A Novel and Efficient Model of Coronary Artery Ligation and Myocardial Infarction in the Mouse

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Rationale: Coronary artery ligation to induce myocardial infarction (MI) in mice is typically performed by an invasive and time-consuming approach that requires ventilation and chest opening (classic method), often resulting in extensive tissue damage and high mortality. We developed a novel and rapid surgical method to induce MI that does not require ventilation.

Objective: The purpose of this study was to develop and comprehensively describe this method and directly compare it to the classic method.

Methods and Results: Male C57/B6 mice were grouped into 4 groups: new method MI (MI-N) or sham (S-N) and classic method MI (MI-C) or sham (S-C). In the new method, heart was manually exposed without intubation through a small incision and MI was induced. In the classic method, MI was induced through a ventilated thoracotomy. Similar groups were used in an ischemia/reperfusion injury model. This novel MI procedure is rapid, with an average procedure time of 1.22±0.05 minutes, whereas the classic method requires 23.2±0.6 minutes per procedure. Surgical mortality was 3% in MI-N and 15.9% in MI-C. The rate of arrhythmia was significantly lower in MI-N. The postsurgical levels of tumor necrosis factor-α and myeloperoxidase were lower in new method, indicating less inflammation. Overall, 28-day post-MI survival rate was 68% with MI-N and 48% with MI-C. Importantly, there was no difference in infarct size or post-MI cardiac function between the methods.

Conclusions: This new rapid method of MI in mice represents a more efficient and less damaging model of myocardial ischemic injury compared with the classic method. (Circ Res. 2010;107:1445-1453.)

Key Words: myocardial ischemia ■ myocardial ischemia/reperfusion injury ■ cardiac injury ■ cardiac dysfunction ■ mouse model

Cardiovascular disease represents the leading cause of morbidity and death in developed countries. Coronary heart disease, which is the single largest cause of cardiovascular disease, is the narrowing of arteries over time caused by atherosclerotic plaques or the acute occlusion of the coronary artery by thrombosis, both of which lead to possible myocardial infarction (MI) and the eventual development of heart failure.1,2 Protection from coronary heart disease–induced damage of the myocardium during myocardial ischemia/reperfusion (I/R) injury has been a target of investigation for the development of innovative cardioprotective therapies.3–7

The increase in the availability of various types of genetically manipulated mice has brought about the need for more efficient ways to induce myocardial damage for both molecular mechanistic studies and potentially therapeutic interventions. Two of the most common models used by researchers are permanent left main descending coronary artery (LCA) occlusion to induce a MI and also temporary coronary artery occlusion to induce I/R injury.8,9 The I/R model is generally used to examine the short-term consequences of ischemic injury, whereas the MI model is usually used to investigate myocardial changes such as remodeling that occur over an extended period of time. Although a variety of surgical manipulations have been used during the past decade to induce the ischemic event, ligation of the LCA is still the most commonly practiced method.5,6–11 However, most investigators still use a method requiring ventilation and wide opening the chest (referred to as the classic method), which can cause extensive tissue damage, high surgical-related death and can also be quite time consuming for most surgeons.12–15

Over the last few years, we have developed a new MI approach in mice that does not require ventilation.11,16–18 Complete characterization and description of this model has
not been performed, including a direct comparison with the classic MI method to determine whether this novel method is indeed more efficient and safer for mice while not compromising the extent of cardiac injury. Thus, this study comprehensively and technically introduces this novel MI method in mice and compares this relatively less invasive surgical procedure to the classic MI and I/R injury. Parameters analyzed were surgical time, mouse postsurgical recovery procedure to the classic MI and I/R injury. Parameters analyzed were surgical time, mouse postsurgical recovery time, tumor necrosis factor (TNF)α and myeloperoxidase (MPO) levels as markers of inflammatory damage from the procedures, arrhythmias, and peri-surgical and overall survival. In addition, we assessed both methods for induction of left ventricular (LV) infarction and in vivo post-MI cardiac function.

Methods

An expanded Methods section is available in the Online Data Supplement at http://circres.ahajournals.org and includes information regarding telemetry ECG recording in conscious mice, in vivo echocardiographic and hemodynamic measurements, determination of LV infarct size in MI models, determination of LV area at risk (AAR) and infarct size in I/R models, measurement of plasma level of TNFαs and tissue level of MPO, and statistical analysis.

Experimental Protocols

Experiments were carried out according to the NIH Guidelines on the Use of Laboratory Animals, and all procedures were approved by the Thomas Jefferson University Committee on Animal Care. A total of 497 (384 mice for MI and 113 for I/R) male 8- to 10-week-old C57/B6 mice were used for this study. For the MI model, mice were subjected to permanent coronary artery ligation using either the new (N) method or the classic (C) method. Mice were randomly assigned to 4 groups: new method of MI (MI-N) or sham (S-N) and classic method of MI (MI-C) or sham (S-C). There were 119 mice used for survival study. Some of the mice that survived at the end of 28 days were also used for echocardiographic, hemodynamic, and infarct size studies as indicated in each study. The rest of 232 mice that survived all kinds of 265 procedures (33 mice died) were used for 24-hour infarct size measurement (32 mice), Masson’s trichrome stain (18 mice), arrhythmia analysis (28 mice), MPO (81), and TNFα (73) assays. In I/R model, mice were subjected to 30 minutes of myocardial ischemia, followed by 24 hours of reperfusion. Mice were also divided into 4 groups: new method of I/R (I/R-N, n=41) or sham (S/I/R-N, n=16) and classic method of I/R or sham I/R (I/R-C [n=40], S/I/R-C [n=16], respectively). All animals were monitored after the surgery and received 1 dose of buprenorphine (0.3 mg/kg) within 6 hours after surgery, and another dose was administered the following morning. No further analgesia was given thereafter.

New Method of Permanent Coronary Artery Occlusion Without Ventilation

In this new method of MI induction, mice were anesthetized with 2% isoflurane inhalation with an isoflurane delivery system (Viking Medical, Medford, NJ) but not ventilated. Of course, before perfecting this surgical model, we recommend using ventilation in mice until the surgeon can routinely perform the LCA ligation in <3 minutes. A small skin cut (1.2 cm) was made over the left chest, and a purse suture was made as shown in Figure 1A through 1C. A small hole was made at the fourth intercostal space with a mosquito clamp to open the pleural membrane and pericardium (Figure 1E). With the clamp slightly open, the heart was smoothly and gently “popped out” through the hole, as shown in Figure 1F. The LCA was located, sutured, and ligated at a site ~3 mm from its origin using a 6-0 silk suture (Figure 1H and 1I). The ligation was deemed successful when the anterior wall of the LV turned pale (Figure 1J). After ligation, the heart was immediately placed back into the intrathoracic space followed by manual evacuation of air and closure of muscle and the skin, by means of the previously placed purse-string suture (Figure 1K through 1L). The mouse was then allowed to breathe room air and monitored during the recovery period, which was generally complete within 3 to 5 minutes. No artificial respiratory aid was required during the recovery time. The sham group underwent the same surgical procedure except that the LCA was not occluded. A movie of this new method of MI can be found in the Online Data Supplement.

Classic Method of MI Induction

The more invasive classic method of MI in mice has been fully described by previous investigators. We have only changed the method of anesthesia. See details in the Online Data Supplement.

New Method of Induction of Myocardial I/R Injury Without Ventilation

This I/R injury procedure in mice is essentially the same as the procedure for inducing MI except that a slipknot is tied around the LCA 2 to 3 mm from its origin with a 6-0 silk suture, as shown in Figure 2A. The heart is then quickly placed back into the thoracic space, followed by manual evacuation of air and the skin closing (Figure 2B). The internal needle end of slipknot suture is cut as short as possible, and the other end of the suture is ~0.8 cm long and remains outside of the chest (Figure 2C). After 30 minutes of ischemia, the slipknot is released by pulling the long end of slipknot suture smoothly and gently until a feeling of release is sensed; at which time, the myocardium begins reperfusing. This outside-the-skin suture knot-releasing method should only be attempted by the experienced surgeon. Alternatively, the mouse can be reanesthetized with 2% isoflurane inhalation, the chest reopened, and the slipknot released by pulling the long end of slipknot suture smoothly and gently, followed by manual evacuation of the pneumonia and chest closure. As above for the MI model, ventilation is recommended until times are fast enough to perform this procedure without ventilation.

Classic Method of I/R Injury

The procedure for the classic method of I/R (I/R-C) is very similar to MI-C method except that a slipknot is made around the LCA. During the 30-minute ischemic period, the mouse was ventilated.
continuously and the incision was covered with a piece of saline-soaked gauze. After 30 minutes of ischemia, the slipknot was released followed by closing the chest in layers.

Results

Novel Method of MI or I/R Is More Efficient Than Classic Method With Shortened Recovery Time and Less Inflammation

As shown in Figure 3A, the surgical procedure time needed for MI in the classic method, even in the hands of an experienced surgeon, is almost 21 minutes for the sham procedure and 23 minutes for full ligation of the LCA. This includes the time needed for intubation (2.13 ± 0.22 minutes, in MI-C) and extubation (1.25 ± 0.2 minutes, MI-C). In contrast, the time that a skilled surgeon can complete the entire procedure of a full LCA ligation with our novel method is <1.25 minutes (Figure 3A). Therefore, compared with the classic approach that needs intubation, our new method of MI has an ≈20-fold improvement in efficiency (Figure 3B). This increased efficiency is even more pronounced in I/R injury model because the classic procedure includes 30 minutes of ischemia while ventilated. Our new method externalizing the suture can significantly decrease this surgical time to <2 minutes and represents a relatively less invasive method of I/R injury.

To further evaluate the 2 methods of murine myocardial ischemic damage, we also recorded the postsurgery recovery time. In the new method, this is defined as the interval from surgical completion until the animal is fully conscious and walking freely in the cage. For the classic method, this is the interval from time of extubation until the animal is fully conscious and walking freely. The recovery time was 3.92 ± 0.15 minutes for the new sham procedure and 5.52 ± 0.28 minutes with a full MI using our new method (Figure 3A). This time is significantly shorter than the classic method, which was ≈19 minutes (Figure 3A). The postsurgery recovery period for the I/R procedure using our quick method showed similar efficiency because the time for recovery was 4.01 ± 0.23 minutes in the SI/R-N group and 4.42 ± 0.18 minutes in I/R-N group, which were significantly shorter than either sham (14.61 ± 0.86 minutes) or I/R (15.07 ± 0.55) using the classic surgical procedure.

To investigate the inflammatory damage/response of these 2 methods, the plasma level of TNFα was measured at 24 hours and 7 days after surgery. As shown in Figure 3C, MI in both methods significantly increased the plasma level of TNFα compared with their sham groups. Importantly, the sham procedure of the new method did not significantly raise circulating TNFα levels compared with a control mouse with no surgery (Co, 12.26 ± 0.51 pg/mL versus 9.78 ± 0.72 pg/mL). Furthermore, this level is significantly lower than TNFα induced by simply intubating and performing the sham procedure classically (33.33 ± 2.13 pg/mL). When MI was actually carried out, the new procedure resulted in significantly lower 24-hour postsurgical TNFα levels, which were actually equivalent (27.34 ± 1.56 pg/mL) to the sham procedure of the classic method. The highest level of TNFα was seen in the MI-C group (Figure 3C). The increased TNFα seen in plasma normalized at 7 days after MI (Figure 3D). To further investigate the inflammatory state in these hearts, we measured cardiac tissue levels of MPO. Twenty-four hours after MI, cardiac levels of MPO in both groups were significantly increased compared with sham-treated mice.
Novel Method of MI or I/R Reduces Mortality and Improves Survival

A total of 119 mice were used in the MI survival study. First, during the peri-surgical period, which is the time from the beginning of the surgery to 6 hours after surgery, only 1 of 43 mice in the MI-N group died because of the bleeding (2.3%; Figure 4A). In contrast, 7 of 44 peri-surgical deaths were seen in the MI-C group (15.9%; Figure 4A). Among those 7 mice, 1 died from an intubation accident, 1 died from bleeding, 1 died from pneumothorax, and 4 died of sudden cardiac arrest. All of the animals in the sham group survived regardless of the surgical method. After the peri-surgical period, there were 13 and 16 deaths in the MI-N and MI-C groups, respectively. The reason for those deaths is summarized in Online Table I. The most common reason for post-MI death in both groups was ventricular rupture (46.2% in MI-N and 43.8% in MI-C) that occurred between days 4 and 8 after MI. Some of the mice died of unknown causes, although we assume that this was attributable to either cardiac arrhythmia or heart failure because autopsy of these mice showed no blood in the thoracic cavity. The overall survival rate (including surgical-

Figure 4. Survival curves in MI or I/R models. Mice were subjected to MI or sham with either the new or classic method and followed for 6 hours (peri-surgery survival curve) (A) and 28 days (overall post-MI survival curves) (B). **P<0.05 MI-N vs MI-C group. C, I/R peri-surgery survival rate in all groups. *P<0.05 I/R-N vs I/R-C group.
related death) 28 days after surgery was 100% in S-N, 93.75% in S-C (1 dead of 16 resulting from an intubation accident), 47.72% in MI-C, and 67.5% in MI-N (Figure 4B). The overall survival rates were 70% in MI-N and 56.82% if perisurgical death was not included. These data confirm an improved outcome using this quicker method of MI.

A total of 113 mice were used in the I/R survival study. As shown in Figure 4C, there was no significant difference in survival rates between sham groups (SI/R-N, 100%; SI/R-C, 93.75%). However, as in the MI study, the survival rate was significantly higher in the I/R-N group (95.12%) compared with the I/R-C group (77.5%). In most cases, the periprocedural death that occurred in the I/R-C group was attributable to arrhythmia during the 30-minute ischemia time.

**Novel Method of MI Has Less Cardiac Arrhythmia**

With the classic method of MI, one of the most frequent causes of perisurgical death could be arrhythmia. To confirm this, we used the telemetry electrocardiographic technique to record ECG during and after MI surgery. After MI, there was a typical elevation of the S-T segment in both MI groups, as shown in Online Figure II. Although there was a diverse occurrence of the types of arrhythmias found (mostly in MI-C; Online Figure III), the most commonly seen arrhythmias were AV block (AVB) and premature ventricular contraction (PVC), as shown in Figure 5A and 5B. The frequency of AVB was not different among all surgical groups during the first 30 minutes (Figure 5C) but was significantly increased during the remaining 7-day data collection periods in MI-C compared with the MI-N and S-N but not the S-C groups (Figure 5E). Although the frequency of PVC was not different between MI-N and MI-C, which trended higher, but was significantly lower in S-N compared with S-C in the first 30 minutes (Figure 5D), indicating the intubation and classic thoracotomy provokes more arrhythmias. Similarly, the frequency of PVC in MI-N and S-N was significantly lower than that of MI-C in the 7 days of recording (Figure 5F).

**MI- and I/R-Induced Injury and Subsequent Cardiac Dysfunction**

Echocardiography was used as a noninvasive method to measure cardiac function 4 weeks after permanent coronary artery occlusion. As shown in Figure 6A, both MI groups exhibit severely impaired and akinetic anterior wall motion when...
compared with sham hearts. Both MI groups had significantly increased LV diastolic and systolic inner chamber dimensions and accordingly decreased LV ejection fraction and fractional shortening, as shown in Figure 6B through 6E. Consistent with the echocardiographic observations, hemodynamic measurements at 4 weeks after MI demonstrated that both MI groups had significantly decreased contractility (measured as +dP/dt), relaxation (measured as -dP/dt) and LV end-diastolic pressure at baseline and on β-adrenergic stimulation, when compared with sham groups (P<0.05; Figure 7B through 7D). There were no differences between the S-N and S-C nor between the MI-N and MI-C groups. Furthermore, heart rates were similar among all of the groups (Figure 7E). These data importantly demonstrate that the new method of MI induces the same degree of cardiac dysfunction as the classic method. We also measured cardiac function via echocardiography and LV catheterization in the post-I/R (24 hours) injured animals and also found that regardless of the method used, cardiac function was lower in the I/R groups (Online Table II).

To further evaluate the reliability of the new method of MI and I/R, we measured LV infarct size, which we found to be statistically unchanged based on method. The LV size of infarction was virtually identical regardless of the size of ischemic area in the LV, was unchanged between the 2 methods (Figure 8G).

**Discussion**

We have developed a novel and efficient surgical procedure for inducing ischemic damage in mouse myocardium that eliminates the need for intubation. This procedure can be accomplished in significantly less time than the classic approach of mouse MI. Of note, this quicker procedure results in less tissue damage and inflammation, with lower occurrence of cardiac arrhythmias and much faster postsurgery recovery. Perhaps most importantly, this novel procedure fully recapitulates the degree of myocardial injury and subsequent post-MI cardiac dysfunction than the classic method.

Although we have published previous studies using this novel method, we have not comprehensively detailed this procedure nor have we directly compared this method with the classic surgical MI procedure to prove that this quicker method is indeed an improvement on the method that could be used more routinely for all investigators in this field. We have used this new method to investigate the mechanism of MI and I/R injury by using both pharmacological tools17,18,20–25 and genetic manipulation.5,11,26–33 We have also used this quicker method involving a “heart pop-out” approach of mouse MI. Of note, this quicker procedure results in less tissue damage and inflammation, with lower occurrence of cardiac arrhythmias and much faster postsurgery recovery.
procedure in stem cell and gene therapy studies (unpublished data), in which we injected stem cells or adenovirus directly into the border zone of the ischemic heart while the heart was exposed. Our experience with this new and rapid method proves that it is extremely reproducible. We believe that this method is an advancement in studies of murine ischemic injury.

Compared with the classic method of MI, one of the advantages of this model is less tissue damage, as indicated by the lower level of acute phase of circulating TNF, a proinflammatory factor, after the surgery. The fact that we found no differences between TNF levels in the S-N and no-surgery control group, coupled with the fact that TNF levels were 3 times higher in S-C compared with S-N, indicates a minimal degree of tissue damage using the new MI procedure. These reduced acute phases of plasma TNF levels in groups of S-N or MI-N are probably attributable to the shortened procedure time and the less invasive surgical procedure, which should further benefit postsurgical recovery and prognosis because there was no difference in plasma levels of TNF among groups chronically. More interestingly, the MPO level in MI-N group was significantly lower than that of MI-C at 7 days after MI, indicating extended, more local inflammation in the classic method of MI.

Efficiency is another advantage of this new model of MI over the classic ventilated approach. The time saving is attributable to the simplified surgical procedure and elimination of ventilation. In our surgery core, we typically perform this procedure in <2 minutes. This quicker time is what is possible in the hands of an experienced and skilled mouse surgeon, but we believe that even without the number of cases performed in our hands (>20 000), this procedure can still be performed more quickly by anyone trained in the classic method. However, we also agree that a skilled surgeon could carry out the classic method of MI more quickly by placing fewer sutures. We would caution starting with this method without ventilation, however, because practice is needed to perfect the “popping-out” technique to quickly ligate the LCA. Once the procedure can be performed in <3 minutes after ventilation is started, it would probably be safe to start using our full surgical method to gain full advantage. Because we found that this procedure creates identical LV infarcts and cardiac dysfunction compared with the classic method, we recommend this procedure to limit damage and improve survival for MI and I/R studies.

Limitation and Methodological Consideration

The procedure of murine model of MI or I/R injury without ventilation is technically demanding and challenging. Critical to the success of the new model is keeping the time that the heart is outside of the body to a minimum. Based on our experience, the time allowed for heart externalization should be no more than 30 seconds to limit global hypoxia. This will give the best prognostic result.

Bleeding could be one of the factors contributing to the increased mortality of the “heart exteriorization” MI and I/R mouse models, especially when the researcher is first learning the procedure.

Pneumothorax is another contributor to early postsurgical death and occurs when the operator forgets either to displace...
air before closing the suture or forgets to keep the hole open when manually evacuating air of the thoracic cavity. One of the advantages of this new procedure is that the pectoral muscles remain intact. This is very important because intact pectoral muscles are necessary to cover the opening (the minithoracotomy) once the heart has been returned to the thoracic space and eliminates the suturing of the muscle, which is one of the limitations of classic thoracotomy. In fact, it is possible to keep pectoral muscles intact while performing a classic MI using the technique we described here, and in doing so, the likelihood of pneumothorax is decreased, and the inflammatory response and the time needed to complete the procedure could be further reduced.

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Disclosures

None.

References

Novelty and Significance

What Is Known?

- Coronary artery ligation in mice to induce myocardial infarction (MI) is typically achieved by an invasive and time-consuming approach that requires ventilation (classic method) and can often result in extensive tissue damage and high mortality.
- An increase in the availability of various types of genetically altered mice has brought about the need for more efficient ways to induce myocardial ischemic damage.

What New Information Does This Article Contribute?

- The article describes a new surgical method of MI and ischemia/reperfusion (I/R) injury that is less invasive and less time-consuming and compares this approach with the more invasive classic method.
- The novel surgical MI procedure is more efficient than the currently used surgical approach.
- The novel MI procedure is less invasive. It creates reproducible infarcts and improves surgical mortality.

In this study, we describe a novel murine model of inducing a MI or I/R injury. In addition to providing all technical aspects of this quicker mini thoracotomy procedure, we have directly compared this with the classic surgical approach that includes intubation, ventilation, and more invasive thoracotomy. Our results demonstrate that compared with the classic approach, this novel method of MI is quicker, less invasive, produces higher survival rates, and induces reproducible infarcts that lead to significant left ventricular dysfunction and remodeling. Accordingly, this animal model may benefit cardiovascular researchers and speed translation from basic science to clinic practice.
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Supplemental Methods

Experimental procedure of permanent coronary occlusion with ventilation (Classic method of MI, MI-C)

The classical, ventilation-based method of MI in mice has been fully described by previous investigators\textsuperscript{1-3}. Only the method of anesthesia was changed. Briefly, mice were initially anesthetized with 3% isoflurane inhalation and then intubated with a 20G intravenous catheter and ventilated with a mixture of O\textsubscript{2} and 1.5-2\% isoflurane, using a rodent ventilator (Harvard Apparatus, Boston). The stroke volume was 0.2 ml and the respiratory rate was 120 breaths/min. Animals were placed in a supine position and the body temperature monitored and kept at 35-36\ºC with a temperature controlled surgical table. A left thoracotomy was then performed through the 4\textsuperscript{th} intercostal space by cutting pectoralis muscles transversely to expose the thoracic cage. The thymus was retracted upward, and the left lung partially collapsed. After the pericardium was opened, the LCA was located and ligated with a 6-0 silk suture 2-3 mm from origin. The ligation was confirmed successfully when the anterior wall of the LV turned pale. The lungs were then inflated to displace air and the thoracotomy site closed in layers. After about 2-5 min ventilation with the room air, the animal was gradually weaned from the respirator once spontaneous respiration resumes and remains in a supervised setting until fully conscious. Sham treated animals underwent the same surgical procedures except the LCA was not occluded.

Telemetry electrocardiographic (ECG) recording in conscious mice

A total of 28 mice (S-N, n=5, S-C, n=5, MI-N, n=7, and MI-C, n=11) were involved quantification of arrhythmias ECG was recorded using the Data Sciences International radiotelemetry data acquisition system (Dataquest A.R.T. version 2.2, Data Sciences International, St Paul, MN) as previously described\textsuperscript{4}. Briefly, 8-week-old male mice were anesthetized with isofluorane. A midline incision of about 1.5 cm was made on the dorsal back cranial to the hind limbs for subcutaneous placement of the implantable transmitter (EA-F20). The two biopotential leads of the transmitter were placed subcutaneously in the lead II configuration (negative lead positioned at the right shoulder and the positive lead positioned about 2cm to the left of the xyphoid process and caudal to the rib cage). Mice were housed in individual cages with unrestricted access to standard chow and drinking water. ECG was monitored during MI and lasted 30 min after the procedure. ECG was recorded once an hour for 1 min for 7 days. Arrhythmias were counted for each animal using Dataquest A.R.T. analysis program. ECG was monitored and recorded before and during procedures and lasted for 30 min. After that, ECG was recorded for 1 min once an hour for 7 days. Arrhythmia was counted for each animal during the first 30 min and for 10 seconds every hour for 7 days using Dataquest A.R.T. analysis program.

Measurement of Plasma Level of TNF\textalpha and Tissue Level of MPO:

The plasma level of TNF\textalpha and heart tissue levels of MPO were measured at 24h and 7d postsurgery by using a TNF\textalpha (BD Biosciences, San Diego, CA, U.S.A.) and MPO ELISA kit (Hycult Biotechnology, HK210) following the manufacturer’s instructions. For the determination of tissue MPO levels, murine hearts were harvested at 24 h and 7 days after surgical MI. LV’s were directly homogenized in lysis buffer [200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% glycerol, Leupeptin 1\µg/ml, Aprotinin 28 \µg/ml]. Protein concentration was determined by BCA assay. MPO levels were measured using a commercially available kit according to the manufacturer’s instructions (Mouse MPO ELISA Kit, Hycult Biotechnology, cat #HK210).

\textit{In vivo} hemodynamic measurements
We assessed *in vivo* cardiac hemodynamic function in total of 38 animal (n=9 in S-N or S-C and n=10 in MI-N or MI-C groups respectively) following 4 weeks of MI. Mice were anesthetized with a 2% Avertin and the right common carotid artery was isolated and cannulated with 1.4 French micromanometer (Millar Instruments, Houston, TX). LV pressure, LV end-diastolic pressure (LVEDP) and heart rate (HR) were measured by this catheter advanced into the LV cavity, and data was recorded and analyzed on a PowerLab System (AD Instruments Pty Ltd., Mountain View, CA) as we have previously described5-7. These parameters as well as maximal values of the instantaneous first derivative of LV pressure (+dP/dt\text{max}, as a measure of cardiac contractility) and minimum values of the instantaneous first derivative of LV pressure (-dP/dt\text{min}, as a measure of cardiac relaxation) were recorded at baseline and after administration of the β-adrenergic receptor (βAR) agonist, isoproterenol (Iso, 0.1 to 10 ng) as described5-7.

**In vivo echocardiographic measurements**

We assessed in vivo cardiac function in total of 40 animals (n=8 in sham MI-N or sham MI-C and n=12 in MI-N or MI-C groups respectively) at 4 weeks after permanent coronary occlusion and MI by using echocardiographic imaging system (Vevo 770, VisualSonic, Toronto, Canada). Mice were anesthetized with a 1.5% isoflurane and two-dimensional echocardiographic views of the mid-ventricular short axis were obtained at the level of the papillary muscle tips below the mitral valve. LV wall thickness and internal dimensions were measured and the LV fractional shortening (LVFS) and LV ejection fraction (LVEF) were calculated as previously described6, 8.

**Determination of LV infarct size in MI model**

A total of 32 and 26 mice were used for either acute phase (24 hrs) or chronic (28 d) infarct size measurement as indicated the Fig. 8 legend. Myocardial infarct sizing was determined as previously described6, 8. Briefly, at the end of 24 hrs or 4 weeks post-MI, mice were sacrificed and the heart was quickly excised and sliced into five 1.0 mm thick sections perpendicular to the long axis of the heart. The sections were then incubated with 1% triphenyltetrazolium chloride (TTC, Sigma) in phosphate solution (pH 7.4) for 15 min at room temperature and then digitally photographed. For infarct size at 24 hours post-MI, TTC-stained area, and TTC-negative staining area (infarct myocardium) were measured using the computer-based image analyzer SigmaScan Pro 5.0 (SPSS Science, Chicago, IL). Myocardial infarct size was expressed as a percentage of the total LV area. For infarct size at 4 weeks post-MI, the length of the scar and the circumference of LV were measured, the infarct size was expressed as the percentage of the circumference of LV.

**Determination of LV area at risk and infarct size in I/R model**

I/R induced myocardial infarction was determined by Evans blue/TTC double staining as described previously5, 7. Briefly, following 24 hours of reperfusion, the ligature around the LCA was re-tied through the previous ligation and 0.2 ml 2% Evans blue dye was injected into the right ventricle. The dye was circulated uniformly and distributed in the heart where it was not limited by the occluded coronary artery. The heart was then quickly excised, frozen with try ice and sliced into five 1.2 mm-thick slices that were perpendicular to the long axis of the heart. The unstained portion of myocardium (area at risk, AAR) was separated from the Evans blue-stained portion of the myocardium (area not at risk, ANAR). The slides were then incubated in PBS containing 1% triphenyltetrazolium chloride (TTC, Sigma) at room temperature for 15 min and then digitally photographed. The Evan’s blue–stained area (ANAR), TTC-stained area, and TTC-negative staining area (infarcted myocardium) were measured using the same software as describe above. Myocardial infarct size was expressed as a percentage of the
infarct area (I) over AAR (I/AAR) and the size of AAR was expressed as the percentage of AAR over total LV area (AAR/AAR+ANAR) as described previously\textsuperscript{7,9}.

**Statistical Analysis:**

All values in the text and figures are presented as mean ± SEM of independent experiments from given n-sizes. Statistical significance was determined by one-way or two-way ANOVA followed by the Bonferroni post-hoc test when appropriate. For the survival study, Kaplan-Meier analysis was used. The arrhythmia data was analyzed with Kruskal-Wallis test followed by Dunn test if it is necessary. Probabilities of 0.05 or less were considered to be statistically significant.
Supplemental Data

### Online Table I

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Number</th>
<th>Post-MI day</th>
<th>Number</th>
<th>Post-MI day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleeding</td>
<td>1</td>
<td>1-2</td>
<td>2</td>
<td>1-2</td>
</tr>
<tr>
<td>Unknown reason 1</td>
<td>3</td>
<td>3</td>
<td>4</td>
<td>1-3</td>
</tr>
<tr>
<td>Ventricular rupture</td>
<td>6</td>
<td>4-7</td>
<td>7</td>
<td>4-8</td>
</tr>
<tr>
<td>Unknown reason 2</td>
<td>3</td>
<td>9-18</td>
<td>3</td>
<td>8-16</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13</strong></td>
<td></td>
<td><strong>16</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Online Table I.** The reason of death for mice subjected to MI-N and MI-C after 6 hours peri-surgical period.

### Online Table II

<table>
<thead>
<tr>
<th>Condition</th>
<th>SI/R-N</th>
<th>SI/R-C</th>
<th>I/R-N</th>
<th>I/R-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)DP/dt (mmHg/s)</td>
<td>7563.25±304.6</td>
<td>7352.41±323.61</td>
<td>5428.51±284.26*</td>
<td>5111.13±361.12*</td>
</tr>
<tr>
<td>(-)DP/dt (mmHg/s)</td>
<td>-7355.10±481.64</td>
<td>-6974.31±332.46</td>
<td>-4945.47±284.26*</td>
<td>-4802.22±380.06*</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.12±0.92</td>
<td>2.48±0.43</td>
<td>8.13±0.82*</td>
<td>8.81±1.42*</td>
</tr>
<tr>
<td>HR (beat/min)</td>
<td>459.24±16.09</td>
<td>477.06±21.85</td>
<td>494.81±13.34</td>
<td>515.27±17.97</td>
</tr>
<tr>
<td>MABP (mmHg)</td>
<td>69.91±2.43</td>
<td>65.93±4.04</td>
<td>55.24±2.18</td>
<td>57.33±5.7</td>
</tr>
</tbody>
</table>

**Online Table II.** Cardiac function determined by hemodynamic measurement using Millar catheterization at 24 hr post surgery in sham I/R and I/R groups. Data are mean ± SEM, n=7-8 per groups, *p<0.05 vs. sham groups.
Supplemental Figure legend

**Online Figure I.** A) Hematoxylin and Eosin (H&E) staining of lung tissue at 24 hrs post-MI in control, sham and MI surgical groups.

**Online Figure II.** A) Figure 2. Representative electrocardiograms from MI-N and MI-C groups at baseline, 5 and 15 min after MI.

**Online Figure III.** Representative abnormal electrocardiogram from MI groups.

**Online Figure IV.** Representative Masson’s trichrome stained tissue sections from sham and MI-N and MI-C groups at 24 hour and 7 day post-MI.
References


Online Figure I. Hematoxylin and Eosin (H&E) staining of lung tissue at 24 hrs post-MI in control (Sham) and MI surgical groups.
Online Figure II. Representative electrocardiogram from MI-N and MI-C groups at baseline, 5 and 15 min after MI.
Online Figure III. Representative abnormal electrocardiogram from MI groups.
Online Figure IV. Representative Masson’s trichrome stained tissue sections from sham and MI-N and MI-C groups at 24 hour and 7 day post-MI.