In vivo and ex vivo functional characterization of left ventricular remodelling after myocardial infarction in mice

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Abstract

Aims The interest in cardiac remodelling (REM) has steadily increased during recent years. The aim of this study was to functionally characterize REM following myocardial infarction (MI) in mice using high-end in vivo and ex vivo methods.

Methods and results Myocardial infarction or sham operation was induced in A/J mice. Six weeks later, mice underwent cardiac magnetic resonance imaging and were subsequently sacrificed for ex vivo measurements on the isolated heart. Thereafter, hearts were trichrome stained for infarction size calculation. Magnetic resonance imaging showed significantly reduced ejection fraction (P < 0.01) as well as increased end-systolic and end-diastolic volumes (P < 0.01) after MI. The mean infarct size was 48.8 ± 6.9% of left ventricle. In the isolated working heart coronary flow (time point 20′: 6.6 ± 0.9 vs. 13.9 ± 1.6 mL/min, P < 0.01), cardiac output (time point 20′: 17.5 ± 2.6 vs. 36.1 ± 4.3 mL/min, P < 0.01) and pump function (80 mmHg: 2.15 ± 0.88 vs. 4.83 ± 0.76, P < 0.05) were significantly attenuated in MI hearts during all measurements. Systolic and diastolic wall stress were significantly elevated in MI animals.

Conclusion This two-step approach is reasonable, since data quality increases while animals are not exposed to major additional interventions. Both the working heart and magnetic resonance imaging offer a reliable characterization of the functional changes that go along with the development of post-MI REM. By combining these two techniques, additional information such as wall stress can be evaluated.

Keywords Animal model; Working heart; Heart failure; Myocardial infarction; Magnetic resonance tomography

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Introduction

Coronary artery disease (CAD) is the major health burden in civilized countries. The American Heart Association published that one out of six deaths was on account of coronary heart diseases in the USA in 2010. They estimated a number of 150 000 silent myocardial infarctions (MIs) per year and one American suffering from a coronary event every 34 s, meaning that every minute, one patient dies from a heart attack.1,2 In Europe, data are similar: 1.8 million deaths are on account of CAD, 22% of women and 20% of men die from CAD.3 While MI leads to myocyte loss on the one hand, on the other hand, the non-infarcted myocardium has to take over the work of the infarcted region. The remote area of infarction is supported by immediate release of various humoral factors, such as endothelin, and activation of the renin–aldosterone–angiotensin system that increases cardiac output (CO) to facilitate the survival of the individual. The long-term consequences are left ventricular dilation and wall thinning in the infarcted area and compensated hypertrophy and fibrosis of the remote area. This process is generally known as post-MI remodelling (REM), a process
that is still not yet fully understood since functional impairment is conversely discussed in literature.\textsuperscript{4–6}

For evaluation of cardiac function, various animal models have been developed during recent years. Langendorff\textsuperscript{7} and the working heart model (WH)\textsuperscript{4} have gained major attention for more than 100 years.\textsuperscript{8} While Oskar Langendorff used hearts of cats, rabbits, and dogs for his experiments, today’s scientific community mainly focuses on rodents such as rats and mice. The recently increasing availability of genetically modified mice has turned the isolated heart model into an even more powerful tool in cardiovascular research.\textsuperscript{4}

Additionally, \textit{in vivo} magnetic resonance imaging (MRI) with high-resolution imaging has become feasible for rodents, because elaborated computer programs can cope with the high heart rate (HR) of these animals. The aim of our study was to combine \textit{in vivo} and \textit{ex vivo} analyses to characterize post-MI REM in the mouse heart.

**Methods**

**Myocardial infarction model**

Male A/J mice (#000646, The Jackson Laboratory, USA) underwent MI or sham operation (sham: \(n = 10\); MI: \(n = 14\)). For cardiovascular stable conditions, inhalative isoflurane anaesthesia was performed for surgery. Prior to experiments, analgesic therapy was applied subcutaneously with buprenorphine 0.1 mg/kg body weight (BW). Afterwards, mice were anaesthetized with 4% isoflurine 0.1 mg/kg body weight (BW). Afterwards, mice were anaesthetized with buprenorphine 0.1 mg/kg i.p. In addition, mice were intubated with a 22 gauge cannula and ventilated in volume controlled mode with 1.5% isoflurane (HSE Minivent 845, Hugo Sachs Elektronik, Germany). Afterwards, left lateral thoracotomy was performed, the pericardium was torn apart and the left anterior descending coronary artery (LAD) exposed. The LAD was ligated below the left atrial auricle with an 8-0 polyethylene suture. Ischaemia was confirmed by immediate myocardial paling below the suture and ST-segment elevation continued. Animals were extubated when spontaneous breathing was observed. For post-operative regeneration, mice were kept under infrared lamps and supplied with oxygen. Sham animals underwent the same procedure without LAD occlusion. Mice received piritramide as post-operative pain medication (0.1 mg/kg i.p.).

All data were recorded with Labchart (v7.3.2) on a Powerlab System (8/30, both ADi Instruments, Spechbach, Germany). The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1985). The animal ethics commission of the Medical University of Vienna approved all experiments.

**Cardiac magnetic resonance tomography**

Six weeks post-MI, mice were anaesthetized with isoflurane 1.5% via a respiration cone, and cardiac MRI was performed on Medspec 3T MR system (Bruker, Biospin, Rheinstetten, Germany). The system worked with a BGA-12 microgradient insert (200 mT/m) and a 38 mm 1H tuned resonator. Haemodynamic analyses were performed under spontaneous breathing and steady control of vital parameters (breathing rate, temperature, and ECG) by a 1025 MR-compatible Small Animal Monitoring and Gating System (SA Instruments Inc., Stony Brook, NY, USA). Body temperature was kept stable at 37°C with a constant stream of heated air. Cardiac function was visualized with a prospective ECG-gated cine gradient echo-based flow compensated magnetic resonance sequence as implemented in ParaVision 3.1 (Bruker Biospin, Ettlingen, Germany) in a long [TE (echo time) = 4.5 ms, TR (repetition time) = 15 ms, slice thickness = 1.5 mm, NA (number of acquisition) = 4, FOV (field of view) = 50 × 30 mm, matrix 128 × 96, 10 frames] and short [TE = 3.8 ms, TR = 14 ms, slice thickness = 1.5 mm, NA = 4, FOV = 25 × 35 mm, matrix 64 × 96, 7–10 frames] axes orientation, with 4–5 imaging levels across the short axes from the apex to the base of the heart. Each imaging session lasted less than 40 min per animal. All mice survived this procedure.

For assessment of the LV function Segment—Software for Quantitative Medical Image Analysis (v1.8 R1172, Medviso AB, Lund, Sweden) was used.\textsuperscript{9} Edges of LV endocard were traced with inclusion of papillary muscle in the multi-slice short axes images. The following parameters were quantified: ejection fraction (EF), end-systolic volume and end-diastolic volume, stroke volume, and CO.

**Haemodynamics**

\textit{Ex vivo} haemodynamic evaluation analysis was performed with the isolated WH (IH-1/IH-SR, Hugo Sachs, March-Hugstetten, Germany). After 6 weeks, mice underwent cardiac MRI under isoflurane anaesthesia. Subsequently, deep anaesthesia was performed with ketamine (100 mg/kg BW i.p.)
and xylazine (12 mg/kg BW i.p.) for organ excision. Characterization on the isolated WH was successfully performed in eight sham and eight MI mice.

Therefore, the beating heart was quickly excised after heparinization (100 I.U./10 g BW i.v.) and fixed on the aortic cannula. Immediately, retrograde perfusion (LD = Langendorff mode) of the aorta was initialized at constant pressure of 50 mmHg. After 15 min of stabilizing in LD, the left atrium was tightly cannulated via a pulmonary vein and the WH mode started, leading to anterograde perfusion of the left atrium and left ventricle. Thereafter, the LD pump was switched off, and afterload was constantly elevated to 50 mmHg. Pump function was evaluated after 25 min in WH mode: Afterload was given rise from 30 to 120 mmHg in 10 mmHg steps while CO was recorded.

During the WH experiment, we recorded continuously the HR, ECG, aortic pressure, left ventricular pressure (LVP), arterial pressure, and coronary pressure (CF). In order to measure the LVP, a microtip catheter (SPR-1000, Millar Instruments, Houston, USA) was inserted into the left ventricle via the aortic valve. External heart work (EHW) was calculated by CO × systolic LVP (LVPsys) for each time point. Systolic and diastolic wall stress were computed accordingly: LVP × LV radius/(2 × wall thickness).

**Organ preparation**

For immunohistochemistry, hearts were immersed in 10% buffered formaldehyde immediately after WH procedure. After 48 h, hearts were cut at mid-papillary level and embedded in paraffin. Sections of 4 μm were stained with Goldner trichrome for MI size calculation.

**Masson–Goldner trichrome stain**

A Masson–Goldner trichrome kit (Carl Roth GmbH, Karlsruhe, Germany) was utilized to quantify fibrous connective tissue, which turns blue after staining and represents the myocardial scar. Therefore, microscope slides were treated in Xylol (3 min), ethanol 100%, 96%, and 70% (3 min each) and subsequently washed in demineralised water before hemalm staining for 1 min and blueing for 5 min. The slides were then stained with all three Goldner solutions (I: 1.5 min, II: 2 min, and III: 5 min), rehydrated, and covered with Aquatex (Merck Millipore, Darmstadt, Germany).

**Statistical analyses**

Statistical testing was performed with SPSS® Statistics (Version 21, IBM® Corporation, New York, USA). Analysis of variance for repeated measurements was performed for *ex vivo* analyses over all time points. Both groups were compared for each time point with unpaired Student’s *t*-test. Correlations between *in vivo* and *ex vivo* results were tested with linear regression. *Ex vivo* results are presented as means ± standard deviation, all other data as means ± standard error of the mean.

**Results**

**Animal characteristics**

Values are depicted in Table 1. Animals underwent cardiac MRI and were sacrificed for *ex vivo* analyses with the WH apparatus 6 weeks after surgery. Masson–Goldner Trichrome staining showed a mean infarction size of 48.8 ± 6.9% (*Figure 1*). Total heart weight was significantly higher in MI vs. sham (222.5 ± 9.0 vs. 157.2 ± 4.3 mg; *P < 0.01*). The total heart weight to BW ratio was significantly augmented in the MI group (8.24 ± 0.30 vs. 6.54 ± 0.21 mg/g; *P < 0.01*). Lung and liver wet–dry ratios did not show any differences.

**Post-operative mortality**

In the MI group, three mice died within the first 7 post-operative days, the overall post-MI mortality was 23%. No animal died in the sham group or during MRI. Dropout rate during WH was 35% in MI and 30% in sham. All in all, eight sham and eight MI hearts completed *ex vivo* and *in vivo* measurements.

**In vivo haemodynamic data by magnetic resonance imaging**

Data are depicted in Table 2. Six weeks after MI or sham operation, haemodynamics were recorded by MRI under isoflurane sedation. HR was homogenous in all two groups and did not show any significant differences. EF was significantly decreased in MI compared with sham (24 ± 2 vs. 67 ± 2%; *P < 0.01*). End-diastolic volume (619.1 ± 82.4 vs. 239.7 mL) was tightly augmented in MI vs. sham (35% in MI compared with sham (24 ± 2 vs. 67 ± 2%; *P < 0.01*). The total heart weight to BW ratio was significantly augmented in the MI group (8.24 ± 0.30 vs. 6.54 ± 0.21 mg/g; *P < 0.01*). Lung and liver wet–dry ratios did not show any differences.

### Table 1. Animal characteristics

|                | Sham (n = 8)     | MI (n = 8)     |
|----------------|-----------------|----------------|
| BW, g          | 25.32 ± 0.59    | 26.56 ± 0.54   |
| THW, mg        | 157.2 ± 4.3     | 222.5 ± 9.0**  |
| THW/BW, mg/g   | 6.54 ± 0.21     | 8.24 ± 0.30**  |
| Tibia, mm      | 18.48 ± 0.34    | 18.94 ± 0.37   |
| Lung w/d       | 4.48 ± 0.21     | 4.67 ± 0.14    |
| Liver w/d      | 3.05 ± 0.05     | 3.20 ± 0.04    |
| Infarct, %     | n.a.            | 48.8 ± 6.9     |

BW, body weight (g); infarct (% of left ventricle); MI, myocardial infarction; n.a. not available; THW, total heart weight (mg); THW/BW, THW to BW ratio; tibia, tibia length (mm); w/d, wet/dry weight ratio. All values in means ± standard error of the mean.

*P < 0.05.

**P < 0.01 indicates differences between MI and sham (Student’s *t*-test).
and end-systolic volume (484.2 ± 71.9 vs. 75.4 ± 10.0 μL/g; P < 0.01) were significantly increased in MI. Systolic (4.0 ± 0.2 vs. 1.8 ± 0.1 mm; P < 0.01) and diastolic (5.1 ± 0.2 vs. 3.2 ± 0.1 mm; P < 0.01) LV diameters were likewise enlarged significantly in MI.

Table 2. Haemodynamic magnetic resonance imaging results

|                    | Sham (n = 8)  | MI (n = 8)  |
|--------------------|---------------|-------------|
| HR, b.p.m.         | 489 ± 12      | 480 ± 14    |
| EDV, μL/g          | 239.7 ± 14.9  | 619.1 ± 82.4** |
| ESV, μL/g          | 75.4 ± 10.0   | 484.2 ± 71.9** |
| EF, %              | 67 ± 2        | 24 ± 2**    |
| FS, %              | 46 ± 3        | 16 ± 1**    |
| Systolic LVd, mm   | 1.8 ± 0.1     | 4.0 ± 0.2** |
| Diastolic LVd, mm  | 3.2 ± 0.1     | 5.1 ± 0.2** |
| LVEDP, mmHg        | 3.2 ± 0.1     | 4.9 ± 0.3** |
| ∂sys (20 min), mmHg| 37.7 ± 5.8    | 67.3 ± 5.0** |
| ∂ed (20 min), mmHg | 6.5 ± 1.5     | 12.3 ± 0.4** |

EDV, end-diastolic volume (μL/g heart weight); EF, ejection fraction (%); ESV, end-systolic volume (μL/g heart weight); FS, fractional shortening (%); HR, heart rate (b.p.m.); LVd, left ventricular diameter; LVEDP, left ventricular end-diastolic pressure (mmHg); MI, myocardial infarction; ∂ed, end-diastolic wall stress (mmHg); ∂sys, systolic wall stress (mmHg).

All values in means ± standard error of the mean.

*P < 0.05 indicates differences between MI and sham.

**P < 0.01 indicates differences between MI and sham groups of same strain (Student’s t-test).

Ex vivo haemodynamic data by working heart

Values are depicted in Figure 2. All flow values were normalized per gram wet heart weight. HR was similar in both groups during the entire WH examination. LVPsys (Figure 2B) was significantly decreased in MI (70.3 ± 6.3 vs. 81.3 ± 6.2 mmHg; P < 0.05). MI hearts showed a significantly elevated LV end-diastolic pressure (LVEDP) compared with sham at 20 min of WH (4.9 ± 0.3 vs. 3.2 ± 0.1 mmHg; P < 0.01). CO (mL/min/g wet heart weight; Figure 2C) was significantly decreased in MI (P < 0.01 for all time points). CF (8.1 ± 1.9 vs. 14.3 ± 4.5 mL/min/g wet heart weight; P < 0.01; Figure 2D) and EHW (CO × LVPsys; 1312 ± 561 vs. 2871 ± 1127; P < 0.01; Figure 2E) were significantly lower during the entire observation period in MI hearts.

Wall stress (∂, Figure 2F and G) was calculated for every time point of WH mode. MI showed significantly higher systolic and end-diastolic values than sham (e.g. time point
20 min: \( \partial \) systolic: 37.7 ± 5.8 vs. 67.3 ± 4.9 mmHg; \( \partial \) diastolic: 6.5 ± 1.5 vs. 12.3 ± 0.4 mmHg; both \( P < 0.01 \). Values remained stable throughout 25 min of evaluation. Finally, the pump function (area under the curve) showed a homogeneous picture: for every given LV systolic pressure, MI hearts developed less CO than sham (Figure 3).

*In vivo* data (fractional shortening) showed a significant correlation with *ex vivo* parameters, namely LVPsys \( (P < 0.01) \), CO \( (P < 0.01) \), CF \( (P < 0.01) \), and EHW \( (P < 0.01) \) (Figure 4).

**Discussion**

In the present study, we underline the positive subsidiary effect of *in vivo* and *ex vivo* analyses to describe the functional changes taking place during post-MI REM. Besides, we wanted to give a detailed description of both methods in order to facilitate their use for other scientists.

The foundation of our study was a reproducible infarction model in mice. We showed that gaseous anaesthetics such as isoflurane guarantee stable conditions for surgery. The mortality rate of 23% in the MI group is comparable with current...
literature and 0% in the sham group underline the stability of the anaesthetic regimen.

Working heart and MRI are both comparatively demanding, expensive, and time-consuming methods, but they allow an exact and synergistic evaluation of post-MI REM. Data derived from the two independent methods allow to calculate wall stress, a major parameter to evaluate the extent of REM. Until recently, echocardiography was the gold standard in terms of geometrical measurements. The main disadvantage of conventional echocardiography is the conversion from single-dimensional measurements into three-dimensional assumptions. In addition, an experienced specialist is obligatory for echocardiography in mice to gain reproducible results. MRI has become available for small animals in recent years. It guarantees detailed in vivo haemodynamics and clear visualization of cardiac dimensions, and it can be repeated several times to describe changes over time. Besides, MRI can be used to simultaneously measure cardiac metabolism, if equipped with specific software and hardware upgrades. In our study, MRI described in detail the development of post-MI remodeling in mice: EF and LV dimensions and volumes were significantly increased in MI. The subsequent WH measurements revealed additional facts: CF, CO, LVP, EHW, and the pump function were significantly reduced in MI during all time points. Furthermore, the significant correlation of in vivo and ex vivo data underlines clearly the synergy of both methods. Fractional shortening correlated directly with systolic LVP, CO, CF, and EHW in both groups (time point 20’, Figure 4).

The WH is one of the most promising tools to satisfy the increasing interest in haemodynamics of genetically engineered mice. Together with steady technical progress in cardiovascular imaging, the WH has reached scientific relevance of highest impact within recent years. In conclusion, this study provides evidence that the combined characterization by MRI and WH gives a comprehensive picture of the functional changes in post-MI REM.

Limitations of the study

Cardiodynamics are measured isolated from the body under stable conditions within a relatively short time in the isolated WH. A general restriction is that the rodent heart cannot be directly transferred to the human. However, for the description of mechanisms, differences between rodent and human myocardium are acceptable, for example, the mouse has an HR of 400–500 beats/min, which is around 10 times higher than in humans. The fact that these measurements are performed totally isolated from the body must be taken into account.

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Conflict of interest

None declared.

Appendix

Pitfalls

Experiments as MI or WH demand an experienced team for obtaining reproducible results. Here are a few pitfalls, which might help in the beginning:

Myocardial infarction model

Intubation of mice is one of the most difficult tasks in this model. Intubation failure highly increases the risk of intraoperative mortality. Use a self-made intubation rack, so that intubation direction is top-down. The intubation cannula must not be forced into the trachea, otherwise you might place it paratracheal. You should feel a typical rumbling when passing the cartilage of the trachea.

Due to thoracotomy, ventilation should only be performed with a post-end-expiratory pressure valve. Post-end-expiratory pressure should be shortly increased during the last thoracal suture to avoid pneumothorax.

If mice die surprisingly after a trouble-free extubation, respiratory insufficiency due to morphines might be a reasonable explanation.

Working heart model

When changing from LD into WH mode, the afterload must not be increased too fast.

At the end of the experiment, the heart should never be cut down from the aortic cannula, since there still might be expensive tip catheters rising from the aorta into the left ventricle.

We recommend the use magnifying glasses for the mouse WH. The mouse heart should be kept warm in an isolated chamber during the whole experiment.
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