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Review

In Vivo Models for Heart Failure Research

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Abstract. *The medical treatment of heart failure (HF) is associated with 50% survival at 5 years, thus being one of the major causes of mortality in Western countries. An understanding of the pathophysiology of HF is essential for the development of novel efficient therapies. Consequently, the use of animal models is indispensable. In addition, the development of new in vivo models of HF is critical for the evaluation of treatments such as gene therapy, mechanical devices and new surgical approaches. However, every animal model has advantages and limitations and none of them is suitable to study all aspects of HF. Besides the technical determinants of a model, species, strain and gender affect the pathophysiology of a given heart pathogenesis and, therefore, have to be considered in each animal model. The most common in vivo models used in cardiology research and in particular in HF remodeling are presented.*

Congestive heart failure (CHF) is one of the leading causes of cardiovascular morbidity and mortality. Indeed, CHF is associated with 5-year mortality above 50% and is the reason for at least 20% of all hospital admissions among persons older than 65 years (1, 2). The leading cause of CHF is coronary heart disease (CHD): more than 50% of all cases are attributed to CHD (3). Other than CHD, conditions that cause CHF include: pressure overload, volume overload and cardiomyopathies. CHF is the final manifestation of various pathological insults, each of which ultimately results in an inability of the left ventricle (LV) to maintain a stroke volume (SV) sufficient to meet metabolic demands.

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Irrespective of the etiology, CHF is a complex framework of abnormal LV function and structure. Deterioration in LV pump function prompts adaptive and initially compensatory local and systemic alterations in an attempt to maintain adequate SV. Among these are: the Frank-Starling mechanism to maintain cardiac output by an increase in preload, myocardial hypertrophy of unaffected myocardium, activation of the sympathetic adrenergic system and activation of the rennin-angiotensin system (RAS), (collectively termed the *neurohormonal axis*), and the cytokine system. At the cellular and molecular level, CHF stimuli lead to a complex of events including cellular changes, the alteration of excitation–contraction coupling, the alteration of contractile apparatus, changes in gene expression, changes in the quantity and nature of the interstitial matrix and cell death. In the short term, the above alterations restore cardiovascular function to an almost normal range. Eventually, these events result in further pump dysfunction and increased wall stress, thereby promoting pathological remodeling (4, 5).

In order to prevent and manage CHF more effectively, it is necessary to understand the pathophysiological mechanisms underlying this disorder. Therefore, *in vivo* models closely mimicking the structural and functional characteristics of human CHF are indispensable. However, every animal model has advantages and limitations and none of them is suitable to study all aspects of CHF. Besides the technical determinants of a model, species, strain and gender affect the pathophysiology of the manipulated heart and, therefore, have to be considered when an animal model is being established. The most common *in vivo* models used to study the remodelling of the heart and HF are presented.

In Vivo Models for Studying Ischemia Stress in the Myocardium

The main modeling techniques used to produce CHD are the coronary artery ligation, coronary artery embolisations and models of incomplete narrowing of coronary arteries. The

Table I. *Animal models of chronic HF, causes, advantages and limitations.*

Causes of HF	Experimental method	Species	Advantages	Limitations
Myocardial ischemia	Coronary artery ligation	Rat, mouse, sheep, dog, pig	The rat model has a low cost and is a relatively simple procedure	Rats differ from humans in terms of electrophysiology, coronary circulation and cardiac protein isoforms
	Coronary artery embolisations	Dog, pig, sheep	Stimulates the clinical etiology of chronic HF	Control of the exact location and length of coronary artery occlusion
Tachycardia-induced cardiomyopathy	Chronic rapid pacing model	Dog, pig, rabbit, sheep	Relatively uncomplicated and requires simple instrumentation. Produces a well defined clinical syndrome which mimics the human state	The mechanical and neurohormonal alterations induced are reversible in a few days after stopping the treatment. The myocardial damage is homogeneous
Pressure overload	Aortic banding Spontaneous hypertension (SHR)	Rat, mouse, sheep, dog, pig	The relatively low mortality rate and the high success of induction of LVH. Also, a good model to evaluate diastolic dysfunction. Is suitable for studying the progression of LVH and HF. Induces a pattern of LV strain similar to that of aortic stenosis. SHR is a good model to reproduce hypertension-induced HF. It does not require surgery and pharmacologic intervention	Considerable differences in the function of subcellular systems exist between rat and human myocardium. SHR: long duration until the development of HF
Volume overload	Arterial – venous shunt Mitral regurgitation	Dog, rabbit, rat	Mitral regurgitation is minimal invasive	These models do not have alterations in the myocardial structure observed in congestive HF due to ischemia, or hypertrophy

species mainly used are the pig, dog, rabbit, sheep, mouse and rat (Table I).

Coronary artery ligation. Coronary artery ligation was first applied in dogs (6). In brief, following orotracheal intubation and left thoracotomy, the proximal left anterior descending coronary artery (LAD) is ligated, inducing myocardial infarction (MI). Despite the fact that numerous discoveries regarding MI and HF have been made with the use of canine models, recent investigations have used alternative animal species due to various disadvantages with dogs. The mortality of this procedure in the acute phase was reported to be more than 50% due to malignant ventricular tachycardias. Moreover, in most cases MIs were small (averaging 21% of the LV) due to a high number of collaterals in dogs (6). In addition, the model is time consuming, expensive and is facing increasing criticism.

An alternative animal model is that of the rat. In brief, after anaesthesia, orotracheal intubation and thoracotomy, the heart is rapidly exteriorized and the LAD is ligated in the proximal segment using a thin thread. The occlusion of the artery can be recognized by blanching of the tissue distal to the ligation. Rats with MI greater than 45% develop CHF after 3 weeks with elevated LV filling pressures, reduced cardiac output and a minimal capacity to respond to preload and afterload stress. The degree of impairment of LV function is directly related to the extent of myocardial loss (7). The mortality seems to be strain dependent. Indeed, in a comparative study the mortality of Sprague Dawley rats was 36%, whereas in Lewis inbred rats it was significantly lower, 16% (8). In the mouse, the surgical procedure used to induce MI is similar to the rat model (9), however, a microscope is required to accurately detect and ligate the relatively small LAD. In both the mouse and rat, mortality associated with MI is about 35-50%, occurring within the first hour after MI, due to ventricular fibrillation and severe acute HF (10, 11).

The noninfarcted territory of the myocardium is susceptible to forces that induce morphological alterations and dilatation

of LV. According to the Laplace law, as the LV chamber enlarges, wall stress increases providing an internal load on the LV. This excess load leads to additional dilatation, hypertrophy (compensatory hypertrophy), and increased wall stress, contributing to further reduction of the LV function. In addition, regional hypertrophy and LV enlargement of myocardium is induced by neurohumoral activation which is positively correlated with LV function (12).

Increased neurohumoral activation in the rat MI model is detected in the plasma and the cardiac tissue. The circulating levels of atrial natriuretic peptide (ANP), tumor necrosis factor-α (TNF-α), brain natriuretic peptide (BNP) and endothelin are elevated after MI. At tissue level, increased angiotensin-converting enzyme (ACE) activity is inversely associated with LV pressure. In addition, the local expression of TNF-α and vascular endothelial growth factor (VEGF) in the inflamed border zone of infarcted myocardium plays an important role in heart dysfunction and remodelling (13, 14). The increased expression of interleukin-1β (IL-1β), IL-6, TNF-α and inducible nitric oxide synthase (iNOS) also confirms the important role of inflammation in cardiac post-ischemic dysfunction and remodeling (15). Indeed, in the early post-MI period a hemodynamic deterioration was most prominent and it was parallel to the expression of iNOS and TNF-α. Additionally, IL-1β expression was correlated with collagen deposition in the non-infarcted region in the rat model (15). In addition, experimental data have shown that the bioregulation system of parathyroid hormone-related peptide (PTHrP/PTH.1R) locally expressed in the ventricular myocardium participates in cardioprotection due to ischemic injury (16, 17).

The magnitude of an MI is the most critical determinant of subsequent LV remodelling and HF. It has also been suggested that apoptosis may be responsible for a significant amount of cardiomyocyte death during both the acute and chronic stages. In this animal model, cardiomyocytes with fragmented DNA were found among the apparently surviving myocytes at the border zone of infarcted tissue (18). Apoptosis occurs rapidly in the rat infarct model, with significant increases apparent 24 hours after MI in the infarct area and border zones. Sustained apoptosis is associated with LV enlargement and increased cardiac fibrosis after MI (19, 20).

The transition from compensated left ventricular hypertrophy (LVH) to HF seems to be calcium dependant. Indeed, sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA)-2α levels decrease during compensated severe LVH, making this a major candidate for mediating the transition from compensated LVH to HF (21). However, SERCA-2α gene down-regulation and the expression of the hypertrophy gene program are not necessarily linked. Indeed, 12 weeks post-MI, the decreased SERCA-2α levels in the myocardium adjacent to the infarct region were correlated with increased β-myosin heavy chain, alpha-skeletal actin and Na^{+}–Ca^{2+} exchanger levels in remote regions of infarction (22).

The advantages of this model include: easy care for rats, cost and simplicity of the ligation procedure. However, a limitation is that the rat differs from the human in terms of electrophysiology, coronary circulation and cardiac protein isoforms. Moreover, the time of MI evolution in the rat differs significantly from that in the human. Indeed, human cardiac necrosis is visible from 6 h after an ischemic episode, increases up to three days, then gradually diminishes and disappears after two weeks (23). In comparison, in rats cardiac necrosis reaches its maximum at day 4 and disappears by day 11. Similarly, in humans the inflammatory polymorphonuclear infiltration appears immediately, with a maximum at day 4, and then diminishes, disappearing at two weeks. In comparison, in rats it drastically diminishes at day 8. Blood vessels as well as fibroblast proliferation begin in the human at day 4 and terminate two months later, while blood vessel proliferation in the rat occurs faster (from 2-11 days) and fibroblast proliferation starts at the same time as in the human, but terminates earlier (up to three weeks) (24). The above indicate a faster onset of healing and of termination processes in rats. Therefore, data obtained from this model must be interpreted with caution.

Coronary artery embolizations. Coronary artery micro-embolizations can produce numerous micro-infarcts throughout the myocardium due to mechanical occlusion of the coronary arterioles in combination with local microvascular vasoconstriction. Micro-infarction would not necessarily result in transmural ischemia or necrosis. However, the cumulative effect of repeated micro-infarctions is the deterioration of cardiac and endothelial function, resulting in HF.

Such a model of HF can be produced by multiple sequential intracoronary embolizations with polystyrene latex microspheres (70 to 100 μm in diameter). With three to ten embolizations performed one to three weeks apart, LV function can be significantly altered. Access is through the femoral artery by placing a catheter in the target coronary artery under fluoroscopy. Embolizations are discontinued when the LV ejection fraction (EF) is less than 35% (25). The species mainly used for the micro-embolization model are the dog, sheep and pig (Table I).

In this model, the drop of LVEF is accompanied by an increase in LV end diastolic pressure (LVEDP) and a significant rise of pulmonary artery wedge pressure and systemic vascular resistance. Twelve weeks after the last embolisation, macro- and microscopic examination of the myocardium show patchy fibrosis and hypertrophy. Observation of this model for up to 2 years has shown that ventricular remodeling would subsequently continue as a maladaptive compensatory mechanism until death. The remodeling process in both the viable and infarcted myocardium may include: infarct expansion, volume

overload hypertrophy, dilation of noninfarcted myocardium, myocyte lengthening, slippage of myocytes and increased wall stress (25). After 16 weeks, the mechanical and hemodynamic compensatory mechanisms that maintain cardiac output fail to match the rate of myocyte loss. This leads to increased LV plasticity with consequent chamber dilation and wall thinning. In contrast to dilation being the end-product of a series of events, it has also been proposed that dilation may be a survival strategy for the preservation of SV and redistribution of wall stress (26).

At the neurohumoral level increased plasma levels of ANP and norepinephrine were documented. Moreover, cardiac dysfunction was parallel to the expression of NO located upstream of TNF-α (27). In addition, the Frank–Starling mechanism was exhausted in the failing heart, which thus became unable to adequately respond to volume overload (28-30). However, this model is characterized by a relative stable degree of LV dysfunction and neurohormonal activation devoid of the critical features of CHF (25). Furthermore, the number of β-adrenergic and L-type calcium channels decreases and the activity of cardiac SERCA drops (31, 32).

This model is characterized moreover by apoptotic cardiomyocytes mainly distributed at the borders of the infarction scars in dogs (33). In sheep, the development of HF is associated with caspase-3 activation and an increased number of TUNEL-positive cardiomyocytes (34). Apoptosis in the subendocardial regions of hibernating areas of the pig heart emphasizes the importance of apoptotic cell death in this model (35).

The advantage of this model is that it simulates chronic HF and the natural history of remodelling in the human. Moreover, it resembles the clinical situation of acute coronary syndrome due to the embolization of atherosclerotic and thrombotic debris into the coronary microcirculation, as well as the situation of patients with diffuse coronary artery disease. Thus, micro-embolization models have the potential to provide insight into mechanisms of ischemic HF. Another advantage of the model is the low risk of infection and inflammatory complications, since coronary artery embolisations are induced percutaneously and it does not require surgical interventions such as thoracotomy.

A limitation of the embolization technique is that the exact location and length of the coronary artery occlusion is not known. To overcome the above limitation, recently a minimally invasive approach to induce MI was recently developed in which a flexible body comprising an open-cell foam sponge is percutaneously placed at a distinct position in the coronary artery (36). However, the embolization model has a few more disadvantages. Firstly, creation of the model requires serial surgical interventions that are time consuming and secondly, the technique may elicit malignant arrhythmias, at a rate of 30% . Thirdly, the canine model fails to manifest the complete CHF phenotype.

Chronic myocardial ischemia. In the rat, a model of incomplete narrowing of coronary arteries similar to the coronary artery occlusion model has been established. After thoracotomy, a probe or copper wire is placed onto the epicardium along the LAD, and the LAD together with the probe is ligated 1-2 mm from its origin followed by removal of the probe, resulting in a reduction in luminal diameter by 42% (37, 38). The actual level of constriction measured at death varies from 18% to 69% (37). In rats, 45 minutes after the non-occlusive constriction, the ischemic heart exhibits increased LVEDP, whereas the LV volume, thickness of LV wall and cardiac cell damage decrease (37). After one week, the ischemic heart exhibits decreased peak systolic pressure, SV and total peripheral resistance. The maximal resting coronary blood flow decreases by 43% and fibrosis, myocytolytic necrosis and myocyte hypertrophy are detected (39). The limitation is that during the procedure of coronary ligation it is inevitable to include some muscle mass within the ligature, which may affect vessel stenosis.

In pigs, the hydraulic occluder technique has been used to cause partial coronary artery stenosis providing a model of chronic myocardial hibernation (40, 41). The LAD or left circumflex (Lcx) coronary artery is isolated and the hydraulic occluder is placed around the target vessel. The inflation or deflation of the banding ring can be carried out percutaneously on the conscious animal. If coronary blood flow is to be measured, a transonic flow probe can be implanted distal to the occluder. Using this technique, myocardial hibernation can be reproduced as was shown by positron-emission tomography and dobutamine stress echocardiography (42, 43). Indeed, the loss of contractile material within cardiomyocytes with the space previously occupied by the myofilaments filled with glycogen, small mitochondria scattered throughout the myolytic cytoplasm, and tortuous nuclei with uniformly dispersed heterochromatin were observed (44). These changes are more prominent in the subendocardial regions. Triphenyl tetrazolium chloride (TTC) staining as well as light and electron microscopic techniques demonstrate little to no subendocardial infarct within the area at risk (42). The major advantage of the hydraulic occluder model is the consistent degree of ischemia within the area at risk. This model is also associated with a low incidence of animal loss. However, one limitation is that the model is technically demanding from both a surgical standpoint of dissecting out the coronary artery for the occluder and the flow probe placement. Another limitation is that it is time consuming, due to chronic animal maintenance.

In pigs, ameroid constrictors have also been used to provoke chronic coronary artery stenosis (45). Most commonly, these constrictors are placed on the Lcx, which is the smallest of the three coronary vessels in swine, supplying ~20% of the LV myocardium. The constrictors are constructed of the hygroscopic material casein encased

within a steel sleeve. When the device is implanted around an artery, the constrictor absorbs water and swells, compressing the artery and producing total coronary occlusion. Closure is more rapid during the first 2-4 weeks after transplantation. Complete occlusion is usually achieved in 4-5 weeks. The choice of ameroid constrictor size is based on vessel diameter. Since swine, unlike dogs, have minimal pre-existing coronary collaterals, they tolerate the acute coronary occlusion poorly. Therefore, this model is very useful for studying chronic myocardial ischemia. The major advantage of the ameroid constrictor model is its simplicity. In addition, the slow and gradually increasing stenosis allows for the formation of collaterals, therefore mimicking the situation seen in humans. It is, thus, a very good model for examining in detail the development of a collateral circulation. However, collateral vessels in the pig appear to develop rapidly and restore myocardial blood flow and function at rest to normal levels by 3-7 weeks after occlusion. A limitation of the occluder technique is the inability to control the degree and progress of stenosis (45). In addition, it causes mechanical trauma that may lead to endothelial damage, platelet aggregation, thrombus formation and local scar formation (46).

In conclusion, while the models of chronic myocardial ischemia may provide a consistent, stable level of myocardial stunning with normal resting coronary blood flow and some degree of LV dysfunction, they do not reproduce the congestive HF phenotype (including activation of the neurohormonal axis) as effectively as acute coronary occlusion. In addition, the fixed stenosis model is very demanding in large animals (pigs).

In Vivo Model of Chronic Rapid Pacing

It is well known that chronic tachycardia can alter cardiac structure and function inducing cardiomyopathy. Patients with atrial fibrillation with elevated ventricular response exhibit cardiomyopathy. Experimental tachycardia-induced cardiomyopathy was first described in 1962 (47). In the dog, atrial pacing at over 330 beats/min can induce symptoms and signs of CHF. The species mainly used for models of chronic rapid pacing are the dog, pig, sheep and rabbit (Table I).

The severity of cardiac dysfunction directly correlates with the rate of pacing. Rapid pacing is characterized by a significant decrease in systolic and diastolic function, followed by decreased cardiac output. Rapid pacing also increases LV end diastolic pressure, mean arterial pressure, pulmonary artery pressure and wall stress. These changes occur as soon as 24 h after rapid pacing. Deterioration of ventricular function occurs at 3-5 weeks resulting in end-stage HF. However, the changes in LV geometry and function are not accompanied by significant changes in LV mass and hypertrophy. Indeed, cardiac chamber dilatation is accompanied by little or no cardiac hypertrophy (48, 49). Thus, chronic pacing tachycardia has subsequently emerged as a method for inducing dilated cardiomyopathy and CHF in animals.

At the neurohumoral level, an early sympathetic activation and attenuation of parasympathetic tone has been observed. In addition, plasma ANP and BNP levels are elevated early in the development of LV dysfunction. However, the hemodynamic and renal responses to the exogenous administration of ANP in the paced dog is blunted suggesting that increased ANP production may not be an important compensatory mechanism to counteract the initial stages of HF. Furthermore, systemic activation of the renninangiotensin system (RAS) is seen with progressive pump failure (50). Plasma levels of both endothelin-1 and TNF-α are consistently elevated (51). Similar to human HF, pacing-induced alterations in the neurohormonal system appear to be time dependent. Indeed, during the early development of pacing-induced LV dysfunction, plasma levels of catecholamines first increase and then plateau (52). Similarly to elevations in plasma endothelin levels, pacing-induced alterations in beta receptor density and function parallel those observed in patients with CHF (53). However, plasma elevations of endothelin and renin occur in more advanced stages of LV dysfunction (54). Clinical studies have also demonstrated that plasma endothelin levels were increased only in the patients with moderate to severe CHF (55). Thus, plasma markers of neurohormonal activation in pacing models of CHF closely parallel those observed in patient populations. As far as calcium homeostasis is concerned, the levels of SERCA are lower while those of the sarcolemmal Na/Ca exchanger are significantly increased (56).

Morphometric analysis of the myocardium has revealed a significant loss in the number of myocytes, suggesting that cell loss may be a major component of canine pacing-induced cardiomyopathy. Indeed, apoptosis has recently been reported in the canine model of pacing-induced CHF and was associated with enhanced p53 DNA-binding activity to the *Bax* promoter, increased expression of *Bax* protein and attenuation of *Bcl-2* (57).

An advantage of this model is that it is relatively uncomplicated and requires simple instrumentation. It produces a well-defined clinical syndrome of biventricular failure mimicing the human state. The neurohumoral and the circulatory alterations also closely resemble that observed in human HF. In addition, HF evolves over a period of several weeks permitting sequential observations (48). This may allow the study of the transition from a compensated state of LV dysfunction to overt failure. However, the main limitation of this model is that the mechanical and neurohormonal alterations are reversible in a few days after stopping the pacing. Within 48 h after stopping pacing, the hemodynamic variables approach control levels, and the LVEF shows significant recovery with subsequent

normalization after 1-2 weeks. Within a few hours after stopping pacing, circulating ANP drops 60%. Unlike hemodynamic dysfunction and neurohormonal activation, chamber dilatation persists even after pacing is terminated. Another limitiation is that the myocardial damage induced by fast pacing is homogeneous and thus those events that are dependent upon the typical mechanical and electrical dyshomogeneity of the failing heart cannot be assessed in this model. Furthermore, unlike clinical forms of CHF, the development of CHF by chronic rapid pacing is not associated with hypertrophy or increased collagen content. The loss of collagen support may considerably contribute to ventricular remodeling. Thus, it must be recognized that rapid pacing models fail to manifest the complete spectrum of CHF.

In Vivo Model of Pressure Overload (PO)

PO is associated with the development of LVH (developing and compensatory hypertrophy) that ultimately transits to HF. PO leads to an increase in the ratio of LV wall thickness to chamber radius. This adaptive response is called concentric hypertrophy and is characterized by lateral expansion of the myocytes by the addition of new sarcomeres, in parallel. The mitochondria increase in number, but decrease in relation to the overall cell volume. Myocardial hypertrophy represents a compensatory mechanism, which attempts to normalize myocardial wall stress (58). Although beneficial, concentric hypertrophy may increase the elastic stiffness of the myocardium, thus reducing LV chamber distensibility. In addition, progressively, the increased afterload outstrips all adaptive mechanisms, preload reserve becomes exhausted despite LV chamber dilation and basal contractility becomes mismatched to the level of afterload (59). Concomitant elevation of both the LV end-diastolic pressure and left atrial mean pressure gives rise to pulmonary capillary and alveolar congestion, while the reduced systolic fractional shortening is associated with an inadequate cardiac output both at rest and exercise (59).

PO is induced by conditions such as hypertension or LV outflow obstruction (such as: aortic stenosis or hypertrophic cardiomyopathy). In the Framingham study, LVH coincided with an increased incidence of cardiac morbid events, such as sudden cardiac death, cardiac failure, myocardial infarction and stroke. Thus, LVH is an independent cardiac risk factor and a major determinant of patient outcome. However, reverse remodeling with normalization of LV mass can be anticipated after corrective therapy, for example, aortic valve replacement in patients having severe aortic stenosis. Reverse remodeling has been clinically documented by echocardiographic follow-up studies (60). However, the underlying cellular and molecular changes in the myocardium are not yet completely understood.

The animal models mainly used to impose a PO are: the supravalvular aortic stenosis model and spontaneous hypertensive rats (SHR). The pulmonary artery banding model is used to induce PO of the right ventricle. The species mainly used for the PO model are the dog, rabbit, cat, pig, mouse, sheep and rat (Table I).

The model of supravalvular aortic stenosis is most commonly used in rats. It is produced by dissecting the ascending aorta free from the pulmonary artery and by placing a Dacron patch (1.5-2.0 cm wide and 6-7 cm long) to encircle it. The diameter of the aorta is reduced by 50% producing a systolic pressure gradient (50-60 mmHg) between the aorta and the LV. This model leads to marked LVH and HF, which are associated with increased β-myosin heavy chains. Progression through LV dilatation and LV failure stages correlated with a significant increase in LVID and decrease in EF. Interestingly, decline in hemodynamic function has been found to correlate with decreases in SERCA expression, glucose uptake, coronary effluent adenosine concentration and increased cardiomyocyte microtubule density. Decreases in SERCA expression occurred in the LV myocardium from failing animals after 20 weeks of banding, suggesting that SERCA may be a marker of the transition from compensatory hypertrophy to HF (61).

At the neurohormonal level, during compensated hypertrophy, catecholamine levels are normal and there is activation of the local myocardial RAS (62). Marked LVH and HF are also associated with increased ANP. Recently, the bioregulator system of PTHrP/PTH which is locally expressed in the ventricular myocardium (16) was shown to be associated with PO hypertrophy (63). With the development of HF, plasma catecholamine levels increase (64). The increased expression of the pro-apoptotic factors, bax and Fas, is associated with no significant changes in expression of the anti-apoptotic bcl-2, indicating that up-regulation of the apoptotic cascade is associated with the transition to HF in this model. In addition, this model is related to collagen deposition and matrix remodeling. Ca^{2+} has also been shown to be an important second messenger in cell growth and survival. Indeed, in response to growth stimuli, cytosolic Ca^{2+} increases, and calcineurin is activated, resulting in dephosphorylation of transcription factors, which then regulate expression of specific genes. Although controversial, calcineurin appears to be a requisite mediator of myocardial hypertrophy (61).

The advantages of this model are that the mortality rate of the banding procedure is relatively low, approximately 10% and that there is a high success of induction of consistent LV hypertrophy. Furthermore, the alteration of LV stiffness and reduction of relaxation make such models important for the evaluation of diastolic dysfunction, which is an important factor in the development of LV failure

(65). In addition, this model is suitable for studying the progression of hypertrophy to HF (66). However, it should be kept in mind that considerable differences in the function of subcellular systems exist between animal (rat) and human myocardium.

A naturally occurring model of PO is that of SHR. Introduced in 1963, the SHR presents a model of genetic systemic hypertension that leads to HF with ageing. Vascular lesions attributed to hypertension in SHR develop early around 6-7 weeks. The severity and distribution of affected arteries is greater in SHR males than in females. Following a stable period of compensated hypertrophy with preserved contractility at 12 months of age, SHR progress to HF with senescence, at 18-24 months characterized by reduced myocardial performance and increased fibrosis. In end-stage HF, severe contractile dysfunction is present in SHR.

In this model, although altered calcium cycling has been observed, no decrease in SERCA was found during the transition from compensated hypertrophy to HF (67). It was suggested that the transition to HF is associated with significant alterations in the expression of genes encoding the extracellular matrix (68). Furthermore, an increased number of apoptotic myocytes was observed, suggesting that apoptosis might be a mechanism involved in the reduction of the myocyte mass that accompanies the transition from stable compensation to HF. Thus, in this model contractile function is compromised by increased collagen, rather than by reduced availability of intracellular calcium.

SHR is a good model for reproducing hypertension-induced HF in humans and for studying the transition from hypertrophy to HF (69). The advantage of this model is that it does not require surgery and pharmacological intervention for induction and thus it is not complicated by mortality due to surgery or drug interventions. However, this model is limited by the long duration until the development of HF over a period of approximately two years; thus, it is a time consuming model and therefore expensive.

In Vivo Model of Chronic Volume Overload (VO)

VO leads to LV chamber dilatation and remodelling in an eccentric manner. Eccentric remodelling occurs by cardiomyocyte elongation and hypertrophy that serves to normalize LV end-diastolic dimension to wall thickness ratio. Progressively, chronic VO induces HF with severe ventricular dilation. The causes of VO are: hypervolemia (such as, valvular heart disease), excessive venous return (such as arteriovenous fistulae), or decreased peripheral vascular resistance. It is also observed in conditions such as hyperthyroidism, beriberi (vitamin B1 deficiency) and severe anemia. The surgical techniques mainly used to induce VO is the creation of a shunt between the arterial and venous system. Usually a shunt is created between the aorta and vena cava, the femoral artery and vein or the carotid artery and internal jugular vein. In addition, a VO model can be induced by mitral valve regurgitation (MR) (70-72). The species mainly used for the VO model are the dog, rabbit and rat (Table I).

During the cervical arteriovenous (A-V) shunt procedure, the internal jugular vein and the left carotid artery are exposed. Both vessels are mobilized over a length of 5 cm and ligated distally. The jugular vein is clamped proximally and transected. The left carotid artery is clamped and an end-to-side anastomosis is performed between the free end of the vein and the side of the carotid artery. After removal of the clamps, the patency of the fistula is confirmed by pulsatile filling of the jugular vein. In this model, an early (30 min after opening of the shunt) 40% increase in cardiac output is observed. A gradual increase in LV end-diastolic diameter and LV end-diastolic volume is found, with a peak at eight weeks. One advantage of this model is that it is possible to evaluate the patency of the shunt by palpating the neck. The shunt is also easily accessible for ultrasound evaluation. However, one limitation is the relative high risk of injuring to the vagus nerve that runs along the common carotid artery. Another limitation is that neither clinical nor hemodynamic signs of overt HF occurred with this relatively small shunt. Thus, this model is considered a compensatory overload dilation model rather than a HF model.

In contrast to the relatively small cervical shunt, a larger shunt can be induced by an infrarenal aorto-caval fistula. This model has been shown to increase LV end-diastolic pressure producing clinical signs of HF (73). A simple technique to create an aorto-caval shunt is the commonly applied needle technique in rats. The inferior vena cava and the abdominal aorta are exposed through a laparotomy. The aorta is punctured caudal to the left renal artery with an 18 gauge disposable needle, which is advanced into the aorta, perforating the adjacent wall between aorta and vena cava and penetrating the latter. A vascular clamp is placed across the aorta cephalad to the puncture, after which the needle is withdrawn and the aortic puncture wound is sealed with a drop of cyanoacrylate glue. The clamp can be removed 30 seconds later. Patency of the shunt is verified visually by swelling of the vena cava and mixing of arterial and venous blood (74).

After creation of an aorto-caval fistula, compensated hypertrophy occurred between two and eight weeks, with normal or only mild depression in hemodynamic function. The LV end-diastolic pressure was increased approximately 5-fold and 2-fold at first and fourth week, respectively. Severe volume overload (VO) from a large aorto-caval fistula initially leads to depressed LV function

followed by a compensatory hypertrophy and near normal function at fourth week (75). Decompensated hypertrophy or HF occurred between 8 and 16 weeks after aorto-caval shunting and was characterized by a decline of cardiac function and a shift in myosin heavy chain isoenzyme expression. At 16 weeks, heart rate, LV systolic pressure, LV dP/dtmax and systolic aortic pressure were not changed in rats with fistulas as compared to the controls. However, cardiac output, SV, LV end-diastolic pressure and all the measured parameters in the right ventricle were significantly increased (75). Moreover at 16 weeks, failing hearts showed a significant increase in beta (1)-receptor density. Basal adenylyl cyclase activity as well as isoproterenol- and forskolin-stimulated adenylyl cyclase activities were increased, indicating up-regulation of beta-adrenoceptor signal transduction as a unique feature of hypertrophy and HF induced by VO (76). Interestingly, not only the duration but also the size of the shunt will determine the onset and severity of HF following the creation of an aorto-caval shunt in rats. Indeed, a small shunt will yield only a moderate degree of HF as opposed to a large shunt. LV end-diastolic pressure is elevated only in the overt HF group caused by a large shunt for a period of 4 weeks. Cardiac function was decreased significantly in the large shunt model with a decrease of dP/dtmax (77). The advantage of this VO model of HF is that the procedure can be accomplished relatively fast and is usually well tolerated. However, a limitation is that it requires a laparotomy.

MR has been induced in dogs with a transvenous approach, resulting in significant MR and dilation of the LV. Under general anesthesia, an introducer sheath is placed into the LV through the carotid artery. Flexible rat-tooth forceps inserted through the sheath are used to cut the chordae. The chordae are cut until the pulmonary capillary wedge pressure increases by 20 mmHg, cardiac output drops by 50% and arterial pressure decreases (78). This model results in LV hypertrophy and dilatation, and, the development of overt clinical HF within 12 weeks. The MR model has the advantage of being a minimally invasive highly reproducible, simple and rapid method of developing high output HF and hypertrophy. It has been used mostly to test the medical treatment of HF, such as the influence of chronic β-adrenoceptor and angiotensin-II blockade on myocyte and LV function (79, 80). It is also very useful for studying the effectiveness of mitral annuloplasty in patients with dilated cardiomyopathy. Limitations of this model are that it induces anatomic changes in the mitral valve and does not have the alterations of the myocardial structure observed in congestive HF due to ischemia.

The neurohumoral activation of VO models includes local activation of the RAS which is associated with depressed myocardial function (79, 81). Recently it was found that ANP expression is a more sensitive marker for VO than PO (82). Moreover, induced myocyte lengthening, alterations in myofilament architecture, and reductions in myocyte contractile function were observed (79, 83). In addition, in the VO model of HF, long-term overexpression of SERCA2α can preserve systolic function, and potentially prevent diastolic dysfunction and improve LV remodelling (84).

In Vivo Model for Studying the Pathophysiology of Cardiomyopathies

Myocarditis. Myocarditis is defined as inflammation of the myocardium associated with cardiac dysfunction. Despite this clear-cut definition, diagnosis and etiologic treatment continue to create considerable debate. Viral infections are frequent causes of myocarditis, while the evidence of persistent viral infection is associated with poor prognosis in different subtypes of cardiomyopathy. Several animal models of myocarditis have been developed and progression to dilated cardiomyopathy and HF occurs in some of them. Indeed, CHF develops after an acute phase of myocarditis induced by the M variant of the encephalomyocarditis virus (85). Myocyte necrosis and biventricular dilatation occur during the phase of viremia and signs of CHF were observed at 7 to 14 days after inoculation.

Altered myocardial function is associated with neurohumoral activation. Potential mechanisms contributing to the progression of CHF include a persistence of the viral RNA within the myocardium, viral-mediated cytokine production with continued myocytolysis, a prolonged immune response and continued fibrosis and abnormalities in microcirculatory function. Interestingly, TNF was elevated in this model and an exogenously administered anti-TNF antibody improved survival and reduced the myocardial lesion, suggesting the importance of TNF in the pathogenesis (86). Nuclear factor (NF)-κB activation in a retrovirus model of encephalitis and myocarditis in mice showed protection against virally-mediated apoptosis with preserved NF-κB expression in the presence of interferon-β. Lack of either of these protective factors in the myocardium leads to pronounced viral infection and subsequent cell death (87).

An autoimmune myocarditis model has been developed in different species by using an immunization process. The model resembles human giant cell myocarditis. Indeed, it was shown that myocarditis and hemodynamic deterioration developed within three weeks after immunization of rats with cardiac myosin. This was associated with increased activity and expression of iNOS, and an inhibitor of iNOS effectively attenuated the histopathological changes. Accordingly, it was concluded that NO may play an important role in mediating the pathophysiological changes in myocarditis of

autoimmune origin (88, 89). Autoimmune myocarditis has also been induced with various intracellular antigens. Indeed, when mice were immunized with a monoclonal anti-dog SERCA antibody myocarditis developed (90).

Toxic cardiomyopathies. The deterioration of cardiac function can be produced by various cardiotoxic agents such as catecholamines, adriamycin or ethanol. It was noted that excessive doses of catecholamines produce diffuse myocardial destruction with myocyte loss and necrosis as well as extensive fibrosis in animals. Such changes may also be noticed in patients treated with high doses of catecholamines or those with pheochromocytoma (91). Various mechanisms have been postulated to be involved in the pathogenesis of this form of cardiomyopathy including the production of highly cytotoxic radicals from the catecholamine metabolism, catecholamine induced coronary vasoconstriction with radical generation resulting from ischemia, deleterious high-energy phosphate deficiency by excessive activation of calcium-dependent intracellular ATPases, and impairment of the phosphorylating capacity of the mitrochondria (91).

In rats, subcutaneous application of isoproterenol induced a dose-dependent impairment of cardiac function and neurohumoral activation (92). Recently, it was demonstrated, that treatment with isoproterenol resulted in LV dilatation and hypertrophy after 2 and 12 weeks. Furthermore, LV filling pressures and right atrial pressures were found to be elevated whereas aortic blood pressures and flow velocities were reduced. Renal blood flow was not significantly altered indicating preserved autoregulation as is usually found in mild HF. Furthermore, neurohormonal systems were found to be activated in the animals that had received isoproterenol suggesting system alterations in response to the primary cardiac insult.

The advantage of this model is that it is characterized by an extraordinary technical simplicity and excellent reproducibility as well as an acceptably low mortality. Therefore, isoproterenol-induced HF compares favourably, at least in these regards, with experimental myocardial infarction, the currently most widely used experimental model. However, this model does not seem suitable for inducing an overt state of HF because higher doses of catecholamines are associated with a high mortality rate of up to 80% (92).

Gene-targeted Models of HF

With the advancement of molecular biology, animals can be genetically manipulated to study the impact of overexpression or deletion of specific genes involved in the pathophysiology of HF. It is possible to breed animals with specific gene defects that cause different types of HF. Transgenic mice with overexpression or deficiencies of specific myocardial receptors help to generate a better understanding of the molecular biology of HF. Using transgenic mice with specific overexpression of the β (1)-adrenergic receptor it was shown that this receptor system not only plays a central role in modulating heart rate and LV contractility, but is also involved in the development of HF. As compared to 12-week-old wild-type mice with identical cardiac performance as well as contractile reserve, the ratio of phosphocreatine to ATP and total creatine content were significantly reduced in transgenic mice with overexpression of the β-1 adrenergic receptor. In addition, there was a significant decrease in creatine transporter content and mitochondrial and total creatine kinase activity as well as citrate synthase activity, indicating impaired oxidative energy generation in the transgenic mice and demonstrating that changes in myocardial energetics play a central role in the deterioration of cardiac function after chronic beta-adrenergic stimulation (93).

Issues Dependent on Species, Strain and Gender of Animal Models

Apart from the choice of technique to induce the type of HF-related disorder to be investigated, the most suitable animal species also has to be selected. The 'ideal' animal model should meet various requirements to mimic human HF such as: cardiac, hemodynamic, neurohumoral and peripheral aberrations. However, none of the models can fulfil all of these criteria and limitations of animal models with respect to their applicability to human HF are common (Table I).

Small laboratory rodents have the advantages of being cheap, easy to breed and generate less criticism regarding animal protection. Recently, the mouse has become the most important laboratory rodent species because of the availability of a large and increasing number of transgenic strains, offering the opportunity to analyse the significance of certain gene functions for the etiology and pathophysiology of HF. However, there are significant physiological differences between the hearts of laboratory rodents and humans. For example, cardiomyocyte action potentials of the rat and mouse are characterized by a very short duration normally lacking a plateau phase; the resting heart rate is about five times higher than in humans and the force–frequency relation is inversly proportional (94). Furthermore, calcium removal from the cytosol is predominated by the activity of SERCA in human and rodents, while the Na^+/Ca^{2+}-exchanger activity is less relevant in rodents than in humans (95). Additionally, in normal rat myocardium, the α-myosin heavy-chain isoform predominates and a shift towards the β-myosin isoform occurs with hemodynamic load or hormonal changes (96).

Compared with rodents, the hearts of large animals are more similar to human hearts, both in anatomy and

physiology. In the dog myocardium, the β-myosin heavy-chain isoform predominates and excitation–contraction coupling processes seem to be similar to the human myocardium (97). Furthermore, in pigs, the anatomy and distribution of THE coronary arteries are similar to those of humans. The coronary vasculature is right dominant as in 80% of human hearts. Therefore, the pig hydraulic occluder or ameroid model is often used to study ischemic heart disease. Large animals also better allow chronic instrumentation. On the other hand, large animal models are costly and require substantial resources with respect to housing and care.

In addition, huge differences exist among species. The coronary circulation in canines is different from that in other species in that there is a natural collateral circulation between the LAD and the Lcx. While this makes the canine model ideal for revascularization trials on the beating heart, such as endoscopic coronary artery bypass grafting (98), the unpredictable extent of myocardial necrosis following acute coronary occlusion makes it an unreliable model for studying the course of LV dysfunction following MI.

Differences exist not only between species but also between the individuals of single species. It has been reported that the branching and position of the LAD in Sprague-Dawley outbred rats is more heterogeneous than in Lewis inbred rats. Accordingly, LAD ligation resulted in more uniform infarct sizes in the latter, indicating that the use of inbred animals is favourable over that of outbred animals to keep the degree of variance between animals as low as possible (8).

Besides inter- and intra-species specific variability affecting the outcome of animal studies gender-specific effects on HF have to be considered. The cardio-atheroprotective effect of estrogens is well known from various hypercholesterolemic animal models (99). Estrogens lead to a reduction in plasma cholesterol (100), inhibit vessel wall low-density lipoprotein (LDL) accumulation (101) and reduce vascular smooth muscle cell proliferation and extracellular matrix deposition after endothelial damage (102). The targets of estrogens may also include the immuno-inflammatory components of atherogenesis (103). Consequently, an influence of estrogens on the inflammatory processes and remodelling following MI cannot be excluded. Thus, male animals may more easily develop HF secondary to CHD, particularly in atherosclerotic animal models. However, modern gender medicine requires the investigation of both males and females.

Finally, many protocols have a sudden onset of HF due to surgical- or drug-related interventions, whereas human HF will usually develop progressively in a time course of years. Most models also use young, adult animals, whereas HF in humans is a disease of the elderly. In addition, human HF is often associated with atherosclerosis, obesity, diabetes or hypertension, but the development of atherosclerosis is unusual in animals, especially in most strains of rats.

Conclusion

Because of the epidemiological and socioeconomic impact of HF, extensive efforts to counteract this disease are required. Since the understanding of disease mechanisms is essential for the development of efficient therapies, the use of *in vivo* models is indispensable. However, every animal model has advantages and limitations and none of them is suitable for studying all aspects of HF. Besides the technical determinants of a given model, species, strain and gender affect the pathophysiology of the manipulated heart and, therefore, have to be considered when an *in vivo* model is being established. At present, the most widely used animals in cardiology models are the rat and mouse. The mouse in particular has the advantage of offering numerous transgenic and gene-targeted strains. Nevertheless, large animal models provide the advantage of a high degree of anatomical and physiological similarity to humans.

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