Video Article

Acute Myocardial Infarction in Rats

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Abstract

With heart failure leading the cause of death in the USA (Hunt), biomedical research is fundamental to advance medical treatments for cardiovascular diseases. Animal models that mimic human cardiac disease, such as myocardial infarction (MI) and ischemia-reperfusion (IR) that induces heart failure as well as pressure-overload (transverse aortic constriction) that induces cardiac hypertrophy and heart failure (Goldman and Tarnavski), are useful models to study cardiovascular disease. In particular, myocardial ischemia (MI) is a leading cause for cardiovascular morbidity and mortality despite controlling certain risk factors such as arteriosclerosis and treatments via surgical intervention (Thygesen). Furthermore, an acute loss of the myocardium following myocardial ischemia (MI) results in increased loading conditions that induces ventricular remodeling of the infarcted border zone and the remote non-infarcted myocardium. Myocyte apoptosis, necrosis and the resultant increased hemodynamic load activate multiple biochemical intracellular signaling that initiates LV dilatation, hypertrophy, ventricular shape distortion, and collagen scar formation. This pathological remodeling and failure to normalize the increased wall stresses results in progressive dilatation, recruitment of the border zone myocardium into the scar, and eventually deterioration in myocardial contractile function (i.e. heart failure). The progression of LV dysfunction and heart failure in rats is similar to that observed in patients who sustain a large myocardial infarction, survive and subsequently develops heart failure (Goldman). The acute myocardial infarction (AMI) model in rats has been used to mimic human cardiovascular disease; specifically used to study cardiac signaling mechanisms associated with heart failure as well as to assess the contribution of therapeutic strategies for the treatment of heart failure. The method described in this report is the rat model of acute myocardial infarction (AMI). This model is also referred to as an acute ischemic cardiomyopathy or ischemia followed by reperfusion (IR); which is induced by an acute 30-minute period of ischemia by ligation of the left anterior descending artery (LAD) followed by reperfusion of the tissue by releasing the LAD ligation (Vasilyev and McConnell). This protocol will focus on assessment of the infarct size and the area-at-risk (AAR) by Evan's blue dye and triphenyl tetrazolium chloride (TTC) following 4-hours of reperfusion; additional comments toward the evaluation of cardiac function and remodeling by modifying the duration of reperfusion, is also presented. Overall, this AMI rat animal model is useful for studying the consequence of a myocardial infarction on cardiac pathophysiological and physiological function.

Video Link

The video component of this article can be found at https://www.jove.com/video/2464/

Protocol

1. Preparation of the Operating Area

1. The operating surface is first prepared by disinfecting the area with 70% ethanol.
2. All surgical instruments are to be sterilized with a hot bead sterilizer before surgery (and in between individual rat surgeries). These instruments include: surgical scissors (2), forceps (1), curved forceps (1), needle holder (2), and a chest rectractor.
3. Cotton gauze and applicators are to be available in order to manage bleeding.
4. Adjust the temperature of a homeothermic blanket system (heating pad), to be used to maintain the body temperature of the animal at 37±1°C, in order to prevent a dramatic fall in body temperature; the size of an infarct is dependent on the duration of occlusion as well body temperature (Tarnavski *et al.*).
5. Prior to anesthesia, each animal will receive a dosage of buprenorphine (0.1-2.5 mg/kg SQ) and carprofen oral tablets (Rimadyl, Bioserv tablet; 1 tablet) the day before the surgery. At the time immediately prior to the surgery, each animal will again receive a dosage of buprenorphine (0.1-2.5 mg/kg SQ).
6. Note: For studies involving the assessment of cardiac function and cardiac remodeling over a period of time (7- to 28-days and beyond), a dosage of buprenorphine (0.1-2.5 mg/kg SQ) will be administered, every 6-hours after surgery for 24-48 hours post surgery and carprofen oral tablets will be provided once daily (see "Post-Operative Recovery").

2. Preparation and Intubation of Rats

1. Prior to the induction of anesthesia, animals will be examined to identify any pre-existing conditions that may complicate surgical outcome; physical examination will include visual inspection and measurement of heart rate, respiratory rate, and body temperature and body weight.
2. If the animals are determined to be healthy, 250-300 gram male rats (e.g. Sprague Dawley) will be weighed and identifying characteristics will be recorded.
3. Rats are then anesthetized with 80 mg/kg of pentobarbital; the correct level of anesthesia is verified through the limb withdrawal response by applying pressure of the rat nail bed (toe-pinch reflex) and periodically throughout the procedure.
4. Buprenorphine (0.1-2.5 mg/kg; SQ) will be administered at the time of surgery.
5. An electric shaver is then used to shave the fur form the neck and chest areas. Note: For studies involving the assessment of cardiac function and cardiac remodeling over a period of time (7- to 28-days and beyond), fur must be completely shaved away from the surgical area to maintain sterility.
6. The shaved areas are to be scrubbed and disinfected with betadine solution followed by wiping the area with 70% alcohol; this betadine and alcohol scrub and disinfection procedure is to be repeated three times.
7. The anesthetized animal is then placed in a supine position on a homeothermic blanket system (heating pad) in order to maintain body temperature.
8. Endotracheal intubation is then performed by first carefully exposing the trachea, to enhance visualization of intubation, by making a 5 mm mid-neck incision and retraction of muscle tissue, just above the trachea.
9. This allows better visualization for insertion of the endotracheal tube (polyethylene size 90; PE 90) where the tubing is beveled on the edge for ease of entrance through the larynx.
10. The endotracheal tube is inserted into the trachea, with visualization aided by using a surgical stereo microscope, by carefully manipulating the tongue while viewing the trachea and the entrance of the endotracheal tube.
11. Mechanical ventilation is then achieved by connecting the endotracheal tube to Kent Scientific ventilator (TOPO Dual Mode) cycling at 80 breaths per minute and a tidal volume of 1.2 ml per 100 gram body weight.
12. A sterile drape is then placed over the rat, leaving only the surgical area exposed, thus minimizing potential contamination during the operation.
13. The animal surgeon and assistants are to wear mask, gown, and gloves; with gloves being changed between individual rat surgeries.

3. Transient Left Anterior Descending (LAD) Artery Ligation

1. Once steady breathing is established, the chest is opened at the left fourth intercostal space, by using hemostats or round end scissors to open the space, but without cutting the tissue so that the risk of bleeding can be reduced.
2. A chest retractor is then positioned within the fourth intercostal space; in order to spread the ribs so that the left ventricle (LV) is exposed, taking maximal care not to damage the lung.
3. The pericardium is then opened, but left in place if possible.
4. The proximal left anterior descending (LAD) artery will be identified (Figure-1), with the use of a surgical stereo microscope, and the LAD is then transiently ligated (or can be tied with a slipknot) using a 6-0 prolene suture for a 30-minute ischemic period (Figure-1), but without exteriorization of the heart.
5. To allow cardiac reperfusion, microsurgical scissors are used to cut the knot in the ligature (or by releasing the slipknot), made by the 6-0 prolene suture, 30-minutes following ligation of the LAD.
6. In sham control rats, the procedure is identical, except the LAD is not transiently ligated.
7. The outflow is then briefly (1-2 seconds) pinched off on the respirator to allow re-inflation of the lungs.
8. The chest retractor is then removed and the ribs are drawn together using a 2-0 prolene suture with an interrupted suture pattern.
9. Once the ribs are closed, the outflow of the ventilator is again briefly (1-2 seconds) pinched off to ensure proper breathing.
10. The skin is closed using 6-0 prolene suture with a continuous suture pattern.

4. Confirmation of Successful Ligation of the LAD Artery

1. Proper ligation of the LAD will be confirmed by observing blanching of myocardial tissue distal to the suture as well as dysfunction of the anterior wall; as observed during the transient LAD ligation.
2. Reperfusion will be verified by the return of red color to the myocardial tissue and the demonstration of some recovery of anterior wall motion; as observed immediately following the transient LAD ligation.
3. Note: For studies involving the assessment of cardiac function and cardiac remodeling over a period of time (7- to 28-days and beyond), an echocardiogram will be performed 24-hours post LAD ligation, in order to determine the extent of the induced myocardial infarction. All animals with a left ventricular fractional shortening (LV FS) < 20%, as determined from an echocardiogram, as well as anterior wall motion dysfunction consistent with the infarct, will be identified and used for follow-up cardiac function and remodeling studies. Sham control rats are expected to have a normal LV FS between 40-45% (del Monte et al).

5. Post-Operative Recovery

1. Once the surgery is complete, the sterile drape over the animal will be removed. The body temperature (by a rectal thermometer), respiratory rate (by visual inspection), heart rate (by palpitation), and abnormal signs of pain are to be monitored until the animal becomes ambulatory.
2. The rat will be removed from the ventilator and then be placed in an animal cage and allowed to recover from anesthesia. In this cage, one end of the cage will be warmed by a heating pad to allow the rat to seek warmth. Also, water will be available to the rat during this observation period.
3. If signs of dehydration are observed after surgery, 0.5 ml of sterile saline will be injected intraperitoneally (IP).
4. Buprenorphine (0.1#2.5 mg/kg; SQ) will then be administered immediately after surgery, and during post-operative recovery.

5. Note: For studies involving the assessment of cardiac function and cardiac remodeling over a period of time (7- to 28-days and beyond), a dosage of buprenorphine (0.1-2.5 mg/kg SQ) will be administered, every 6-hours after surgery for 24-48 hours post surgery. Carprofen oral tablets (Rimadyl, Bioserv tablet) will also be provided (1-tablet daily) for at least 48-hours post surgery.

6. Histological Evaluation of the Ischemia Reperfusion

1. Following completion of the 4-hour reperfusion period, rats are to be anesthetized again with 80 mg/kg of pentobarbital and the LAD is ligated once again (or the slipknot can be retied) (Huang et al and Kaiser et al).
2. 1 ml of 5 % Evan's Blue (in phosphate-buffered saline) is then injected into the right ventricular chamber and allowed to perfuse for 2-minutes.
3. After 2-minutes, 1 ml of saturated potassium chloride solution is then injected into the right ventricular chamber in order to stop the heart from beating.
4. The heart is then rapidly excised, embedded in 2% agarose phosphate-buffered saline, and then sectioned in 1-2 mm slices.
5. The viable myocardium is then stained with 2% triphenyl tetrazolium chloride (TTC) for 20-minutes and images of each slice is captured. The images are then quantified in order to identify the amount of myocardial area: (1) that is not-at-risk (blue sections), (2) area-at-risk (AAR; red and white sections), and (3) area that is infarcted (white "unstained" sections) (Figure-2).

7. Representative Results

For studies involving the assessment of infarct size and the area-at-risk (AAR), by Evan's blue dye and triphenyl tetrazolium chloride (TTC), following 4-hours of reperfusion, proper 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; as observed during the transient LAD ligation. Reperfusion can be verified by the return of red color to the myocardial tissue and the demonstration of some recovery of anterior wall motion; observed immediately following the transient LAD ligation. For studies involving the assessment of cardiac function and cardiac remodeling over a period of time (7- to 28- days and beyond), proper ligation of the ischemia followed by reperfusion can also be confirmed by blanching of the tissue, dysfunction of the anterior wall and the return of red color with some recovery of the wall motion following reperfusion. In addition, impaired cardiac function can be determined by an echocardiogram being performed 24-hours post LAD ligation, in order to determine the extent of the induced myocardial infarction. All animals with a left ventricular fractional shortening (LV FS) < 20%, as determined from an echocardiogram, as well as anterior wall motion dysfunction consistent with the infarct, will be identified and used for follow-up cardiac function and remodeling studies. Sham control rats are expected to have a normal LV FS between 40-45% (del Monte).

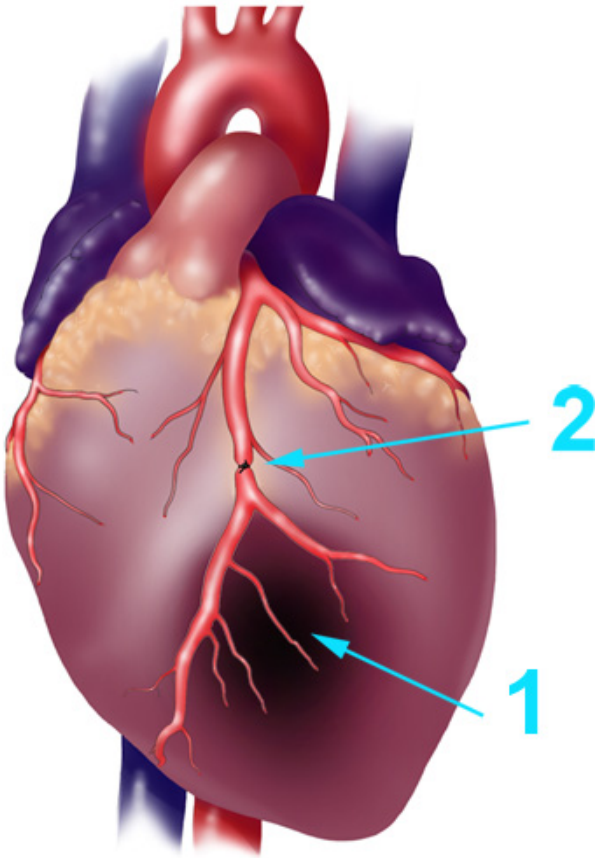

Figure 1. Schematic Diagram of a Myocardial Infarction and LAD Ligation. Schematic diagram of (1) myocardial infarction and (2) ligation of the left ventricular anterior descending (LAD) artery using a suture, used to mimic the myocardial infarction caused by plague formation.

Figure 2. Representative Image of a Rat Heart Following AMI. Representative image of the rat heart showing the area in blue (non-occluded coronary perfusion area), area in red (occluded coronary perfusion area indicating the area-at-risk (AAR) for infarction, and area in white/yellow (infarcted zone within the AAR).

Discussion

An acute myocardial infarction (AMI) in rats, also referred to as ischemia-reperfusion (IR), is commonly used to mimic human ventricular remodeling leading to heart failure. The progression of LV dysfunction and heart failure in animal models (rats and mice) is similar to that observed in humans who sustain a MI, survive and then subsequently develops heart failure (Goldman). This procedure outlined one version of this technique and also contained additional notes describing methodological variations. Additional such variations to the acute myocardial infarction (AMI) procedure include the duration of ischemia and the duration of reperfusion. Also, there are variations regarding the surgical procedure used to gain access and ligate the LAD. In regard to the duration of the ischemic period, the time of the LAD ligation to induce a myocardial infarction can be restricted acutely (30-minutes to 60-minutes; AMI), or alternatively, restricted chronically (permanent; MI). In regard to the duration of reperfusion following this initial ischemic period prior to myocardial assessment (i.e. the subsequent reperfusion period), can be short (4-hours to 24-hours) in order to evaluate infarct size and area-at-risk (AAR) by Evan's blue dye and triphenyl tetrazolium chloride (TTC), or alternatively, prolonged (7-days to 28-days and beyond) in order to evaluate cardiac function and remodeling. This pathological cardiac remodeling (apoptosis, necrosis and LV dysfunction) will vary depending on the chosen duration of ischemia and reperfusion. As an alternative to the surgical procedure of minimally exposing the heart in order to ligate the LAD within the fourth intercostal space and without exteriorization of the heart (see Part 3: Transient Left Anterior Descending (LAD) Artery Ligation), an incision through the sternum, to create a larger access and view of the heart, may be performed. Briefly, an incision is made through the ventral chest skin to mid-thorax after which the thorax is opened to mid-sternum. This partial thoracotomy is followed by retracting the sternal edges with a retractor. The proximal LAD artery is then identified, with the use of a surgical microscope, and either transiently ligated (or can be tied with a slipknot) using a 6-0-prolene suture for a 30-minute ischemic period (AMI) or permanently ligated using a 6-0-prolene suture (MI). For AMI procedures, microsurgical scissors will be used to cut the knot in the ligature 30-minutes following LAD ligation. The outflow is then briefly (1-2 seconds) pinched off on the respirator to allow re-inflation of the lungs. The retractor is removed and the ribs are drawn together and sutured using 2-0 prolene with an interrupted suture pattern. Once the chest is closed, the outflow is briefly pinched off again to ensure proper breathing and the skin is closed using 6-0 prolene sutures with a continuous suture pattern. Overall, the acute myocardial infarction (AMI) animal model described in this protocol mimics human ischemic heart disease and serves as an important tool to help elucidate the molecular signaling mechanisms involved in cardiac remodeling and heart failure.

Disclosures

No conflicts of interest declared.

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