Monitoring reperfused myocardial infarction with delayed left ventricular systolic dysfunction in rabbits by longitudinal imaging

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Background: An experimental imaging platform for longitudinal monitoring and evaluation of cardiac morphology-function changes has been long desired. We sought to establish such a platform by using a rabbit model of reperfused myocardial infarction (MI) that develops chronic left ventricle systolic dysfunction (LVSD) within 7 weeks.

Methods: Fifty-five New Zealand white (NZW) rabbits received sham-operated or 60-min left circumflex coronary artery (LCx) ligation followed by reperfusion. Cardiac magnetic resonance imaging (cMRI), transthoracic echocardiography (echo), and blood samples were collected at baseline, in acute (48 hours or 1 week) and chronic (7 weeks) stage subsequent to MI for in vivo assessment of infarct size, cardiac morphology, LV function, and myocardial enzymes. Seven weeks post MI, animals were sacrificed and heart tissues were processed for histopathological staining.

Results: The success rate of surgical operation was 87.27%. The animal mortality rates were 12.7% and 3.6% both in acute and chronic stage separately. Serum levels of the myocardial enzyme cardiac Troponin T (cTnT) were significantly increased in MI rabbits as compared with sham animals after 4 hours of operation (P<0.05). According to cardiac morphology and function changes, 4 groups could be distinguished: sham rabbits (n=12), and MI rabbits with no (MI_NO_LVSD; n=10), moderate (MI_M_LVSD; n=9) and severe (MI_S_LVSD; n=15) LVSD. No significant differences in cardiac function or wall thickening between sham and MI_NO_LVSD rabbits were observed at both stages using both cMRI and echo methods. cMRI data showed that MI_M_LVSD rabbits exhibited a reduction of ejection fraction (EF) and an increase in end-systolic volume (ESV) at the acute phase, while at the chronic stage these parameters did not change further. Moreover, in MI_S_LVSD animals, these observations were more striking at the acute stage followed by a further decline in EF and increase in ESV at the chronic stage. Lateral wall thickening determined by cMRI was significantly decreased in MI_M_LVSD versus MI_NO_LVSD animals at both stages (P<0.05). As for MI_S_LVSD versus MI_M_LVSD rabbits, the thickening of anterior, inferior and lateral walls was significantly more decreased at both stages (P<0.05). Echo confirmed the findings of cMRI. Furthermore, these in vivo outcomes including those from vivid cine cMRI could be supported by exactly matched ex vivo histomorphological evidences.

Conclusions: Our findings indicate that chronic LVSD developed over time after surgery-induced MI in rabbits can be longitudinally evaluated using non-invasive imaging techniques and confirmed by the entire-heart-slice histomorphology. This experimental LVSD platform in rabbits may interest researchers in the field of experimental cardiology and help strengthen drug development and translational research for the
Introduction

Myocardial infarction (MI) is the leading cause of morbidity, mortality, and disability worldwide and remains one of the greatest challenges in biomedical research (1). The evaluation of cardiac dysfunction after MI is essential for the prediction and diagnosis of heart failure (HF). The common fundamental defect in cardiac dysfunction after MI is a gradually decreased ability of the heart to provide sufficient cardiac output to support the normal functions of organs due to impaired ejection of the left ventricle and blood perfusion to tissues. Severe left ventricle systolic dysfunction (LVSD) will lead to irreversible congestive HF. Therefore, LVSD is most frequently studied to elucidate the physiological and pathophysiological mechanisms of HF and to develop new diagnostic and therapeutic approaches for HF after MI (2-5). Several factors such as infarct size, LV remodeling, stunned or hibernating myocardium, and mechanical complications, may influence the appearance of LVSD after MI (6-8). Among these factors, ventricular remodeling is the most important (8). The process of ventricular remodeling refers to alterations in ventricular architecture, associated with an increased volume and an altered chamber configuration, which are driven by a combination of pathologic hypertrophy and apoptosis of myocytes, myofibroblast proliferation, and interstitial fibrosis (9). Ventricular remodeling is directly implicated in post-infarction development of ventricular dilation, a process that can influence LV function and survival outcomes (10). Multiple imaging approaches have been used to better understand the structural and molecular changes that underlie the progression of LV remodeling from myocardial injury to MI and, ultimately, to congestive HF. Advances in cardiovascular imaging have come at a rapid pace over the last several years (11). Cardiac magnetic resonance imaging (cMRI) and speckle-tracking imaging with ultrasound echocardiography (Echo) have been widely used to monitor LV remodeling after MI. These techniques can (I) provide specific quantitation of cardiac function, accurate chamber volumes and structure, myocardial viability and coronary perfusion in both acute and chronic settings and, (II) exclude mechanical complications (11-14), though often lacking gold standard histopathology proofs.

In translational cardiology, development of appropriate animal models for non-invasive evaluation of cardiac function is important to understand mechanisms of cardiac remodeling, and to provide new therapeutic strategies. Surgical complete coronary ligation to induce irreversible myocardium damage and subsequent remodeling has been extensively used and described (15-17), but often with high mortality due to severe ventricular tachycardia and ventricular fibrillation in the acute phase (18-20). While small animal MI models in rats and mice (19,21) display marked differences compared to the human heart (22), larger animals such as the dog, sheep and swine are very labor intensive and expensive (23,24). We experienced that the rabbit coronary occlusion/reperfusion model meets the requirements of the ideal experimental model (25). A medium sized rabbit heart has many similarities to the human heart in terms of cardiovascular anatomy, ventricular performance, cardiac metabolism, electrophysiology, coronary artery distribution, and collateralization after an acute event. In addition, with the lower phylogenetic scale, longer life span, strain-specific characteristics and low cost, the rabbit is a suitable species for cardiac research (13,25,26). Technically, the dimension of a rabbit heart is large enough to study with clinical imaging scanners and small enough to just fit in a standard glass slide for entire-heart-slice histomorphologic studies, both of great translational significance.

In the clinic, approximately 40% of MI patients suffer from LVSD (8). However, a good animal model of LVSD has not been proposed thus far. Most experiments have focused on acute or subacute MI over hours and days, but not on a MI model that systematically progresses to chronic LVSD. In this study, we established a rabbit model of reperfused MI that evolves to chronic LVSD.
over the time course of 7 weeks, and developed an imaging platform for longitudinal monitoring and evaluation of cardiac morphology and contractile function. This experimental platform combines in vivo cardiac MRI and echocardiography with postmortem immunohistochemical analysis.

Methods

Experimental protocol

All animal procedures were approved by the Ethical Committee of the KU Leuven. Fifty-five New Zealand white male rabbits (3–4 kg) were obtained from the Animal Center of KU Leuven (Heverlee, Belgium). Animals were randomized into two groups: one group was subjected to open-chest surgery without manipulation of the heart (sham group), while the other group received induction of MI (MI group). To monitor cardiac function, cMRI and echocardiography were performed at baseline, 48 h (cMRI only), 1 week (echo only) and 7 weeks post MI on each animal. After 7 weeks, rabbits were killed for further multiple histological processings.

MI model

All surgical procedures were performed in a sterile manner in the animal operating suites. The rabbit model of acute reperfused MI was previously described in details (13). Briefly, rabbits were sedated, endotracheally intubated and mechanically ventilated. After intravenous (iv) access was established, rabbits received 40 mg/kg/h sodium pentobarbital to maintain anesthesia. After disinfection of the chest, skin and subcutaneous tissues were cut open by layers along the left sternal border. Subsequently, the 4th and 5th intercostal space cartilages were cut and the pericardium was opened to expose the left circumflex artery branch (LCx). The LCx was ligated by a detachable knot using 2-0 silk at 2 mm below the left atrial appendage, of which the pullable end was left outside the thorax after closure of the thoracic cavity by layered suture. Reperfusion was induced by pulling the exteriorized end of the suture in a closed-chest condition after 1 h of coronary occlusion. Similar procedures were applied for sham-operated animals, except for the LCx ligation. The pericardium was opened to expose the left circumflex artery branch (LCx). The LCx was ligated by a detachable knot using 2-0 silk at 2 mm below the left atrial appendage, of which the pullable end was left outside the thorax after closure of the thoracic cavity by layered suture. Reperfusion was induced by pulling the exteriorized end of the suture in a closed-chest condition after 1 h of coronary occlusion. Similar procedures were applied for sham-operated animals, except for the LCx ligation. In the event of sustained ventricular fibrillation during coronary occlusion or reperfusion, animals were given 2% XYLOCAINE (1 mg/kg iv; lidocain, Eurovet Animal Health B.V.). After reperfusion, animals were allowed to recover on a warming blanket and were ventilated further until their own respiration took over.

Serum cardiac troponin T (cTnT) measurements

To determine the optimal time point of peak cTnT concentrations, serum samples at different time points post MI (baseline, 30 min, 1, 2, 4, 8 and 24 h) from a rabbit were collected from the ear vein without anesthesia. Serum cTnT levels were evaluated using a routine laboratory assay.

Cardiac magnetic resonance imaging

Using a 16-channel phased array knee coil, cMRI was performed on the anesthetized rabbit at a 3.0T clinical Siemens MRI scanner (Trio, Siemens, Erlangen, Germany) with a maximum gradient capability of 45 mT/m, triggered by ECG and gated by respiration using a small animal monitoring and gating system (SA Instruments, Inc. Stony Brook, NY, USA). The two ECG electrodes were attached to the shaved thorax skin with an apical pulse and to the left leg. The respiration sensor was attached to abdomen of the rabbit, which was placed supinely in a holder and gas-anesthetized with 2% isoflurane in the mixture of 20% oxygen and 80% room air, through a mask connected via a tube to a ventilation instrument (Harvard Apparatus, Holliston, MA, USA). All images were acquired during free breathing of the animal. Eight short-axial slices of the heart were collected with a slice thickness of 3.0 mm without gap to cover the entire LV. Turbo spin echo sequence of black blood imaging was applied for cardiac morphology with the parameters: TR of 621–750 ms, TE of 15–74 ms, FOV of 240×195 mm², FA of 180°, and in-plane resolution of 0.9×0.9 mm². The cine-MRI images were acquired on gradient echo in the short-axis, vertical long-axis and horizontal long-axis planes for displaying cardiac contraction. Each cine-MRI consisted of 25 frames/cycle, and the scan parameters: TR of 23 ms, TE of 3.7 ms, FOV of 240×195 mm², FA of 12° and spatial resolution of 1.3×0.9 mm². The delayed contrast enhancement (CE) images were acquired by a 3D segmented k-space inversion recovery turbo fast low angle shot sequence 20 minutes after an iv bolus injection of meglumine gadoterate (Gd-DOTA, Dotarem®, Guerbet, France) at 0.2 mmol/kg with parameters: TR of 396 ms, TE of 1.54 ms, TI of 360 ms, FOV of 240×180 mm², FA of 15° and in-plane resolution of 1.1×0.8 mm².
Analysis of cMRI

cMRI images were read using an off-line workstation with dedicated software (SyngoMR A30, Siemens). The assessment and quantification of MI size and global LV function in CE and Cine-MRI images were made using the software SEGMENT (Medviso AB, Lund, Sweden). The endocardial and epicardial borders were manually traced in the end-diastolic and end-systolic short-axis Cine images. Papillary muscles were included in the myocardium. Global LV functions including end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), ejection fraction (EF), cardiac output (CO) and mass were measured according to standard methods (4). Regional LV function was also assessed by measuring wall thickening from end-diastolic phase to end-systolic phase in six clockwise sectors on the mid-ventricle section of Cine images.

Transthoracic echocardiography

Transthoracic echocardiographic examinations were performed on anesthetized rabbits using a 10S transducer (4.4–10 Mhz) (GE Healthcare, Machesen, Belgium) on a Vivid 7 ultrasound machine (GE Healthcare). LV internal diameter at end-diastolic (LVIDd) and end-systolic phase (LVIDs), muscle thickness in diastole (IVSd) and in systole (IVSs) and LV posterior wall thickness in end-diastole (LVPWd) and end-systole (LVPWs) were measured at three levels: at the level of the mitral valve (mv), papillary muscle (pm) and apex (ap). In addition, the long axis diameter in end-diastole (LAXd) and systole (LAXs) was obtained. EDV [EDV = (LVIDd_mv² + LVIDd_pm² + LVIDd_ap²) xLAXdxII/18] and ESV [ESV = (LVIDs_mv² + LVIDs_pm² + LVIDs_ap²) xLAXdxII/18], EF [EF = [(EDV – EDV)/EDV] x100], and SV (SV = EDV – ESV) were calculated (27). The measurements for all parameters at 1 and 7 weeks post-surgery are reported as a percentage of the baseline measurement for each animal. Subsequently, an average per group was calculated for each parameter.

Histomorphology

After in vivo data acquisition, rabbits were euthanized with an overdose of sodium pentobarbital. The isolated heart and left lung were photographed, and then fixed with 10% formalin for 24 h. The fixed heart was cut into 3 mm short-axis sections, which were paraffin-embedded and cut into 5 µm slices. The heart slices were mounted entirely on standard glass slides, followed by hematoxylin-eosin (HE) and Masson Trichrome (MT) staining for necrosis and chronic fibrosis evaluation. Myocardial macrophage infiltration was evaluated by RAM-11 staining, using a mouse monoclonal anti-rabbit macrophage clone (1/50; M0633; DAKO, Leuven, Belgium) in Tris-NaCl-blocking buffer (TNB) (Perkin Elmer, Boston, USA). RAM-11 signals were subsequently detected with the Tyramide Signal Amplification kit (Perkin Elmer). In addition, the dissected lung was photographed and a small portion of fixed pulmonary tissue was paraffin-embedded and cut into 5 µm slices for HE staining to observe the presence or absence of HF-induced pulmonary congestion.

Statistical analysis

Data are shown as means ± SD for the number of animals studied. Differences between all groups were analyzed using the parametric one-way ANOVA. If a statistical difference was detected (P<0.05), the difference between the individual groups was determined using the Tukey’s multiple comparison test. Correlation analyses between serum cTnT levels and LV infarct sizes and between EF measurements of MRI and Echo were performed using the non-parametric Pearson correlation test. All statistical analyses were performed with GraphPad Prism 6 software (GraphPad, La Jolla, CA, USA).

Results

General characteristics of the rabbits

In 48 out of 55 rabbits, surgery was successful (87.3%). Mortality rate was 12.7% (7/55) or 3.6% (2/55) for the acute or chronic stage respectively. Four groups were distinguished: sham (n=12), MI_NO_LVSD (n=10), MI_M (moderate)_LVSD (n=9), and MI_S (severe)_LVSD (n=15). LVSD and severe LVSD developed in 71% (24/34) and 44% (15/34) respectively of MI animals after 7 weeks.

Body weight was not significantly different between the groups at the start, at 1 and 7 weeks post-surgery (Table 1). Serum cTnT levels were the highest in MI_S_LVSD rabbits and were significantly different from that of sham, MI_NO_LVSD and MI_M_LVSD animals (Table 1). Serum cTnT levels correlated positively with LV infarct size at the acute stage (r=0.89; P<0.0001), while they were negatively correlated with EF (r=-0.95; P<0.01).
Cardiac MRI

MI sizes were less than 20% of the LV in MI_NO_LVSD, 20–42% of the LV in MI_M_LVSD and 43–54% of the LV in MI_S_LVSD (Table 1). Chronic MI sizes by cMRI were smaller than acute MI sizes, and were better correlated with MI sizes determined by histology (r=0.93 versus r=0.86, respectively). As shown in Figure 1, longitudinal evaluation by in vivo CE-MRI of mid-ventricular slices at 48 hrs and at 7 weeks post MI (Figure 1A,B,C,D,E,F,G,H) revealed that the hyper-enhanced MI region in the MI_S_LVSD animals extended to the anterior, lateral and posterior wall at the acute stage (Figure 1D). This range persisted, however the wall became thinner 7 weeks (Figure 1H) post-surgery for MI_S_LVSD rabbits in comparison to the other groups. The corresponding cine images for the MI_S_LVSD (Figure 1L,P) rabbits indicated that the LV dilated and wall motion decreased, as can be demonstrated vividly by the videos of cine cMRI (Figure S1).

For regional cardiac function measured by six segments, there were no significant changes of LV wall thickening between sham and MI_NO_LVSD animals at both experimental stages (Table 2). Wall thickening significantly decreased to about 44% and 42% at the acute and chronic stage, respectively (P<0.05 for each) in only two cardiac segments (inferolateral and anterolateral) of MI_M_LVSD rabbits. But, wall thickening significantly decreased (25–38% at the acute stage; 25–41% at the chronic stage; P<0.05 for each) in up to four cardiac segments (anterior, inferior, inferolateral and anterolateral) of MI_S_LVSD rabbits, as compared to other groups (Table 2).
Echocardiography

Echocardiographic analysis revealed that rabbits with the largest LV infarct size (MI_S_LVSD group) developed a severe cardiac dysfunction 1 and 7 weeks post MI, as indicated by a significant EF reduction as compared to the other groups (Table 3). Acutely, hearts of these rabbits showed a significant increase in ESV as compared to MI_
Figure 2 Bar charts of cardiac functions evaluated by in vivo cine cMRI after the induction of a reperfused MI in rabbits. Data are presented as means ± SD for sham (n=12), MI_NO_LVSD (n=10), MI_M_LVSD (n=9) and MI_S_LVSD (n=15) rabbits. Global functional parameters in panels A-D and % changes of parameters to baseline in panels E-H are shown for baseline, 48 hours (acute stage) and 7 weeks (chronic stage) post MI surgery, respectively. *P<0.05 versus sham; †, P<0.05 versus MI_NO_LVSD; ‡, P<0.05 versus MI_M_LVSD; *, P<0.05 versus baseline and ‡, P<0.05 versus the acute stage according to the parametric one way ANOVA statistical test and Tukey’s multiple comparison test. EF, ejection fraction; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume.
NO_LVSD rabbits, while EDV was not different. This resulted in a significant reduction of the SV in rabbits with severe L VSD versus the other three groups (Table 3). At the chronic stage both EDV and ESV were increased and EF significantly reduced in MI_S_L VSD rabbits as compared to the other animals. A strong positive correlation was found between EF determined by echocardiography and by cMRI at both time points, but the correlation was even stronger at 7 weeks as compared to 1 week post MI surgery (Figure 3).

For MI_S_LVSD rabbits LVID in both phases was also significantly enhanced as compared to the other groups (Table 3). In addition, these animals had significant thinning of the posterior wall at the papillary muscle level in the chronic phase as compared to the other groups; however, this change was more pronounced in the end-systolic phase as compared to the end-diastolic phase (Table 3).

Figure 4 illustrates the echocardiographic changes as shown in Table 3. For sham-operated and MI_NO_LVSD animals, LV volume and wall thickness did not change over time. Hypokinesis and akinesis of the posterior wall was observed for rabbits with a large infarct area (MI_M_LVSD and MI_S_LVSD) at the acute stage. At the chronic stage hypokinesis and akinesis was more severe for the MI_S_LVSD group. MI_S_LVSD rabbits showed the highest dilation of the heart and extreme thinning of the posterior wall in the end-diastolic (Figure 4W) and end-systolic phase (Figure 4X) as compared to the other groups at the chronic stage.

### Postmortem histomorphology

Postmortem heart sections of the four animal groups revealed ventricular remodeling to different degrees among three MI groups (Figure 5). The most extensive infarct lesion was present in MI_S_LVSD animals (whitish region in Figure 5D). The infarct lesion was substantially smaller for animals with moderate (Figure 5C) or no L VSD (Figure 5B) relative to no infarct in sham animal (Figure 5A). The LV cavity was dilated, the thickness of lateral wall was decreased and the papillary muscles were atrophied in rabbits with MI_S_LVSD (Figure 5D) relative to the other groups. The transverse heart slices (Figure 5A,B,C,D), size of the infarct lesion, dilation of the LV cavity and lateral wall thickness corresponded well in-between the macroscopic views of the HE (Figure 5E,F,G,H), MT (Figure 5I,J,K,L) and RAM-11 (Figure 5M,N,O,P) stained heart sections. The isolated wet lung lobes gradually turned dark red from sham animals to rabbits with MI_S_LVSD due to a different degree of pulmonary congestion (Figure 5Q,R,S,T), confirming our findings in the heart.

### Table 2 Longitudinal evaluation of left ventricular wall thickening by cMRI in rabbits with no (MI_NO_LVSD), moderate (MI_M_LVSD) or severe left ventricle systolic dysfunction (MI_S_LVSD) after sham-surgery or with reperfused MI

<table>
<thead>
<tr>
<th>Wall thickening (%)</th>
<th>Anterior</th>
<th>Anteroseptal</th>
<th>Inferoseptal</th>
<th>Inferior</th>
<th>Inferolateral</th>
<th>Anterolateral</th>
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<tbody>
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<td>Sham</td>
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<tr>
<td>48 hours post MI</td>
<td>66±4.8</td>
<td>66±4.3</td>
<td>67±4.0</td>
<td>67±5.6</td>
<td>72±6.8</td>
<td>74±6.8</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>68±5.2</td>
<td>68±8.5</td>
<td>69±6.9</td>
<td>70±5.3</td>
<td>74±7.7</td>
<td>74±6.5</td>
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<td>MI_NO_LVSD</td>
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<tr>
<td>48 hours post MI</td>
<td>66±7.2</td>
<td>65±4.5</td>
<td>66±7.7</td>
<td>66±8.4</td>
<td>69±7.0</td>
<td>70±7.6</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>68±7.6</td>
<td>66±5.2</td>
<td>68±6.8</td>
<td>68±6.2</td>
<td>72±6.6</td>
<td>74±8.2</td>
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<tr>
<td>48 hours post MI</td>
<td>55±4.2</td>
<td>62±3.9</td>
<td>63±3.3</td>
<td>54±4.3</td>
<td>45±5.4*</td>
<td>43±5.9*</td>
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<td>7 weeks post MI</td>
<td>59±4.6</td>
<td>66±3.6</td>
<td>67±4.3</td>
<td>54±4.6</td>
<td>45±6.6*</td>
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<tr>
<td>48 hours post MI</td>
<td>38±6.0**</td>
<td>65±4.3</td>
<td>65±3.8</td>
<td>33±4.9**</td>
<td>26±5.5**</td>
<td>25±4.2**</td>
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<tr>
<td>7 weeks post MI</td>
<td>41±7.2**</td>
<td>69±5.6</td>
<td>69±5.4</td>
<td>33±5.7**</td>
<td>26±6.2**</td>
<td>25±5.1**</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD of 9–15 rabbits. *, P<0.05 versus sham rabbits; †, P<0.05 versus MI_NO_LVSD rabbits; ‡, P<0.05 versus MI_M_LVSD animals according to the one-way ANOVA test followed by the Tukey’s multiple comparison test.
Microscopically (Figure 6), MI_NO_LVSD hearts had more fibrosis (Figure 6F) and more adipocyte infiltration (Figure 6B,F) and macrophage infiltration as compared to the heart of sham animals (Figure 6A,E,I). Heart sections of MI_M_LVSD rabbits showed most severe infiltration of inflammatory macrophages (Figure 6K) and interstitial collagen deposition (Figure 6C,G) and moderate presence of adipocytes (Figure 6C,G,K). Hearts of rabbits with MI_S_LVSD were characterized by excessive presence of adipocytes (Figure 6D,G,K). Hearts of rabbits with MI_S_LVSD were characterized by excessive presence of adipocytes (Figure 6D,G,K). Hearts of rabbits with MI_S_LVSD were characterized by excessive presence of adipocytes (Figure 6D,G,K). Hearts of rabbits with MI_S_LVSD were characterized by excessive presence of adipocytes (Figure 6D,G,K).

<table>
<thead>
<tr>
<th>% of baseline</th>
<th>Sham</th>
<th>MI_NO_LVSD</th>
<th>MI_M_LVSD</th>
<th>MI_S_LVSD</th>
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<tr>
<td>N</td>
<td>12</td>
<td>10</td>
<td>9</td>
<td>15</td>
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<tr>
<td>EDV (mL)</td>
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<tr>
<td>1 week post MI</td>
<td>−1.1±15</td>
<td>−4.8±14</td>
<td>6.6±10</td>
<td>−5.8±13</td>
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<tr>
<td>7 weeks post MI</td>
<td>3.4±22</td>
<td>−2.9±15</td>
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<td>35±30</td>
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<tr>
<td>ESV (mL)</td>
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<tr>
<td>1 week post MI</td>
<td>−1.1±14</td>
<td>−7.5±17</td>
<td>6.5±16</td>
<td>24±27</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>2.4±28</td>
<td>−8.5±19</td>
<td>15±27</td>
<td>79±34</td>
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<td>SV (mL)</td>
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<tr>
<td>1 week post MI</td>
<td>−0.65±22</td>
<td>0.26±28</td>
<td>7.7±16</td>
<td>−30±12</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>5.0±20</td>
<td>5.0±27</td>
<td>−5.8±26</td>
<td>−2.4±25</td>
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<tr>
<td>EF (%)</td>
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<tr>
<td>1 week post MI</td>
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<td>4.3±19</td>
<td>1.0±11</td>
<td>−25±13</td>
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<tr>
<td>7 weeks post MI</td>
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<td>7.2±18</td>
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<td>LVIDd (mm)</td>
<td></td>
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<tr>
<td>1 week post MI</td>
<td>−0.11±6.5</td>
<td>−1.5±6.3</td>
<td>6.7±7.6</td>
<td>0.79±8.3</td>
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<tr>
<td>7 weeks post MI</td>
<td>−0.32±9.6</td>
<td>−3.3±9.5</td>
<td>−1.6±7.6</td>
<td>18±10</td>
</tr>
<tr>
<td>LVIDs (mm)</td>
<td></td>
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</tr>
<tr>
<td>1 week post MI</td>
<td>0.35±11</td>
<td>−2.4±11</td>
<td>1.8±12</td>
<td>12±16</td>
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<td>7 weeks post MI</td>
<td>−4.8±15</td>
<td>−7.1±9.8</td>
<td>−3.0±12</td>
<td>30±11</td>
</tr>
<tr>
<td>LVPWd (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week post MI</td>
<td>4.7±22</td>
<td>7.5±33</td>
<td>39±52</td>
<td>40±47</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>1.0±26</td>
<td>−3.7±24</td>
<td>32±26</td>
<td>−5.0±34</td>
</tr>
<tr>
<td>LVPWs (mm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 week post MI</td>
<td>0.40±18</td>
<td>18±31</td>
<td>25±32</td>
<td>13±32</td>
</tr>
<tr>
<td>7 weeks post MI</td>
<td>8.8±18</td>
<td>19±43</td>
<td>25±27</td>
<td>−19±32</td>
</tr>
</tbody>
</table>

Data are expressed as means ± SD of n rabbits. LVID and LVPW parameters were measured at the level of the papillary muscle level. *P<0.05 versus sham rabbits; †P<0.05 versus MI_NO_LVSD rabbits; ‡P<0.05 versus MI_M_LVSD animals; §P<0.05 versus baseline; ¶P<0.05 versus 1 week post MI according to the one-way ANOVA test followed by the Tukey’s multiple comparison test. LVIDd, left ventricle internal diameter measured in the end-diastolic phase; LVIDs, left ventricle internal diameter measured in the end-systolic phase; LVPWd, LV posterior wall thickness at the end-diastolic; LVPWs, LV posterior wall thickness at the end-systolic phase; EDV, end-diastolic volume; ESV, end-systolic volume; SV, stroke volume; EF, ejection fraction.
Figure 4 Longitudinal evaluation of cardiac function by *in vivo* echocardiography after the induction of a reperfused MI in rabbits. All images are echocardiographic short axis images at the level of the papillary muscle at the end-diastolic (ED) and end-systolic (ES) phase obtained from sham rabbits and reperfused MI-induced rabbits with no (MI_NO_LVSD), moderate (MI_M_LVSD) or severe (MI_S_LVSD) left ventricle systolic dysfunction.

Discussion

In this study we report, for the first time, an occlusion/reperfusion rabbit model that evolved from the acute MI
Figure 5 Post-mortem macroscopic views of the mid-ventricle sections from the sham and induced-MI rabbits with no (MI_NO_LVSD), moderate (MI_M_LVSD) and severe (MI_S_LVSD) left ventricle systolic dysfunction at 7 weeks post-surgery. All groups are shown either as gross sections (A-D) or as corresponding views stained with haematoxylin & eosin (HE, E-H), Masson Trichrome (MT, I-L) and RAM-11 (M-P). Images of the wet lungs are also shown as the different degrees of blood congestion (Q-T). The dashed yellow squares denote the areas that have been microscopically focused in Figure 6 (E-T).
into chronic stage with four different groups (sham, MI_NO_LVSD, MI_M_LVSD, and MI_S_LVSD) by using a combination of cMRI, echocardiography, serum biomarker cTnT test and histomorphology. The highest degree of LV remodeling was observed with the largest infarct size in MI_S_LVSD models at 1 and 7 weeks post MI surgery. EF measured by cMRI in these chronic MI_S_LVSD rabbits was less than 35%, which is consistent with clinical results (28,29). This change was accompanied by a severe dilation of the heart as evidenced by an increase of EDV and ESV by 50% and 150%, respectively after 7 weeks post MI.

Among all MI models, 44% displayed severe LVSD, which is close to the clinical finding that 40% of MI patients suffer from LVSD following MI (7,8). The different degrees of chronic LVSD could be distinguished from sham and MI_NO_LVSD rabbits via imaging and histology.

The different symptoms and/or signs with HF are often subjective, and the threshold for diagnosis may vary widely among clinicians. However, the presence and degree of LVSD can be measured objectively by cardiac imaging techniques. To induce a rabbit model of LVSD is technically challenging and only rarely reported with.
In clinical practice, is more influenced by the degree of
most common parameter of global cardiac performance
acute MI (31), correlated well with infarct sizes. EF, the
S_L VSD rabbits. Acute serum levels of cardiac troponin
surgery: sham, MI_NO_L VSD, MI_M_L VSD and MI_
function measurements, serum troponin T level analysis
chronic stages. We found that cardiac contractile function
by cine cMRI and echocardiography at the acute and
cMRI
systematically investigated in this study. MI sizes after
the follow-up by cMRI and echocardiography over months
have not been reported. Using a non-invasive imaging
platform, MI from acute through chronic stage has been
invasive echocardiography in a rabbit ischemia-reperfusion
model. We showed high-quality 2D cardiac images of
rabbits with different MI sizes, which correlated well with
cMRI at both acute and chronic stages.

So far most of the animal experiments on MI have
focused on acute or subacute MI over hours and days, but
the follow-up by cMRI and echocardiography over months
have not been reported. Using a non-invasive imaging
platform, MI from acute through chronic stage has been
systematically investigated in this study. MI sizes after
surgery were determined by delayed contrast-enhanced
cMRI in vivo, and cardiac contractility was evaluated both
cine cMRI and echocardiography at the acute and
chronic stages. We found that cardiac contractile function
strongly correlated with MI sizes. According to cardiac
function measurements, serum troponin T level analysis
and histology, we distinguished four different groups after
surgery: sham, MI_NO_L VSD, MI_M_L VSD and MI_
S_L VSD rabbits. Acute serum levels of cardiac troponin
T, a marker for myocardial injury used in the diagnosis of
acute MI (31), correlated well with infarct sizes. EF, the
most common parameter of global cardiac performance
in clinical practice, is more influenced by the degree of
LV remodeling than by any other factors (6). Therefore,
we focused on EF changes for evaluation of global cardiac
function and remodeling. At present, echocardiography
remains the predominant clinically applicable non-invasive
test of choice, based on a broader availability. The 2D
echocardiography is well established and has emerged as
an important non-invasive clinical tool for the assessment
of LV systolic and diastolic function after MI (30,32).
However, non-invasive 2D echocardiography on small
animals is challenging. Although echocardiography on
rabbits has been reported, most studies focused on the
complete occlusion coronary artery model (30,32-34).
Only one study reported the regional function in a rabbit
ischemia-reperfusion model, but the authors used open
chest invasive echocardiography (35). In our study, we
longitudinally evaluated global LV function using non-
invasive echocardiography in a rabbit ischemia-reperfusion
model. We showed high-quality 2D cardiac images of
rabbits with different MI sizes, which correlated well with
cMRI at both acute and chronic stages.

Postmortem histology confirmed the different degrees of
LV remodeling in the 3 MI groups. All animals with a large
extended transmural infarction belonged to the MI group
with severe LVSD, while the MI_M_LVSD rabbits showed
smaller infarct areas without transmurality. This may
explain why some rabbits with initially a high reduction in
EF and a large infarct size at the acute stage, do not develop
severe LVSD in the end. At 7 weeks post MI, the LV
architecture changed in the infarcted regions, resulting in
different degrees of fibrosis or collagen deposition, as well
as adipocyte and macrophage infiltration as characterized
using H&E and RAM-11 stainings. We found the largest
quantities of macrophages mixed with fibrotic collagen and
normal myocytes in the moderate LVSD heart, while the
MI region in the severe LVSD heart was replaced by scar
tissue enriched with adipose cells and fibrotic collagen, but
with less macrophages. Larger transmural infarcts were
associated with more infiltrating adipocytes, more collagen
deposition/fibrosis and fewer macrophages as compared to
smaller non-transmural infarcts as the MI transitioned from
acute into chronic stage. In the severe LVSD heart, a large
amount of adipose cells and fibrotic collagen predominantly
formed the infarct scar region during the remodeling
process, which resulted in elongation and thinning of the
infarcted LV. These changes provoked a progressive decline
in ventricular performance. In turn, the LV chamber
became enlarged and the shape of the heart shifted from an
elliptical to a more spherical chamber configuration. No
statistically significant differences were seen in septal wall thickness between the four groups, which may indicate that cardiomyocyte size may not have been affected. The reason may be that seven weeks post MI in rabbits are not long enough to induce the compensatory hypertrophy of normal myocardium. To confirm the different degrees of ventricular damage in our model, we analyzed postmortem wet lungs as indirect evidence. A bigger LV infarct size resulted in LV dysfunction, and ultimately led to accumulation of red blood cells in the capillaries of the pulmonary alveoli. The MI_S_LVSD group did indeed show the highest level of blood congestion as evidenced by both macro- and microscopic findings. This is the first study to report such findings in the rabbit.

MI induced by left anterior descending (LAD) coronary artery ligation results in more uniform infarct sizes, but the mortality is more than 50% due to severe ventricular tachycardia during and after the MI induction (22). In our study, we show that ligation of the LCx in rabbits reduced the mortality rate to 12.7% and 3.6% at the acute and chronic stage, respectively. Although we always ligated the LCx at the same location (2 mm below the left atria), the infarct extent is quite variable. This may be due to the anatomy of the rabbit coronary artery system. This not only differs between species, but may also vary significantly within a single species. In our study, different infarct sizes were obtained, which in turn developed into different degrees of LV dysfunction, thus more closely resembling structural and functional characteristics of the dysfunction in human patients. Combined with the advanced clinical cardiac imaging techniques (36-40), this experimental LVSD platform in rabbits can be easily applied in clinically relevant imaging studies on translational cardiology and can help strengthen drug development and clinical research for the management of cardiovascular diseases. Recently, this platform has already been successfully applied in ongoing cardiac animal experiments (41,42).

There exist certain limitations in this study. The mortality could become higher at the acute stage if the infarct area was made too big. To reduce the mortality we choose LCx instead of LAD ligation, it was difficult to control the progress of MI, thus not all MIs turned transmural and further became cases of S_LVSD. The plasma concentrations of N terminal pro B type natriuretic peptide (NT-proBNP) after MI provide an alternative method of assessing cardiac function, but we could not detect NT-proBNP in our rabbit LVSD models, the reasons might be that the kit is not sensitive to rabbit serum, or seven weeks post MI are not long enough for NT-proBNP detection.

Conclusions

Our study provides a rabbit model with different degrees of chronic LVSD, which could be distinguished from sham and MI_NO_LVSD rabbits via in vivo cMRI, echocardiography and postmortem histology. Our model matches the pathophysiology of systolic dysfunction after MI in human patients and is highly reproducible and cost-effective. It may also be useful for the preclinical testing of treatments targeting myocardial damage following MI and satisfy the needs in preclinical or translational cardiac imaging research.

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Footnote

Conflicts of Interest: The authors have no conflict of interest to declare.

Ethical Statement: All animal procedures were approved by the Ethical Committee of the KU Leuven.

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Figure S1 Videos of *in vivo* cine cMRI display mid-ventricular slices at baseline without MI, at 48 hrs of acute MI phase and at 7 weeks of chronic MI phase on the rabbits of moderate (first line) and severe (second line) LVSD. The arrow indicates the decreased lateral wall motion with different degrees (43). cMRI, cardiac magnetic resonance imaging; MI, myocardial infarction; LVSD, left ventricle systolic dysfunction.

Available online: http://www.asvide.com/article/view/27474

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