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A Novel Mouse Model of Left Ventricular Pressure Overload and Infarction Causing Predictable Ventricular Remodelling and Progression to Heart Failure

Carla J. Weinheimer¹, Ling Lai², Daniel P. Kelly², and Attila Kovacs^{1,*}

¹Center for Cardiovascular Research, Cardiovascular Division, Washington University School of Medicine, St. Louis, Missouri, United States of America

²The Sanford-Burnham Medical Research Institute at Lake Nona, Orlando, Florida, United States of America

Abstract

Mouse surgical models are important tools for evaluating mechanisms of human cardiac disease. The clinically relevant co-morbidities of hypertension and ischemia have not been explored in mice. We have developed a surgical approach that combines transverse aortic constriction (TAC) and distal left anterior coronary ligation (MI) to produce a gradual and predictable progression of adverse left ventricular (LV) remodelling that leads to heart failure (HF). Mice received either sham (SH), MI alone, TAC alone or HF surgery. Infarct size and LV remodelling were evaluated by serial 2-D echocardiograms. TAC gradients were measured by the Doppler velocity time integral ratio between constricted and proximal aortic velocities. At 4 weeks, hearts were weighed and analysed for histology and BNP, a molecular marker of HF. Echocardiographic analysis of segmental wall motion scores showed similarly small apical infarct sizes in the MI and HF groups at day 1 post-surgery. MI alone showed little change in infarct size over 4 weeks (0.26 ± 0.02 to 0.27 ± 0.04 , $p = \text{NS}$), however HF mice showed infarct expansion (0.25 ± 0.06 to 0.39 ± 0.09 , $p < 0.05$). HF mice also revealed progressive LV remodelling with increases in LV volumes ($1d = 36.5 \pm 5.2 \mu\text{l}$, $28d = 89.1 \pm 16.0 \mu\text{l}$) vs. no significant changes in the other groups. Furthermore, systolic function progressively deteriorated in the HF group only (EF, $1d = 55.6 \pm 3.6\%$, $28d = 17.6 \pm 4.1\%$, $p < 0.05$) with an increase of BNP by 3.5 fold. This surgical model of pressure overload in the setting of a small infarction causes progressive deterioration of cardiac structural and functional properties and provides a clinically relevant tool to study adverse LV remodelling and heart failure.

Keywords

experimental model of heart failure; left ventricular hypertrophy; myocardial infarction; echocardiography

*Address for Correspondence: Attila Kovacs, MD, Washington University School of Medicine, Cardiovascular Division, Campus Box 8086, 660 South Euclid Avenue, St. Louis, Missouri, 63110, U.S.A, akovacs@wustl.edu, Phone: (314) 362-8901, Fax: (314) 362-0186.

DISCLOSURES

None.

INTRODUCTION

Despite significant advances in cardiovascular research and clinical therapies, heart failure remains a major public health problem in the United States effecting over 6 million Americans. Heart failure is responsible for approximately 1 million hospitalizations and 300,000 deaths annually (1). This disease state arises from maladaptive progressive cardiac remodelling. The two most common aetiologies for this process include hypertensive and ischemic heart disease. Mouse models of these cardiac conditions have played a major role in advancing our understanding of the molecular mechanisms involved in the ventricular remodelling process. Surgical constriction of the aorta (most commonly the transverse aorta) to impose chronic pressure overload on the left ventricle (LV) is a commonly used animal model to study the molecular pathways involved in the hypertrophic response of the myocardium. However, progression to heart failure using this model is variable and depends on the severity and duration of the constriction, as well as other factors such as mouse strain, age, duration, and location of the band (2). Similarly, surgical ligation of the left anterior coronary artery (LAD) is the model of choice for studying molecular pathophysiologic mechanisms involved in myocardial infarction and subsequent post-infarction LV remodelling. The extent of remodelling is directly related to infarct size and location: small apical infarcts are associated with no or minimal changes in LV shape and function, whereas, moderate to large infarcts result in aneurismal dilatation of an apical scar with markedly impaired cardiac function. These severe conditions routinely induced in animal models are relatively uncommon in current clinical environments where most patients undergo some form of revascularization therapy during the course of their disease.

Clinical evidence shows that the concomitant occurrence of hypertensive and ischemic heart disease may represent an additive risk for adverse cardiovascular events including the development of heart failure (3–5). The simultaneous effects of these comorbidities may involve distinct pathogenic mechanisms compared to pressure overload or myocardial ischemia alone. Studies focused on this important clinical question would be facilitated by the availability of a relevant mouse model. Therefore, we sought to establish a novel surgical model, which combines the application of an aortic constriction and LAD ligation resulting in a gradual and predictable development of pathologic LV remodelling. Importantly, this model has low procedural mortality, a uniform cardiac phenotype, and a high degree of reproducibility.

RESULTS

Study design

We studied four groups of mice: 1) myocardial infarction (MI) alone, 2) aortic banding (TAC) alone, 3) combined myocardial apical infarction plus aortic banding (HF), and 4) sham operation (SH). Sham surgery was identical to the combined procedure, with the exception of tightening of the coronary or aortic ligatures. Postoperatively, mice were studied by non-invasive echocardiography at 1 day, 7 days, 14 days, and 28 days after surgery. After the last echocardiogram, mice were euthanized and hearts were removed. The LV was dissected away from the right ventricle and both were weighed and snap frozen for

tissue analysis. Gravimetric LV weights were indexed to pre-procedural mouse body weights.

Procedural Mortality Post-HF Surgery

A major aim of this study was to establish a new model of progressive adverse remodelling with low mortality. Particularly, since open chest procedures commonly used to produce a heart failure phenotype in rodents, such as proximal LAD ligations and tight aortic banding often cause early death. A total of 23 C57Bl/6 mice were subjected to HF with only 1 death occurring within 24 hours of surgery. This was considered a death from procedural complications, and therefore was not included in the long-term survival figure (Figure 1). No mice were excluded from analysis due to an infarct that was too small or large, or because aortic constriction was not adequate. Post-operative mortality numbers for the 28 days duration of the study included zero deaths in the TAC alone, MI alone, and sham groups; and only 4 mice in the HF group yielding an overall mortality rate of 18%. Of the deaths post-HF, 2 occurred during the first week, 2 during the second week, and none during the remaining two weeks of the study (Figure 1). Therefore, the concomitant surgical application of a small MI and moderate TAC results in a model of heart failure with low surgical and postoperative mortality and no evidence of body weight reduction or clinical signs of congestion.

LV Remodelling Post HF Surgery

We next sought to assess the effects of this procedure on LV remodelling including evaluation of progressive changes in chamber size, shape, function and ventricular mass. Serial echocardiographic studies were used to determine the degree of remodelling for a period of 28 days following surgery (Figure 2).

LV volumes—End-diastolic LV volumes were determined from the parasternal long axis views with echo at 1, 7, 14, and 28 days after surgery in all four experimental groups (Figure 3A). As expected, sham surgery caused no change in LV volumes during the four weeks of the study. Likewise, application of TAC alone resulted in hypertrophied hearts with normal LV chamber sizes, which did not change significantly over the course of the study. Similarly, no significant changes of end-diastolic LV volumes were seen in the group of mice with small apical infarcts alone. This confirms our previous observation that limited myocardial injury (<25% infarct size) does not lead to significant LV remodelling (6). In contrast, concomitant induction of a small LV apical injury and moderate LV pressure overload (HF model) resulted in a marked and progressive increase in end-diastolic LV volume. Specifically, compared to the sham group, LV volumes increased by 80%, 128%, and 154% by 1 week, 2 weeks, and 4 weeks, respectively. Similarly, there was marked increase of the LV end-systolic volumes seen in the HF group at those respective time points ($p<0.05$ for all comparisons). Thus, combined use of LAD ligation and TAC acting synergistically resulted in LV dilatation characteristic of adverse LV remodelling and failure.

LV function—To assess LV function, ejection fraction was determined using 2-D echocardiographic long-axis views by the disc summation method (Figure 3C). Mice with

only aortic banding maintained normal systolic function throughout the study. Animals in the MI only group showed a minimal reduction in ejection fraction without adverse progression when compared with sham and TAC groups. In contrast, mice in the HF group developed progressively worsening LV systolic dysfunction as evidenced by a gradually decreasing ejection fraction (EF) with a relative magnitude decrease of 34%, 46%, and 72% when compared with the sham groups at 1 week, 2 weeks, and 4 weeks, respectively (Figure 3C). With respect to stroke volume, heart rate, and cardiac output (Table), no significant differences were observed among sham, TAC and MI groups. Thus demonstrating that despite significant structural alterations of the LV (hypertrophy in TAC, small apical infarct in MI), these hearts were able to maintain normal hemodynamic performance. In contrast, cardiac output decreased significantly in the HF group at the 4 week time point as the result of significantly diminished stroke volume (Table).

Infarct expansion—Infarct expansion is considered to be the major pathophysiologic mechanism responsible for the early phase of post-MI remodelling. It is driven by increased segmental wall stress, which is a function of infarct size, wall thickness, segmental curvature and LV pressure. 2-D echo has been widely used to characterize major parameters of this process (7). We performed a detailed analysis of infarct size based on a serial short-axis derived global representation of segmental wall motion (Figure 3D). SWSMI (segmental wall motion score index) is a close correlate of infarct size measured by histology (8, 9). In this study, SWSMI confirmed that the MI only group had small apical infarcts, the sizes of which did not change over the course of the study (Figure 3D and Table). Conversely, mice in the HF group began the post-surgical course with the same degree of wall motion abnormalities as those in the MI only group, but developed a significant increase in SWSMI from 1 day to 1 week. This is consistent with the notion that early infarct expansion plays an important role in the adverse remodelling process seen only in mice with combined ischemic injury and pressure overload.

Hypertrophic response

Increased hemodynamic load caused by loss of myocytes or pressure overload elicits a cardiac hypertrophic response that contributes to a pathologic ventricular remodelling process. The pressure gradient induced in the aortic banding model is a major determinant of the hypertrophic response. Therefore, to account for the small but inherent variability in the degree of aortic constriction, as well as the potentially confounding effect of decreased stroke volume on the TAC gradient, we measured Doppler velocity spectra at the site of constriction and at the level of the aortic root and expressed that ratio (VTI). These measurements showed that the VTI ratio in the HF group remained stable over time and was not different from values in the TAC only group (Table). At the conclusion of the study, hearts were harvested and LVs were isolated and weighed. LV weight data from the four experimental groups are shown in Figure 4C. Mice in the MI only group did not exhibit an increase in LV weights, further demonstrating that a small apical infarct is insufficient to trigger a significant hypertrophic response. The moderate pressure overload induced in the TAC only group, on the other hand, resulted in a 38% increase in LV mass in a pattern of concentric hypertrophy. In contrast, mice with combined MI and pressure overload (HF) had 74% greater hypertrophy compared with sham controls, which represents an additional 36%

increase in hypertrophy compared with those in the TAC alone group ($p < 0.05$ for both comparisons, Figure 4C and Table). Figure 4A shows representative cross section images of the LV at the papillary muscle level demonstrating concentric hypertrophy in TAC and marked eccentric hypertrophy in HF. These results provide further evidence that ischemic injury and moderate pressure overload serve as cooperative stimuli for the LV hypertrophic response during the chronic phase of remodelling. Furthermore, the magnitude of hemodynamic changes during LV remodelling is reflected by the extent of neurohormonal changes including release of natriuretic peptides. We measured BNP mRNA levels by quantitative real-time PCR from non-infarcted LV myocardium (Figure 4D). BNP expression was significantly increased in both MI only and TAC only groups compared with sham animals, 1.8-fold and 1.6-fold increase, respectively. In contrast, there was a more than 3-fold increase of BNP expression seen in the HF group ($p < 0.05$ for both comparisons, Figure 4D). In addition, quantitative analysis of picrosirius red-stained basal sections of the LV (remote from the apical infarcts in MI and HF hearts) showed mild increase in collagen deposition in TAC hearts and marked myocardial fibrosis in HF mice (Figure 4B and E) demonstrating that the accelerated pathologic remodelling of HF hearts is associated with an exaggerated fibrotic response of the myocardium.

DISCUSSION

Cardiac remodelling is a progressive process characterized by changes in size, shape and function of the heart (10). The two most common pathophysiologic causes for these changes in humans are myocardial infarction and hypertension (10). Initially, these remodelling changes are an adaptive response to altered loading conditions meant to maintain cardiac output, but ultimately progressive LV dysfunction ensues and the clinical syndrome of heart failure develops. Following myocardial infarction, the loss of viable myocytes sets into motion a process that includes degradation of collagen and other extracellular matrix modifications. The result is thinning and dilatation of the infarcted area, as well as reactive hypertrophy and fibrosis of the non-infarcted myocardium. This in turn leads to global left ventricular dilatation and impaired systolic function (11–13). By contrast, chronic hypertension leads to progressive myocardial hypertrophy, interstitial fibrosis, increased left ventricular stiffness and eventual diastolic and systolic failure (14, 15).

Clinical studies provide ample evidence that the comorbid occurrence of ischemic and hypertensive heart disease poses an incrementally increased risk of cardiovascular morbidity and mortality to patients. A recent meta-analysis involving 17 major studies and over 56,000 participants with MI and antecedent hypertension confirmed previous findings that a history of hypertension in patients with MI is a risk factor for adverse outcomes, including all-cause mortality, cardiovascular mortality, stroke, heart failure, and recurrent MI (3). The precise mechanism for this adverse interaction in patients remains speculative, but increased infarct size, aggravated infarct expansion, and an increased prevalence of multiple other clinical risk factors in this cohort appear to play an important role (3, 16). Furthermore, the pattern of LV hypertrophy is also a major determinant of adverse outcomes, as demonstrated by the echocardiographic cohort of the VALIANT trial showing that concentric remodelling was associated with a 3-fold increase in fatal and nonfatal cardiovascular events following a

high-risk MI (17). LV remodelling remains an important treatment target in patients after MI and with chronic heart failure (10, 15).

The current study sought to model the interaction between two common co-morbid conditions important for the development of heart failure in a novel mouse model. The main characteristics of this HF model are: 1) low procedural mortality, 2) marked, gradual, progressive ventricular dilatation, 3) depressed systolic function, and 4) a uniform and highly reproducible cardiac phenotype.

When examining commonly used rodent models of MI, a number of observations can be made relative to our HF model. The early phase of remodelling in MI is confined to the infarct zone and consists primarily of infarct expansion (18). The main determinants of this process include infarct size and transmural extent, with a large transmural infarct developing greater expansion (6, 16, 19, 20). However, with limited myocardial damage the distending forces are minimal and do not significantly affect infarct healing, scar formation and segmental LV shape. A key feature of our HF model is that, in the presence of pressure overload, the early phase of LV remodelling is exaggerated because increased segmental wall stress leads to disproportionate infarct expansion relative to the size of myocardial injury. Late remodelling after MI involves the LV globally and is associated with progressive dilatation, distortion of ventricular shape, and hypertrophy of the myocardium remote from the site of injury. These changes are historically observed in hearts with moderate and large infarcts. Yet, the results of the current study demonstrate that sustained pressure overload exerts a deleterious effect on late LV remodelling even in hearts with small infarcts because the distending forces exceed the restraining forces of remote myocardium, resulting in progressive LV dilatation. The presence of this imbalance is further supported by the fact that, in the HF group, BNP activation and myocardial fibrosis are even more pronounced than in the MI alone and TAC alone groups. Therefore, this model is more akin to the typical individual with chronic hypertension in the context of a small or “rescued” ischemic event.

When comparing key features of the commonly used aortic banding models (eg. long term TAC) to our findings with the HF model, the following observations can be made. First: The hypertrophy phenotypes of the aortic banding models depend on the site, severity and duration of constriction (21, 22). Mild and moderate degree aortic stenosis induces myocardial hypertrophy with preserved systolic function over time, while severe constriction of the aorta during TAC can lead to LV failure but at the cost of high mortality and acute tissue damage from stretch injury (23). In the current study we implemented a moderate constriction of the transverse aorta using a 26 gauge constrictor (instead of the commonly used 27 gauge needle for this mouse weight). The goal was to achieve a moderate band. As with other models of moderate aortic constriction, our TAC only group developed significant concentric hypertrophy but LV function remained normal up to 4 weeks. Second: In contrast to the common constriction models, this study implemented the same degree of moderate pressure overload, but in combination with a small MI (<25%), which resulted in marked adverse remodelling and myocardial dysfunction. In addition, LV mass was disproportionally higher than expected for the level of pressure overload. These results suggest that the addition of a small degree of ischemic injury can significantly

accelerate ventricular decompensation in the aortic band model. This outcome is likely because LV dilatation is associated with concomitant LV distortion, which results in a mechanically disadvantageous shape, increased regional wall stress and compromised ventricular ejection performance (18). Thus, exposing the LV to a combination of a small loss of contractile muscle mass and a moderate level of pressure overload, neither of which is sufficient in isolation to trigger adverse remodelling, will synergistically lead to a progressively maladaptive process. This process involves a precarious balance destined to be disrupted with the passage of time, causing increased cavity volumes with insufficient compensatory hypertrophy. This evolution leads to loading conditions which promote further myocardial enlargement and dysfunction (7), i.e. “left ventricular dilatation begets left ventricular dilatation” (18).

In recent years the mouse has become the mammalian species of choice for studying the molecular basis of cardiovascular diseases. Parallel with the use of sophisticated gene targeting techniques there have been extraordinary accomplishments made in applying miniaturized phenotyping techniques to characterize the effects of molecular manipulation on cell, tissue and organ function. These developments require the constant re-evaluation of known mouse models and the development of new ones that approximate the human condition to the greatest possible extent. To our knowledge, this is the first description of the simultaneous use of aortic constriction and LAD ligation in mice.

Combined use of ischemic injury and pressure overload has been previously described in other species. Dog studies performed mainly in the 1980's have established the deleterious effect of hypertension on infarct size, infarct expansion, and post-infarct remodelling (24, 25). Spawned largely by these and similar investigations, the beneficial effect of chronic afterload reduction post-MI has been established clinically. ACE inhibition has become the mainstay of pharmaceutical therapy in the prevention and treatment of adverse post-MI remodelling.

In rats, the combination of MI and aortic banding, either at the ascending (AscAC) or the abdominal level (AbdAC), has been described and utilized in multiple studies. In the first description of the model in rats (16), Nolan et al showed a low level of mortality in both MI only and the AscAC/MI groups (27% and 13%, respectively, $p=NS$). Infarct size was significantly increased in the AscAC/MI group compared with the MI only group (39% vs. 28%, respectively). In morphometric analysis, they demonstrated greater infarct expansion as evidenced by increased wall thinning and increased LV volumes compared to hearts with MI or AscAC alone (16). Results of our study are in line with these findings. In a study combining the use of AbdAC with MI, Linz et al. found high postoperative mortality in the hypertensive rats undergoing MI (68%), even though infarct size was small and aortic pressure was only moderately increased (26). The reason for this high mortality is not entirely clear. In a separate study using simultaneous AbdAC/MI, Anthonio et al. demonstrated increased infarct size, impaired LV function, and exaggerated activation of the neurohormonal system compared with infarct only or aortic banding only groups (27). Again, the current study confirms these findings, and is now presented in a mouse model amenable to genetic manipulation. Most recently, in a distinctly different but related model, Chen J et al. (28) used an elegant modification of concomitant hypertrophic and ischemic

stimulus. They sequentially combined LV pressure overload (AscAC), 30 min ischemia followed by reperfusion, and subsequent removal of the aortic band, and demonstrated that sufficiently large and prolonged ischemic injury in combination with a sufficiently long hypertrophic challenge resulted in adverse LV remodelling even after the inciting stimuli were no longer present (28).

In summary, the results from this study indicate that a small ischemic insult in the face of pressure overload synergistically produces a model of heart failure, which is characterized by a gradual and progressive LV dilatation, impaired systolic function, and activation of hypertrophic genes. This novel surgical model combining the use of the two most common aetiologies for myocardial dysfunction will provide a useful platform to investigate cellular and molecular mechanisms of adverse LV remodelling in mice and to explore new heart failure therapies.

MATERIALS AND METHODS

Animals

Eight week old female C57Bl/6 mice (Jackson Labs) were used. All experiments were performed according to the recommendations of the Guide for the Care and Use of Laboratory Animals, 8th Edition (National Research Council. *Guide for the Care and Use of Laboratory Animals: Eighth Edition*. Washington, DC: The National Academies Press, 2011) and were approved by the Animal Care and Use Committee at Washington University.

Surgical procedures

Adult female mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg) administered i.p. A total of 44 C57Bl/6 mice weighing 18.6 $\pm$ 2.1 g were divided into 4 groups: TAC alone, n=6; MI alone, n=6; HF n=23; and SH, n=9. To perform the HF surgery, mice were restrained supine, intubated, and ventilated using a Harvard respirator. Following dissection through the intercostal muscles, the aorta was identified and freed by blunt dissection. 7.0 silk suture was placed around the great vessel, tied around a blunt 26 gauge needle and then rapidly removed (29). Immediately following this procedure, a left thoracotomy was performed, the LV and the left main coronary artery system exposed, and the apical portion of the LAD ligated with a 9-0 silk suture. The surgical incision was closed and the mice were recovered on a warmer until being returned to their cage. Total time to perform this dual surgical procedure was less than 15 minutes.

Echocardiographic studies

Image acquisition—Ultrasound examination was performed using a Vevo 770 Ultrasound System (VisualSonics Inc, Toronto, Ontario, Canada). Mice were lightly anesthetized with an i.p. injection of 0.005 ml/g of 2% Avertin (2,2,2-Tribromoethanol, Sigma-Aldrich, St. Louis, MO). If required, one-fifth of the initial dose was given as a maintenance dose at regular intervals. Hair was removed from the anterior chest using chemical hair remover, and the animals were placed on a warming pad in a left lateral decubitus position to maintain normothermia (37°C), monitored by a rectal thermometer.

Ultrasound gel was applied to the chest. Care was taken to maintain adequate contact while avoiding excessive pressure on the chest. Two-dimensional and Doppler images were obtained by hand-held manipulation of the ultrasound transducer. Complete 2-D long-axis, serial short-axis views (for segmental wall motion score index, SWMSI (8, 9), and Doppler ultrasound examination of the TAC gradient (30) were performed using multiple views. After completion of the imaging studies mice were allowed to recover from anaesthesia and returned to their cages.

LV structure and function analysis—Parasternal long-axis views of the LV were used to measure LV volumes by the disk summation method (31). End-diastolic and end-systolic digital image frames were retrieved off-line on a computer workstation and the LV endocardial silhouette was manually traced using VisualSonics software package (Figure 2). Ejection fraction was calculated as [(end-diastolic volume - end-systolic volume) / end-diastolic volume] x 100 (%).

Quantification of myocardial infarction—Non-invasive evaluation of infarct size was performed by slight modifications of previously validated methods (8, 9, 6). Briefly, 2-D short-axis movie loops of the LV were acquired from base to apex by manually advancing the transducer by 1 mm increments yielding 7–9 movie loops depending on the size of the LV. The extent of abnormal wall motion was assessed visually on each of the short-axis images by dividing the circumference of the short-axis image slices into 12 segments and counting the number of akinetic segments. To derive the SWMSI, the sum of these values from each slice was divided by the total number of segments (12 x the number of short-axis slices). This is a close correlate of histological infarct size (8, 9).

Determination of TAC gradient—Non-invasive evaluation of the pressure gradient across TAC was performed by Doppler echocardiography. The aortic arch along with the surgically constricted transverse aorta was imaged from the right upper parasternal view. Under 2-D guidance, pulse wave Doppler sample volume was placed at the site of the constriction and adjusted so that the highest velocity blood flow jet could be recorded. To account for the potential effect of decreased cardiac output in HF mice, aortic flow velocity was also measured proximal to the constriction at the level of the aortic root. These velocity spectra were digitally traced and velocity time integral (VTI), mean and peak gradients were measured. The ratio of distal VTI/proximal VTI was calculated as an index of TAC gradient across the band.

Histologic Analysis

After completion of the study, hearts were removed, weighed and cut into 5 short-axis slices with orientation corresponding to echo images. The slices were laid into cassettes in the correct orientation and fixed in 10% formalin for further processing. Hearts were processed on an extended time processing schedule using an automated (Tissue-Tek) tissue processor. Hearts were then paraffin-embedded, sliced with a manual microtome (Leica) and stained with haematoxylin/eosin and picrosirius red for histological and pathological examination. Myocardial collagen content was quantified from picrosirius red-stained serial sections

using colour thresholding by ImageJ software. Data were expressed as average percentage of 40 fields.

Quantitative Real-Time PCR

Brain natriuretic peptide (BNP) mRNA levels were measured by quantitative real-time PCR of cDNA as described (32). In brief, total RNA was extracted from non-infarcted LV myocardium consistently taken from the basal segments and reverse transcribed with TaqMan reverse transcription reagents (Applied Biosystems). PCR reactions were performed in triplicate in a 96-well format using a Prism 7500 Sequence Detector (Applied Biosystems). Mouse-specific primer-probe set for BNP was used to detect specific gene expression. Actin (Applied Biosystems) primer-probe set was included in a separate well (in triplicate) and used to normalize the gene expression data.

Data analysis

Data are presented as means $\pm$ standard deviation of the mean. One-way analysis of variance for repeated measurements was used for overall comparison of repeated measurements and One-Way ANOVA followed by post-hoc Tukey's Multiple-Comparison Test was performed to compare experimental groups. $P < 0.05$ was considered statistically significantly.

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Figure 1. Long-term survival in the HF model

Kaplan-Meier curves depict survival data over the course of 28 days of post-operative remodelling. There was a small but significantly increased mortality seen over time in the HF group, whereas the control groups of TAC alone, small MI alone and Sham operation had 100% survival. * $p < 0.05$ compared with Sham.

Figure 2. Echocardiographic appearance and analysis of LV remodelling in HF mice
Representative parasternal long-axis views from an HF mouse depict end-diastolic (top) and end-systolic (bottom) 2-D images of the LV at 1, 7, 14, and 28 days post-surgery, respectively. Endocardial borders are highlighted in white. LV volumes were calculated by disc summation method. Images demonstrate progressive LV dilatation and deterioration of LV function consistent with adverse LV remodelling over time.

Figure 3. Progressive LV remodelling in the HF model measured by non-invasive serial echocardiography

LV volumes: line graphs in upper panels depict changes in LV end-diastolic (A) and end-systolic (B) volumes among the 4 experimental groups over the course of 28 days. Data show marked and progressive LV dilatation in the HF group over time, reaching more than twice the LV chamber size by the end of the study when compared with the control groups.

LV function: line graph in lower left panel (C) shows LV EF values measured in the 4 experimental groups at 1, 7, 14, and 28 days post-surgery and demonstrates a progressive decline in LV systolic function following HF surgery. EF decreased to 1/3 of the control level by the end of the study, while there were no significant changes seen in the Sham and TAC alone groups and only a minimal decline detected in the MI only group at the 28d time point. Panel D shows evidence of significant infarct expansion in the HF group only, measured by the SWMSI. * $p < 0.05$ compared with Sham for all comparisons.

Figure 4. Hypertrophic response in the HF model

A) Representative haematoxylin/eosin stained mid-LV cross sections show concentric hypertrophy in TAC and marked eccentric hypertrophy in HF hearts. **B)** Representative picrosirius red stained basal LV sections show mild collagen deposition in TAC and marked myocardial fibrosis in HF hearts. **C)** Bar graph depicts values for isolated LV weights normalized to body weights across the 4 experimental groups obtained at the conclusion of the study. LV weight index significantly increased in the TAC alone group, and there was a further significant increase seen in the HF group. **D)** Bar graph demonstrates myocardial BNP expression obtained from hearts at study end. BNP expression was significantly increased in TAC alone and MI alone groups, but there was a further significant increase detected in the HF group. * p<0.05 compared with Sham; † p<0.05 compared with TAC or MI. **E)** Bar graph shows quantitative analysis of collagen area fraction across the 4 experimental groups. Myocardial fibrosis mildly but significantly increased in the TAC alone group, and there was a further marked increase seen in the HF group.

TABLE
Evaluation of LV structure and function during remodelling in HF model by serial echocardiography and gravimetry.

	Sham (n=9)			TAC (n=6)			MI (n=6)			HF (n=23)		
	1d	7d	14d	28d	1d	7d	14d	28d	1d	7d	14d	28d
EDV	37±3	36±3	35±3	35±2	37±3	32±4	32±4	30±6	39±3	36±3	36±4	38±4
ESV	15±2	13±2	13±2	12±1	15±2	11±3	12±2	11±3	17±1	16±2	17±2	19±3 [*]
EF	60±2	65±3	64±3	64±4	59±3	65±3	63±3	64±2	55±4	56±3	52±3	49±3 [*]
SV	22±2	23±3	22±2	23±3	22±3	21±4	20±3	19±4	22±2	20±2	19±2	19±2
HR	604 ±18	610 ±20	630 ±17	640 ±22	615 ±28	622 ±25	605 ±24	625 ±19	599 ±30	611 ±28	630 ±22	621 ±31
CO	13±2	14±2	14±2	15±2	13±1	13±2	12±2	12±2	13±2	12±2	12±2	12±3
SWMSI	0	0	0	0	0	0	0	0	0.26 ±0.02	0.28 ±0.03	0.27 ±0.05	0.27 ±0.04
VTI Ratio	0.53±0.09	0.58±0.05	0.51 ±0.02	0.51 ±0.07	4.30 ±0.32	4.55 ±0.37	4.79 ±0.41	4.66 ±0.31	0.54 ±0.06	0.59 ±0.02	0.57 ±0.08	0.60 ±0.03
LVMI				3.4±0.5				4.7±0.5 [*]				3.7±0.1
										0.35 ±0.08 [*]	0.37 ±0.07 [*]	0.39 ±0.09 [*]
										4.43 ±0.31	4.55 ±0.35	5.02 ±0.25
												5.9±0.7 ^{†‡}

EDV: end-diastolic LV volume (μl); ESV: end-systolic LV volume (μl); EF: LV ejection fraction (%); SV: LV stroke volume (μl); HR: heart rate (bpm); CO: cardiac output (ml/min); SWMSI: LV segmental wall motion score index; VTI Ratio: velocity time integral ratio of TAC over proximal aortic flow velocities; LVMI: LV mass index (mg/g). Values are means ± SD.

^{*} p<0.05 compared with Sham;

[†] p<0.05 HF compared with TAC.