arterial with venous blood, which is in contrast to the clinical setting in patients with mitral valve regurgitation. Volume overload in mice causes minimal apoptosis in the absence of pathological remodelling, which is in contrast to the increased afterload following TAC surgery. This indicates that increased preload, i.e. aorto-caval shunt, and increased afterload, i.e. TAC, contribute to different morphological phenotypes, which is important for the design of future HF therapies.

2.2 Toxic cardiomyopathy

The anthracycline compound Doxorubicin (DOX) is a standard anticancer therapeutic agent. DOX causes dilated cardiomyopathy in a dose-dependent manner[92] that is typically irreversible and progressive. DOX has been administered to numerous small animal models[56–60] and promotes the formation of free radicals and mitochondrial dysfunction.[93,94] Juvenile DOX exposure in mice results in no immediate contractile dysfunction, however, impairs the ability to adapt to angiotensin II-induced hypertension later in life, which is restored by co-treatment with resveratrol.[95] Notably, cancer cachexia increases the risk of HF and decreases systemic insulin levels. Chronic insulin supplementation decreases glucose usage by the tumour, normalizes cancer-mediated impairment in cardiac Akt signalling and attenuates contractile dysfunction.[96] Conversely, HF following MI increases tumour growth as reported for APC^{Min} mice that have a mutation in the tumour suppressor gene *Adenomatosis polyposis coli (Apc)* and are prone to multiple intestinal neoplasia (Min) and cancer development.[97]

Chronic stimulation of G-protein-coupled ß-adrenergic receptor signalling with isoproterenol provokes cardiomyocyte hypertrophy and fibrosis in mice[8,61] and rats,[62] which is similar to the progressive HF development in mice with cardiac-specific overexpression of β_1-adrenergic receptors.[98] The mechanisms responsible include an imbalance between the increased energy demand, which is based on the hypercontractility of the myocardium relative to the oxygen and nutrients provided.

Monocrotaline (MCT) is a pyrrolizidine alkaloid obtained from the plant species *Crotalaria spectabilis*, which induces pulmonary hypertension and RV hypertrophy. MCT is converted in the liver to MCT pyrrole and circulates to the lung parenchyma to increase capillary permeability and to trigger interstitial oedema and smooth muscle hypertrophy.[99] These alterations increase pulmonary vascular resistance, RV pressure overload, and RV failure. MCT has been used in rats[63,64] and larger animal models. Importantly, non-specific side effects for MCT have been reported, such as lung and kidney injury,[64,99] which are important to consider when designing future studies.

As previously reviewed, high circulating homocysteine levels are a risk factor for the future onset of HF.[100] Similarly, dietary supplementation with homocysteine increases inflammation, collagen remodelling, and oxidative stress,[65,66,100] and provokes contractile dysfunction in both normotensive and spontaneously hypertensive rats.[65–67] Chronic ethanol ingestion contributes to dilated cardiomyopathy in both rodent models and humans.[101] The underlying mechanisms comprise decreased myocardial contractility as a consequence of altered myofibrillar Mg^{2+}-ATPase activity and cardiomyocyte loss.[68]

2.3 Genetically engineered models

Numerous transgenic animal models of HF have been generated to investigate the impact of genetic modifications, typically gain-of-function or

loss-of-function modifications, on cellular and molecular processes contributing to clinically relevant phenotypes.[102,103] The complex topic of genetic modification for the generation of transgenic mouse models has been reviewed in detail.[104] Transgenic mice with whole body gene deletions have been developed (constitutive knockouts). Confounders that emanate from the deletion of a gene throughout the entire organism resulted in the development of tools to generate conditional knockouts with spatial and temporal gene deletion. Gene deletion can be facilitated by Cre/loxP- or Flippase/FRT-mediated recombination, and tissue-specific Cre and Flippase recombination are achieved by the use of a specific promoter (e.g. *myosin heavy chain 6, Myh6*). Recently, engineered nucleases have been developed to modulate DNA sequences and to generate transgenic mice. The nucleases used for genome editing include transcription activator-like effector nuclease (TALEN), zinc-finger nuclease (ZFN), and clustered regularly interspaced short palindromic repeat (CRISPR)/-associated protein 9 (Cas9). Compared to other nucleases, the CRISPR/Cas9 system is more efficient and the design of constructs easier to perform.[104] Important limitations of the CRISPR/Cas9 system are non-specific off-target effects, which can affect the phenotype of the model generated and necessitate whole-genome sequencing of the generated mouse model. Genetic modification can also be facilitated by adeno-associated virus (AAV)-mediated delivery of DNA constructs, which can be performed by the use of a combination of specific viral serotypes and promotors (e.g. AAV9 and *Myh6*).[105] Compared to the generation of transgenic animals, virus-mediated approaches are usually more time- and cost-efficient. Potential drawbacks include side effects in other tissues following systemic injection and badge-to-badge variability of the virus construct.

3. Small animal models of HFpEF

In the following sections, we will discuss the most common models to investigate classical risk factors for the development of HFpEF, which include hypertension, obesity, diabetes mellitus, and aging. Importantly, systolic contractile dysfunction may also be present in these models, which additionally enables their use as HFrEF models. Additional risk factors for the development of HFpEF in humans include renal dysfunction, chronic obstructive pulmonary disease (COPD) and atrial fibrillation, which have not been studied in detail in small animal models.

3.1 Hypertension

The Dahl salt-sensitive rat, which was generated by inbreeding Sprague-Dawley rats,[71] is one of the most commonly used HFpEF models. When fed with a high-salt diet containing 8% NaCl, this model rapidly develops hypertension, diastolic dysfunction, and HFrEF.[72] Spontaneously hypertensive rat is an inbred strain of Wistar-Kyoto rats with hypertension.[73] Chronic infusion with angiotensin II causes hypertension and cardiomyocyte hypertrophy in mice and rats.[69,70] Major advantages of these models are the slow progression of hypertension and HF, which is also observed in patients with hypertension and is in contrast to the immediate increase in LV workload following TAC surgery.

3.2 Obesity and diabetes mellitus

Numerous small animal models have been generated to investigate the impact of Type 1 (T1D) and Type 2 diabetes (T2D) on the heart.[6] A commonly used model for T1D is the Akita mouse (*Ins2*$^{Akita+/-}$), which exhibits a mutation in the *Insulin2* encoding gene. This results in misfolding of the insulin protein, endoplasmic reticulum stress, and β-cell failure.[74] Hearts from Akita mice show increased inflammation[75] and diastolic dysfunction in the presence of normal systolic function.[76]

The glucosamine-nitrosourea streptozotocin (STZ) is toxic to pancreatic β-cells and has been used to study both T1D and T2D. Because of its structural similarity to glucose, STZ enters pancreatic β-cells via the glucose transporter 2 (GLUT2), causing cellular damage, and impairing insulin production. The STZ-mediated effects on β-cell destruction and hyperglycaemia are dose-dependent. High-dose STZ treatment induces T1D in rodents. In contrast, low-dose STZ protocols have been used to overcome the low penetrance of some high-calorie dietary regimens and to mimic β-cell failure and late stage T2D. Therefore, low-dose STZ treatment has been added to the high-fat diet (HFD) protocols.[6]

Ob/ob[77] and db/db[79] mice are commonly used models of obesity and T2D that are based on leptin resistance or deficiency, respectively. Diastolic dysfunction has been reported for both models.[78,80] Additional models for T2D and insulin resistance include Zucker fatty (ZF) rats, which express non-functional leptin receptors[81] and Zucker diabetic fatty rats, which are an inbred strain of ZF rats with high serum glucose levels.[82]

Numerous dietary treatment regimens are used to induce insulin resistance and T2D in rodents. HFD chow usually contains a total fat content of up to 60%. Rodent 'Western' diets typically contain a high content in fat and sucrose, which makes them a useful tool to study pathologies that have been described by the 'Western' dietary pattern in humans.[106] Depending on the total fat content and duration these dietary treatments may induce contractile dysfunction in rodents. The proposed mechanisms have been recently discussed in detail.[6,102]

3.3 Aging

HFpEF is primarily found in elderly patients. Senescence-accelerated prone (SAMP) mice have been generated by selective inbreeding of AKR/mice with inherited senescence[107] and have been subsequently used to study various effects of aging. SAMP mice develop age-dependent diastolic dysfunction, adverse remodelling, endothelial cell dysfunction, and HFpEF when subjected to a high-salt, HFD. These studies suggest endothelial cell dysfunction as one potential mechanism contributing to the age-dependent increase in HFpEF in patients.[108,109]

4. General advantages and limitations using small animal models

General advantages of small animal models include a lower housing cost compared to large animals, shorter gestation times and reduced costs for pharmacological treatments, which is typically administered proportionally to body weight. The potential increase in sample size improves statistical power. Recent advancements in magnetic resonance imaging, high-resolution transthoracic echocardiography, and micromanometer conductance catheters enable a detailed assessment of contractile function even in small rodents. Major advantages of mouse models compared to rats are the availability of a variety of already existing transgenic strains and readily available tools to generate novel transgenic lines. Therefore, transgenic mouse models also facilitate the investigation of specific genetic modifications in the context of superimposed stressors, for example using a surgical model or dietary treatment. Genetic modifications using viral vectors are typically easier to introduce into the genome of small rodents compared to larger animals. This mainly results from the

amount of virus required for sufficient transduction, which is typically proportional to body weight. Intravenous delivery of AAV9, for example, results in a very low transduction rate of cardiomyocytes in dogs.[110] Based on the technical challenge to transduce myocardial tissue of large animals, surgical and catheter-based approaches have been developed to overcome this limitation. In contrast, AAVs that target the myocardium of small rodents can easily be delivered by intravenous and intraperitoneal injection. Using transgenic gain-of-function models, it is important to consider that high levels of overexpression can cause HF per se, as reported for transgenic overexpression of the biologically inactive green fluorescent protein (GFP).[111]

Despite these advantages, several limitations using small animal models warrant attention. Rodents are typically on the same or very similar genetic background, which does not reflect the genetic heterogeneity of the patient population. Another limitation of small animal models, especially surgical models, is the rapid induction of the stressor, which is in contrast to the typically slow disease progression in patients. HF and coronary artery disease are often associated with atherosclerosis in patients, which is difficult to induce in most small rodent models. Several differences comparing the murine and human heart exist that result from the difference in heart rate. In general, the heart rate and the size of the species are inversely correlated, with about 500–600 beats per minute (b.p.m.) in mice, 350 b.p.m. in rats, 60–80 b.p.m. in humans, 30 b.p.m. in elephants, and 6 b.p.m. in blue whales.[112,113] Human ventricular myocytes predominately express β-myosin heavy chain (MHC). Adult murine cardiomyocytes mainly express α-MHC with rapid ATPase activity, which facilitates a contraction rate of up to ∼600 b.p.m. Action potentials in murine cardiomyocytes exhibit a rapid repolarization phase, lack a prominent plateau phase and have a shorter total duration compared to human cardiomyocytes. This facilitates faster contraction/relaxation compared to larger mammals, which is required to sustain cardiac output (calculation: stroke volume × heart rate) at high heart rates. Based on these contractile kinetics, the ability to increase heart rates in small animal models is impaired relative to humans, which can typically increase by up to approximately three-fold. In contrast, the heart rate of mice can increase by about 30–40% under exercise conditions, which limits cardiac reserve and is an important consideration in the design of exercise studies.

In humans, HF is typically observed in older patients, in contrast to most rodent models, in which HF is commonly induced by various stressors starting at young ages to reduce experimental costs. Depending on the extent and duration of the stressor, rodent models with HFpEF may also develop HFrEF. In contrast, several disease conditions are associated with HFpEF in humans, which typically do not progress to HFrEF, such as hypertensive heart disease.

5. General considerations for the use of small and large animal models

Despite the specific limitations and differences outlined above, myocardial energetics and contraction are overall relatively similar between small rodents and humans. Consequently, numerous proteins share functions across species, which makes small rodent models inevitable tools to rapidly conduct proof-of-principle studies at a large scale and to test for different druggable targets and genetic modifications over a relatively short-time period. However, despite their widespread use and acceptance, studies performed in small rodent models should be interpreted with caution. Different phenotypes for humans with genetic mutations and transgenic mice recapitulating diseases were observed. For example, patients with Duchenne Muscular Dystrophy, which lack the expression of the dystrophin protein, have an average survival rate of about 40 years, with about 10-20% of patients developing HF. In contrast, dystrophin-deficient mice have a normal life span and relatively mild cardiomyopathy (reviewed in Ref.114). Another example is the nonsense T116G mutation in the phospholamban (PLB) gene in patients with dilated cardiomyopathy, which results in severe HF. Conversely, PLB deficient-mice exhibit enhanced cardiac contractility and a normal life span.[115] Several drugs have also been tested in small animal models with beneficial effects observed, despite their failure in humans.[116] Examples include the phosphodiesterase (PDE) 5 inhibitor Sildenafil, which attenuated the onset of HF post-TAC in mice[117] but showed no benefit in chronic HF patients in the RELAX trial.[118] Relaxin and the recombinant protein Serelaxin also attenuated adverse remodelling post-MI in mice[119] but showed no beneficial effects in humans with acute HF in the RELAX-AHF-2 trial.[120]

These examples highlight different adaptations of small animal models with genetic modifications and pharmacological treatments compared to patients. As a result, results from small animal models should be validated in large animals prior to Phase I trials in humans. Swine is a prototypical pre-clinical large animal model. Advantages of swine are a similar expression pattern of MHC isoforms and a similar reserve in heart rate and cardiac output compared to humans. Importantly, animal models are typically subjected to an single-drug treatment in the context of a HF stressor and beneficial effects for a specific drug tested might be observed. However, these effects may not be observed in later clinical trials, in which patients typically receive the drug in addition to the well-established standard treatment for chronic HF. This also provides a potential explanation for the successful bench-to-bedside translation of the very early studies performed by the Pfeffer group using ACE inhibitors and the failure of numerous later clinical studies, which showed efficacy in animal models, but not in patients.

6. Summary and conclusion

Small animal models, especially mice and rats, mimic various aspects of the pathogenesis of HF and help to decipher various underlying contributing mechanisms of the disease. Several limitations for small animal studies exist that warrant the interpretation of the results of the studies performed with caution. Despite these specific limitations, small animal models serve as invaluable tools that have greatly advanced our understanding of the pathogenesis of HF. Based on recent advancements in genome editing, numerous novel transgenic models are likely to be generated in the near future. These models will continue to facilitate the identification of new targets and to develop novel treatment strategies for HF patients.

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