Video Article

A Murine Model of Myocardial Ischemia-reperfusion Injury through Ligation of the Left Anterior Descending Artery

Zhaobin Xu[1], Jenna Alloush[1], Eric Beck[1], Noah Weisleder[1]

[1]Davis Heart & Lung Research Institute, Department of Physiology and Cell Biology, The Ohio State University

Correspondence to: Noah Weisleder at Noah.Weisleder@osumc.edu

URL: https://www.jove.com/video/51329
DOI: doi:10.3791/51329

Keywords: Medicine, Issue 86, Myocardial Ischemia/Reperfusion, permanent ligation, left anterior descending artery, myocardial infarction, LAD, ligation, Cardiac troponin I

Date Published: 4/10/2014

Citation: Xu, Z., Alloush, J., Beck, E., Weisleder, N. A Murine Model of Myocardial Ischemia-reperfusion Injury through Ligation of the Left Anterior Descending Artery. *J. Vis. Exp.* (86), e51329, doi:10.3791/51329 (2014).

Abstract

Acute or chronic myocardial infarction (MI) are cardiovascular events resulting in high morbidity and mortality. Establishing the pathological mechanisms at work during MI and developing effective therapeutic approaches requires methodology to reproducibly simulate the clinical incidence and reflect the pathophysiological changes associated with MI. Here, we describe a surgical method to induce MI in mouse models that can be used for short-term ischemia-reperfusion (I/R) injury as well as permanent ligation. The major advantage of this method is to facilitate location of the left anterior descending artery (LAD) to allow for accurate ligation of this artery to induce ischemia in the left ventricle of the mouse heart. Accurate positioning of the ligature on the LAD increases reproducibility of infarct size and thus produces more reliable results. Greater precision in placement of the ligature will improve the standard surgical approaches to simulate MI in mice, thus reducing the number of experimental animals necessary for statistically relevant studies and improving our understanding of the mechanisms producing cardiac dysfunction following MI. This mouse model of MI is also useful for the preclinical testing of treatments targeting myocardial damage following MI.

Video Link

The video component of this article can be found at https://www.jove.com/video/51329/

Introduction

Animal models of myocardial infarction (MI) are important in research of the complex pathophysiology of ischemic heart disease[1]. Ischemia-reperfusion (I/R) injury is a major contributor the myocardial damage generated during MI. The initial ischemia injury produced by occlusion of the coronary circulation can be minimized in MI patients by the use of angioplasty to restore perfusion in a timely fashion. While this intervention has greatly reduced the number of deaths due to acute MI, restoration of blood flow into the ischemic area results in I/R injury that leads to death of cardiomyocytes. This loss of myocardial mass contributes to decreased cardiac output and progression towards heart failure. Thus, study of the mechanisms that result in cardiomyocyte death from I/R injury is an important line of inquiry in cardiovascular research. Surgical coronary ligation is a useful experimental technique to induce models of MI in various animal types, including the rat, dog and pig. Publications in different laboratories have introduced various methods on the establishment of the mice heart model of I/R injury[2,3]. In order to gain insight into these mechanisms we must have access to reliable animal models that can reproduce several aspects of MI pathology. Development of such models is also essential for testing therapeutic approaches for treatment of MI and associated I/R injury.

Most of the currently available surgical techniques to simulate MI in experimental animals involve surgical dissection into the chest cavity to expose the left anterior descending artery (LAD) that is then occluded by a ligature for defined period in time to produce the ischemic event. Then that ligature can be removed to allow for reperfusion of the ischemic area and generation of I/R injury. One major limitation of these approaches in that the position of the literature on the LAD is not always accurately reproduced, which can lead to variation in the severity of the MI induced by this approach. Most available techniques only generally described the approximate location of the LAD in the anterior wall of the heart. As the branching and direction of the LAD can vary in individual animals the location is not always fixed and can be easily confused[4,5], leading to potential complications during surgery[6]. The consequences of improper placement of the ligature can run from variability in the size of the infarct induced in the left ventricle to completely compromising the specificity of the model. Here we present a modified method for myocardial I/R and permanent ligation in mice that allows for improved accuracy of placement of the ligature on the LAD. By applying specific approaches for the initial incision and internal dissection, as well as the use of manipulations to lift the atria to allow better appreciation of the LAD and the site where it emerges from the aorta. Establishing the position on the LAD and its origin provides the opportunity to ligate the LAD in a reproducible fashion. This model of myocardial I/R and permanent ligation not only decreases the variation in infarct size following surgery, it can also decrease the incidence of excessive bleeding during the operation.

Protocol

This animal protocol was approved by and is in accordance with the guidelines and regulations set forth by the Institutional Animal Care and Use Committee (IACUC) at The Ohio State University. All policies developed by the local IACUC are in compliance with the Animal Experimentation Guide developed by Office of Laboratory Animal Welfare at the National Institutes of Health.

1. Anesthesia and Endotracheal Intubation

1. Autoclave all instruments and surgical supplies before use. Wear sterile, single use surgical gloves throughout the procedure. Maintain a sterile field throughout the procedure. Use of a sterile drape is suggested but not shown in the video to allow for better visualization of anatomical landmarks on the mouse.
2. Place each mouse individually in an induction chamber and provide anesthesia using 5% isoflurane and oxygen with a flow rate of 0.4 L/min until loss of righting reflex and then maintain the animal with 2% isoflurane in 100% oxygen with a flow of 0.4 L/min by means of a nosecone tube connected to the anesthesia apparatus until the tracheal tube is in place. The isoflurane anesthesia machine used should be appropriately vented and equipped with charcoal filters to minimize exposure of the surgeon to isoflurane fumes during the procedure. The nosecone is noted but not shown in the video to allow for visualization of the manipulations to intubate the mouse.
3. Shave the animal's chest with an animal hair clipper in a different location than the surgery platform to avoid contamination of the surgery location.
4. Place the mouse in a supine position on surgery platform for subsequent intubation. A simple small polystyrene foam platform can serve as an operating platform. Cover the platform with a pre-sterilized drape to provide a sterile surface. Place a heating pad between the platform and drape to maintain the body temperature of the mice in surgical procedures.
5. Attach a length of 2–0 silk suture of at least 10 cm to the platform with tape and then loop the suture around the front upper incisors. Position the cone in close proximity (2-3 cm) to the edge of the platform over the nose of the mouse. Pull the mouse taut and secure it to the platform by the tail with a piece of tape.
6. Secure the legs to the sides of the body with strands of tape. It is important that the front limbs are not over-stretched as this can compromise respiration.
7. Prepare the shaved surgical sites with Betadine and alcohol before the neck and chest incisions are made.
8. Place the platform with the mouse head pointing in the direction of the operator. Cut a 0.5 cm median cervical skin incision. Separate the lobes of the thyroid gland at their isthmus to expose the sternohyoideus muscle where the trachea can be seen under the muscle.
9. Remove the inner needle of an 18 gauge trocar so it can be used as an intubation tube. The needle point can serve as a holder and 1 cm of the outer tube can serve as the tracheal tube.
10. Hold the tongue of the mouse with curved forceps in one hand and move it slightly upwards. View the trachea through the cervical skin incision. Use the other hand to gently insert the intubation tube until the tube is seen inside the trachea.
11. As soon as the tube is in the tracheal, move the curved forceps in other hand towards the tube and quickly remove the inner needle. If the tube cannot be inserted into the tracheal, the tube should be pulled out to avoid producing respiratory problems. It is important to point the tip of the tube up when it is close to the throat in order to avoid inserting the tube into the esophagus instead of the trachea.

2. Ventilation and Fixation

1. Provide artificial ventilation with an animal respirator venting 2% isoflurane in oxygen with a flow rate of 0.4 L/min. Use a modified Y-shape connector to connect the intubation tube with the ventilator. The correct positioning of the tracheal tube can be confirmed by judging the symmetrical chest expansion.
2. Set the tidal volume at 260 µl/stroke and ventilation rate is 130 strokes per minute, which can be adjusted to the body weight of a particular mouse if necessary.
3. Remove the tape on the tail and turn the mouse gently to place it in a right lateral decubitus position for the subsequent surgery. Use tape to secure the tail and legs to the platform again.
4. Insert the rectal probe to monitor the body temperature and adjust the warming pad to maintain the temperature around 37 ºC.
5. Secure the probe to the platform using tape. Inject bupivacaine subcutaneously at the incision site to numb the area before the incision is made.

3. Thoracotomy

1. Make an oblique incision that is approximately 1 cm long at a site 2 mm away from the left sternal border in the direction of where the left front leg meets the body (approximately 1-2 mm below where the leg and body join). The superficial thoracic vein is near this site and the incision should be made so that the lateral end of the incision goes up to, but does not cut into, the vein.
2. Cut though the thoracic muscle to expose the ribs underneath. During this step avoid accidental injury of the vessel. If bleeding does occur, use cotton applicators to stop any bleeding before proceeding to the next step[7].
3. Visualize the ribs and inflating lung through the thin and semitransparent chest wall. Open the chest cavity using surgical scissors to make a 6-8 mm incision in the third intercostal space. This incision should be a minimum of 2 mm from the sternal border where the internal thoracic artery is located. Damage to the artery will produce heavy bleeding that is difficult to control.
4. Insert the pre-sterilized homemade chest retractors into the incision and gently pull back to open the incision so that it is about 8-10 mm wide while being careful to avoid the lung. The retractors should be attached to the surgical platform with pins.
5. At this point the heart should be visible, however, the lung will still cover a portion of the heart. Pick up the pericardium gently with curved forceps, pull it apart, and slide the tissue behind the retractors. During this manipulation the lung will lift up and away from the heart.

4. Positioning LAD

1. Locate the LAD on the surface of the heart through a dissection microscope. The LAD runs down the middle of the heart wall from near the apex of the heart down through the left ventricle. The LAD appears bright red and will be pulsing strongly. The vein here is sometimes mistaken for the LAD, however proper lighting can help distinguish the two vessels. If the lighting is too bright it can be difficult to appreciate the color differences between the vessels.
2. Use a sterile cotton ball fragment with a diameter of approximately 1-2 mm to prepare the LAD for ligation. Place the cotton between the left atrium and left ventricular, which will lift the left atrium and help expose the LAD and clarify its position. If the LAD cannot be located, the fragment can be slid further in so the left atrium is lifted even higher to reveal the aorta where the LAD originates.

5. LAD Ligation

1. The ideal positioning for the ligature is approximately 2 mm lower than the tip of the left auricle. The pulmonary trunk can be used as a marker to help identify the left auricle. Alternatively, the ligation position can be visualized as a point 1-2 mm away from the branching of the left circumflex. Use curved forceps to gently apply pressure at a site immediately below the intended ligation point. This will make it easier to see the artery and will also help hold the heart in place and simplify tying the ligature. Do not apply pressure with the forceps for more than 5 seconds at a time and avoid compression of the heart that might alter pumping.
2. Use a tapered needle to pass a 6–0 silk suture underneath the LAD while observing with a dissecting microscope. Insert the needle under the artery with precision as the needle will enter the left ventricle chamber if placed too deeply or damage the LAD if the needle is too shallow. If the LAD is injured remove the needle and suture the LAD to control bleeding, however if bleeding cannot be controlled it is preferable to euthanize the animal.
3. Make a loose double knot with the suture, leaving a 2-3 mm diameter loop through which a 2-3 mm long piece of PE-10 tubing is placed[8].
4. Tighten the loop around the artery and tubing then secure the loop by tying one additional slipknot, taking care not to damage the ventricle wall. For permanent ligation, directly tie the LAD with a knot[9]. Confirm the occlusion of LAD by checking for appearance of a paler color in the anterior wall of the LV that should appear within a few seconds after ligation.
5. Remove the retractor and close the wound temporarily by pinching the skin together with a bulldog clamp. The length of time that ischemia is maintained depends on the experiment design, but is frequently 20, 30, 45 or 60 min. The mouse remains on the ventilator for the duration of the LAD artery occlusion.

6. Reperfusion

1. After the ischemia period remove the bulldog clip and insert the chest retractors to expose the ligature. Untie the knot and remove the PE-10 tubing. Confirm reperfusion by observing a return of the pink-red color of the anterior wall of the LV after 15-20 sec.
2. Leave the suture in place if 2% triphenyl tetrazolium chloride (TTC) and blue staining will be performed after reperfusion. If staining is not necessary, the suture can be removed.
3. The reperfusion time will depend on the experiment design, usually spanning from 1 hr to 24 hr.

7. Chest Closure and Postoperative Care

1. Close the chest cavity by sewing shut the incision in the 3rd intercostal space with 4–0 silk suture. It is important that the lungs are clear of the suture and do not become trapped as the 3rd and 4th ribs are sutured together. While tying the suture knots it is helpful to apply slight pressure to the chest with the needle holder to minimize any room air that might be trapped in the chest cavity.
2. Close all layers of muscle with continuous sutures using 4–0 silk. Use nylon sutures to close the skin with a continuous suture. Alternatively, the skin can be closed with interrupted suture.
3. When suturing is complete cease the flow of isoflurane while oxygen continues to flow. Once the mouse moves its whiskers or tail it, should start making attempts to breathe spontaneously. Remove the mouse from the ventilator with the intubation tube still kept in the trachea.
4. Observe the animal carefully until the mouse resumes a normal breathing pattern and then extubate the mouse. The tube should be removed slowly to avoid aspiration of oral cavity secretions.
5. Confirm the mouse is not in any respiratory distress by observing it for another 3-5 min before returning it to a cage. If signs of dehydration are observed after surgery, provide up to 0.5 ml of sterile saline by intraperitoneal injection.
6. For post-operative analgesia, administer an opioid analgesic (buprenorphine, 0.1 mg/kg) subcutaneously (SC) before the animal is ambulatory and then provide an additional dose every 4-6 hr for the next 24 hr. Check the animal signs of distress at 12 hr after surgery. Simulation of myocardial infarct using survival surgery requires assessment of pain and distress following recovery from the surgery. The current accepted best practice is to provide analgesia for the first 24 hr following an invasive procedure with additional doses given as warranted due to weight loss or signs of pain. For permanent ligation, body weight should be tracked daily to help to gauge the animal's recovery.
7. Ibuprofen (Motrin), a nonsteroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesia and antipyretic activity, or other NSAIDs, may be provided in the animal's drinking water as a 0.2 mg/ml solution for two days before the surgery and up to a 7 days after surgery in along with the buprenorphine to manage any additional pain/distress.

8. Measurement of Myocardial Infarct Size

1. Anesthetize and intubate the mouse at the end of desired reperfusion time. Cut the chest skin in the midline to the xyphoid. Open the abdomen and the diaphragm below the rib cage and from both sides of the midclavicular line.
2. Expose the heart and then re-ligate the LAD in the same location. Cannulate the aorta so 10% Phthalo Blue can be slowly injected directly into the aorta to stain the heart for delineation of the ischemic zone from the nonischemic zone[10].

3. Rapidly excise the heart and wash it in 30 mM KCl (potassium chloride solution) to cease the beating of the heart and allow for more consistent sectioning. Freeze the heart for at least 4 hr at -20 °C and cut the heart into slices of 1 mm using a heart matrix sectioning device[11].
4. Incubate heart slices with 2% TTC at 37 °C for 40 min. The infarct area is demarcated as a white area while viable tissue stains red.
5. Fix the stained slices with 10% formaldehyde overnight, which will help to increase the contrast between the infarct area and the normal tissue. Photograph the slices and calculate the area at risk (AAR), the nonischemic zone and the infarct area using ImageJ software.

9. Measurement of Cardiac Enzyme Levels

Measure cardiac troponin I (cTnI) levels in the serum of mice by obtaining blood from the portal vein and then isolating serum by centrifugation. Serum cTnI levels are then determined with a quantitative rapid cTnI assay[12].

Representative Results

Following 24 hours of reperfusion, analysis of infarct size and the area-at-risk (AAR), by phthalo blue dye and triphenyl tetrazolium chloride (TTC), ligation of the LAD can be confirmed by observing blanching of myocardial tissue distal to the suture as well as dysfunction of the anterior wall. Reperfusion can be verified by the return of red color to the myocardial tissue and the demonstration of some recovery of anterior wall motion.

Infarct areas (white) should be distinguishable from areas at risk (red) and the area not at risk (blue). Application of Phthalo Blue dye (**Figure 1A**) allows for resolution of the area of the heart where occlusion of the LAD, whereas hearts that are not stained with blue dye can only show the area of infarct (**Figure 1B**). Infarct sizes are dependent on duration of ischemia. Importantly, cardiac troponin I (cTnI) levels are low in sham operated animals that underwent all surgical procedures except ischemia and reperfusion as compared to animals which underwent myocardial infarction (**Figure 2**). This indicates the sham surgery did not produce significant cardiac pathology while the ischemia/reperfusion injury was sufficient to produce elevation of this widely used biomarker for MI.

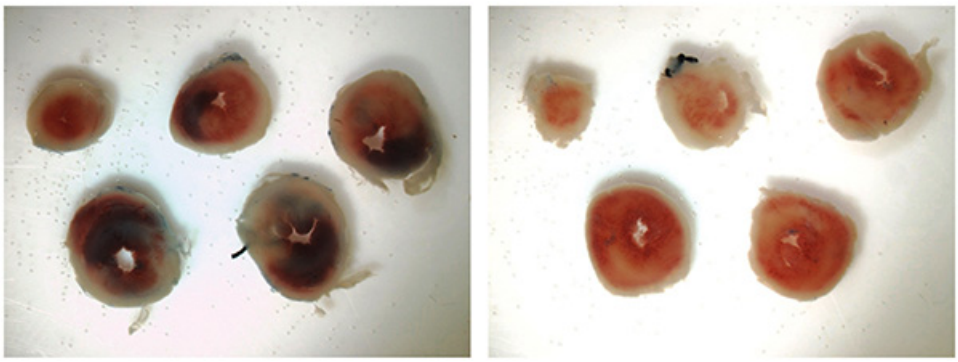

Figure 1: Quantification of the extent of infarct following LAD occlusion surgery. (**A**) Representative image of wild type mouse heart sections from animals subjected to 45 minutes ischemia and 24 hours reperfusion. Injection of blue dye allows for assessment of the non-ischemic zone of the heart that is not at risk for an infarct. (**B**) A representative image of a heart where blue dye is not injected to emphasize the area-at-risk (AAR), which appears red, and the infarct area, which appears white. The areas of each region are calculated as percentages of the total left ventricle (LV) area multiplied by the total weight of that slice. Click here to view larger image.

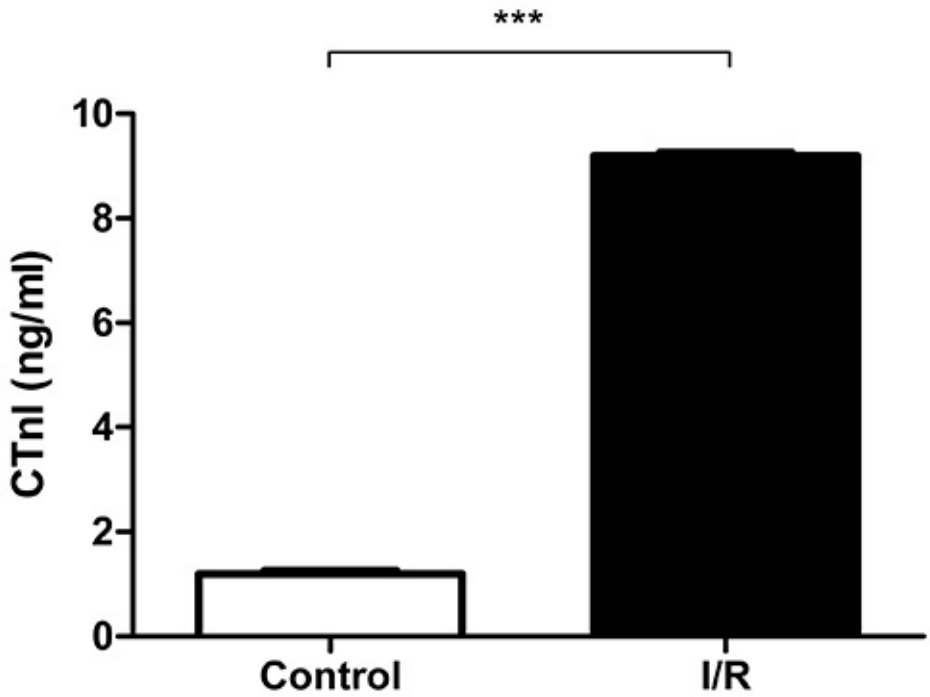

Figure 2: Use of cardiac troponin levels as a measurement of the extent of cardiac infarct. A bar chart of the cardiac troponin I (CTnI) levels in mice subjected to 45 minutes ischemia and reperfusion for 24 hours (I/R) or sham surgery as a control. Blood was collected from the portal vein at 24 hours after surgery from three animals for each group. The levels of cTnI are significantly elevated in animals following I/R injury (9.195 ± 0.07146) compared to the sham control animals (1.195 ± 0.06651). Data is presented as means ± S.E.M. and *** indicates P < 0.0001 comparing sham control and I/R groups by T-test. Click here to view larger image.

Discussion

Mouse myocardial ischemia-reperfusion models are an effective method for cardiovascular research to simulate clinical acute or chronic heart disease[13,14]. Significant effort has been applied to develop and refine surgical approaches that produce ischemic events and reperfusion damage in the hearts of several different animal types. While there are particular advantages to the use of different animals systems, the mouse has characteristics that have led to extensive interest in producing myocardial I/R in the mouse heart. One of the major reasons is the genetic tractability of the mouse system. The extensive selection of genetically modified animals available, and the relative ease by which new models can be generated to address specific questions, have no match in other animal model systems. Another reason for the increasing use of mice in cardiovascular studies is the increasing availability of surgical equipment and other experimental tools specifically designed for use in mice. The relatively low cost of mouse models is also an important contributor to their use in studies. The increasing need for rigor in preclinical studies necessitates the use of additional animals, which can be more realistic when fewer resources are necessary to include the appropriate number of animals. While the use of the mouse model has several advantages there are disadvantages as well, particularly when considering the divergent aspects of mouse and human cardiovascular physiology. Many larger animal models, such as the dog and pig, more closely mimic most aspects of human cardiovascular physiology than the mouse. Another disadvantage is the size of the mouse, manipulation of the smaller heart in the mouse requires a higher degree of surgical skill, particularly in locating the LAD and reproducibly ligating it to produce a consistent infarct area in the left ventricle. The method presented here can provide a significant improvement in identification and ligation of the LAD. Our consistent results in the amount of cTnI release from the heart (**Figure 2**) suggest that we can reproducibly generate infarct of a similar size and level of cardiomyocytes death.

A key aspect of surgeries to induce experimental myocardial infarct is the clear identification and ligation of the LAD. In our approach detailed here we have improved the methodology to identify and access the LAD, allowing for more consistent positioning of the ligation on the vessel. During the surgery, we make use of a small piece of sterile cotton to lift the left atrium up and fully expose the LAD, which clarifies the position of LAD and facilitates the ligation of LAD. This a critical step for the technique and a differentiation point from other approaches. The introduction of these modifications for LAD ligation should allow for more reproducible results during simulation of MI in mouse models. While improved precision in the placement of the ligature should improve consistency in the size of infarct generated it is still important to measure the at risk zone using perfusion of Phthalo Blue dye. This is particularly true during the use of genetic modification mouse lines where the manipulation of gene expression can result in changes in the response of the blood vessels of the heart to ligation.

Another critical step during ligation in confirming that ischemia has been effectively generated by the ligation of the LAD. Observation of a distinct, rapid color change in the area of risk is essential to be certain that ischemic conditions have been produced in the targeted section of the myocardium. The change in color of the myocardium should be seen within a few seconds if the LAD is effectively occluded. Other critical steps in the procedure involve the duration of the ischemic period and the time allowed for reperfusion before experimental endpoints are measured. As mentioned in the protocol, the length of the ischemic period can be varied to produce different degrees of ischemic damage to the heart. Generally a longer period of ischemia will result in more extensive myocyte death throughout the risk zone. The length of reperfusion can have effects on the development of cardiac pathology, including the appearance of fibrotic lesions in the heart as well as the stabilization of cardiac

output and electrophysiological changes. Thus, the specific length of these experimental steps must be tailored to address the specific questions examined in the study. The experimental endpoints should also be selected based on the length of ischemia and reperfusion periods used and the specific questions to be addressed in the experiment. We present the use of TTC staining to measure infarct size and ELISA measurements of serum CTnI levels as endpoints to assess the extent of cardiac damage. These endpoints can be used for any length of reperfusion, however they are particularly useful for shorter reperfusion periods (24 hours) where functional defects may not have stabilized yet. While we do not go into detail here on functional measurements of cardiac output, such as Doppler echocardiography[15] and microsphere measurements of coronary blood flow[16], these approaches are useful to understand the changes in cardiac function during longer term experiments, such as chronic occlusion of the LAD.

While the use of mouse models of MI have great advantages for the study of I/R injury in the heart there are still limitations to these approaches. Since major surgical incisions must be made into the chest cavity the resulting tissue disruptions and associated inflammation can influence the response of the heart to the MI effects. These concerns can be partially addressed through the use of sham surgical control mice, where all the surgical steps are all conducted with the exception of the tightening of the ligature around the LAD. Another issue that is produced by the invasive nature of the surgery is the need to manage the pain and suffering that occurs during and after the procedure. Pain management approaches that conform to current best practices are detailed in this procedure and are necessary to prevent suffering of the experimental animals. It is important to be aware that the use of many different types of anesthetics and analgesics can have cardioprotective effects following their application. Thus, it appropriate to apply these agents to the control mice, even any control mice that are not used for sham surgeries, in order to avoid any complications to interpretation of experimental results. Another limitation to this approach is that it does not provide a perfect simulation of pathology associated with human MI. Frequently the mouse models used for such experiments do not suffer from co-morbidities that underlie the MI in humans, such as coronary vascular disease, diabetes and hypertension. Such complications that are not present in the mouse model could have effects on the pathways being studied in a particular experiment and should therefore be considered when interpreting results. In these cases, the use of genetically modified mice that display some of these underlying pathologies may be appropriate to more effectively model the disease as it would present in human patients. In the future, other aspects of this approach could be modified to more accurately simulate additional aspects of human MI pathology.

Despite these limitations, the methods described here represent an effective approach to produce localized I/R injury in the mouse that simulates much of the pathologic effects of MI in human patients. Our technique allows for easier manipulation of the LAD that can lead to more reproducible results and simplify the surgery. However, mastering this technique still requires significant surgical skill that can only be gained through practice of the procedure. Taking sufficient care when conducting the surgery, particularly at places where this is noted in the protocol, will improve the survival rate of animals as well and the reproducibility of the experimental results. Once the surgical approach is mastered, this protocol will prove quite useful to investigators studying the effect of MI on cardiovascular physiology as well as those interested in testing the efficacy of therapeutic interventions on a mouse model.

Disclosures

Dr. Noah Weisleder is a Founder and Chief Scientific Officer at TRIM-edicine, Inc.

Acknowledgements

Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases, part of the National Institutes of Health, under Award Number R01-AR063084. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

1. Abarbanell, A. M. *et al.* Animal models of myocardial and vascular injury. *J Surg Res* **162**, 239-249, doi:10.1016/j.jss.2009.06.021, S0022-4804(09)00351-5 [pii] (2010).
2. Gao, E. *et al.* A novel and efficient model of coronary artery ligation and myocardial infarction in the mouse. *Circ Res* **107**, 1445-1453, doi:10.1161/CIRCRESAHA.110.223925, CIRCRESAHA.110.223925 [pii] (2010).
3. Virag, J. A. & Lust, R. M. Coronary artery ligation and intramyocardial injection in a murine model of infarction. *J Vis Exp*, doi:10.3791/2581, 2581 [pii] (2011).
4. Salto-Tellez, M. *et al.* Myocardial infarction in the C57BL/6J mouse: a quantifiable and highly reproducible experimental model. *Cardiovasc Pathol* **13**, 91-97, doi:10.1016/S1054-8807(03)00129-7, S1054880703001297 [pii] (2004).
5. Kumar, D. *et al.* Distinct mouse coronary anatomy and myocardial infarction consequent to ligation. *Coron Artery Dis* **16**, 41-44, doi:00019501-200502000-00008 [pii] (2005).
6. Degabriele, N. M. *et al.* Critical appraisal of the mouse model of myocardial infarction. *Exp Physiol* **89**, 497-505, doi:10.1113/expphysiol.2004.027276, expphysiol.2004.027276 [pii] (2004).
7. Shao, Y., Redfors, B. & Omerovic, E. Modified technique for coronary artery ligation in mice. *J Vis Exp*, doi:10.3791/3093 (2013).
8. Klocke, R., Tian, W., Kuhlmann, M. T. & Nikol, S. Surgical animal models of heart failure related to coronary heart disease. *Cardiovasc Res* **74**, 29-38, doi:S0008-6363(06)00514-1, [pii] 10.1016/j.cardiores.2006.11.026 (2007).
9. Nossuli, T. O. *et al.* A chronic mouse model of myocardial ischemia-reperfusion: essential in cytokine studies. *Am J Physiol Heart Circ Physiol* **278**, H1049-1055 (2000).
10. Cozzi, E. *et al.* Ultrafine particulate matter exposure augments ischemia-reperfusion injury in mice. *Am J Physiol Heart Circ Physiol* **291**, H894-903, doi:01362.2005, [pii] 10.1152/ajpheart.01362.2005 (2006).
11. Kim, S. C. *et al.* A murine closed-chest model of myocardial ischemia and reperfusion. *J Vis Exp*, e3896, doi:10.3791/3896, 3896 [pii] (2012).
12. Nagarajan, V., Hernandez, A. V. & Tang, W. H. Prognostic value of cardiac troponin in chronic stable heart failure: a systematic review. *Heart* **98**, 1778-1786, doi:10.1136/heartjnl-2012-301779, heartjnl-2012-301779 [pii] (2012).

13. Borst, O. *et al.* Methods employed for induction and analysis of experimental myocardial infarction in mice. *Cell Physiol Biochem* **28**, 1-12, doi:10.1159/000331708, 000331708 [pii] (2011).
14. Diepenhorst, G. M., van Gulik, T. M. & Hack, C. E. Complement-mediated ischemia-reperfusion injury: lessons learned from animal and clinical studies. *Ann Surg* **249**, 889-899, doi:10.1097/SLA.0b013e3181a38f45 (2009).
15. Benavides-Vallve, C. *et al.* New strategies for echocardiographic evaluation of left ventricular function in a mouse model of long-term myocardial infarction. *PLoS One* **7**, e41691, doi:10.1371/journal.pone.0041691, PONE-D-11-08927 [pii] (2012).
16. Bamberg, F. *et al.* Accuracy of dynamic computed tomography adenosine stress myocardial perfusion imaging in estimating myocardial blood flow at various degrees of coronary artery stenosis using a porcine animal model. *Invest Radiol* **47**, 71-77, doi:10.1097/RLI.0b013e31823fd42b, 00004424-201201000-00012 [pii] (2012).

