Video Article

LAD-Ligation: A Murine Model of Myocardial Infarction

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Abstract

Research models of infarction and myocardial ischemia are essential to investigate the acute and chronic pathobiological and pathophysiological processes in myocardial ischemia and to develop and optimize future treatment.

Two different methods of creating myocardial ischemia are performed in laboratory rodents. The first method is to create cryo infarction, a fast but inaccurate technique, where a cryo-pen is applied on the surface of the heart (1-3). Using this method the scientist can not guarantee that the cryo-scar leads to ischemia, also a vast myocardial injury is created that shows pathophysiological side effects that are not related to myocardial infarction. The second method is the permanent ligation of the left anterior descending artery (LAD). Here the LAD is ligated with one single stitch, forming an ischemia that can be seen almost immediately. By closing the LAD, no further blood flow is permitted in that area, while the surrounding myocardial tissue is nearly not affected. This surgical procedure imitates the pathobiological and pathophysiological aspects occurring in infarction-related myocardial ischemia.

The method introduced in this video demonstrates the surgical procedure of a mouse infarction model by ligating the LAD. This model is convenient for pathobiological and pathophysiological as well as immunobiological studies on cardiac infarction. The shown technique provides high accuracy and correlates well with histological sections.

Video Link

The video component of this article can be found at https://www.jove.com/video/1438/

Protocol

Balb/C mice weighing a minimum of 20g at an age of 8 to 12 weeks are purchased from Charles River (Sandhofer Weg 7, D-97633 Sulzfeld). Mice are housed under conventional conditions, fed standard mouse pellets and water *ad libitum*. Anesthetize mouse with isoflurane (2%) using an induction chamber.

1. Shave the neck area and the left side of the ribcage and disinfect using 80% ethanol.
2. Place the mouse on its back and place a facemask over its nose and mouth to keep up the anesthesia.
3. Check the reflexes pinching the tail and hind feet to be sure that the mouse is sufficient anesthesized.
4. Under microscopic view perform a midline cervical incision separating the skin, muscle and tissue covering the trachea.
5. When the trachea is exposed cut a small hole into the tissue between two cartridge rings below the glottis to insert the endotracheal tube (Figure 1). Insert the endotracheal tube holding the cranial part of the trachea using micro surgical forecepts. Check the thoracic movement to be sure that both lungs are well ventilated.
6. The respiration rate (RR) should be approximately 110 per minute, with an inspiratory pressure of 17 to 18cm H_2O. Turn the mouse carefully, lying on its right side, facing its left side. Perform a leftsided thoracotomy between the 3rd and the 4th rib, and dissect the tissue and muscle carefully, using a cauter to prevent bleeding.
7. Open the thorax carefully, once the thorax is opened, find the heart, without touching the lung with any sharp object. Now remove the part of the pericardial sac that is covering the heart.
8. The LAD is located between the pulmonary artery and the left auricle. Use an 8-0 Prolene suture (Ethicon, Norderstedt, Germany) to ligate the LAD proximal with one single suture (Figure 2).
9. Place a chest tube (28G, venal catheter), between the 4th and the 5th rib.
10. Close the thoracic incision in layers, using 6-0 Prolene running sutures (Ethicon, Norderstedt, Germany) to adapt the ribs and 4-0 Prolene running sutures (Ethicon, Norderstedt, Germany) to close the skin.
11. Drain the thorax with the help of a 2ml syringe carefully (Figure 3). Place the mouse on its back. Now take the endotracheal tube out and adapt the tracheal cartridge rings with one single stitch using 7-0 Prolene sutures (Ethicon, Norderstedt, Germany).

12. Place the face mask on the mouse and close the skin using 4-0 Prolene running sutures (Ethicon, Norderstedt, Germany).

Validation

There are different possibilities to confirm the success of the LAD-ligation.

The troponin test can be performed 6 to 18 hours after surgery, using only 150 l blood without the need to euthanize the animal. The blood is applied to a customized troponin test kit (TROP T Sensitive, Roche, Mannheim) (Figure 4). Troponin is a regulative protein in the actin filaments of the muscle cell, there are different isotypes in the three types of muscle. The isotypes cTnI and cTnT are found in the heart and are released in tissue injury. The standardized test for troponin T (cTnT) is based on two heart specific monoclonal antibodies, one traps the troponin in the blood sample, the second one is a marker.

The infarcted area can be identified macroscopically after 3 days (Figure 5).

TTC (2,3,5-Triphenyltetrazolium chloride) staining measures tissue viability used to evaluate infarct size. Evans blue dye (1,5%, 1.0mL) in phosphate-buffered saline (PBS) is injected into the left ventricular cavity to measure the myocardial ischemic area. The mouse is euthanized and the heart is harvested and sectioned into slices. The tissue slices are incubated in 1% TTC PBS solution, pH 7.4 at 37°C for 20min. Tissues are fixed in 10% PBS-buffered formalin overnight at 2-8°C. TTC is administered ex vivo to dye Evans blue-negative areas (Figure 6).

For histology the mouse has to be euthanized and the heart has to be embedded for further processing. After performing paraffin sections, slides are stained with H&E (hematoxylin and eosin) or trichrome to visualize fibrotic tissue (Figure 7).

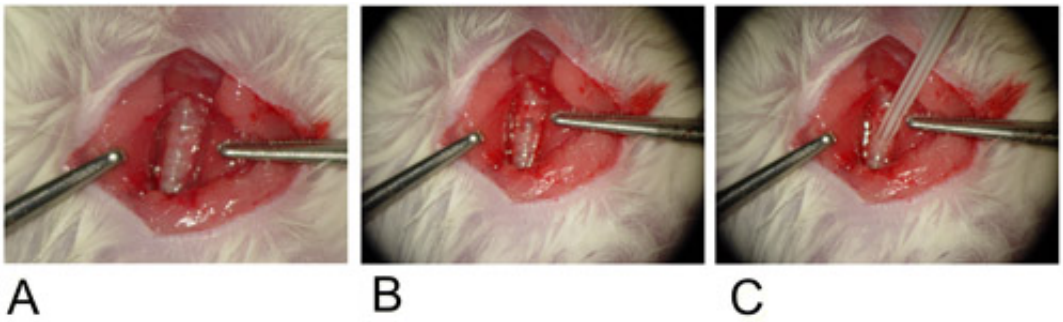

Figure 1: Tracheal tube.
1A: Exposed trachea, after ventral cervical incision.
1B: Trachea is opened two cartilage rings caudal to the larynx.
1C: Tracheal tube is inserted into the trachea.

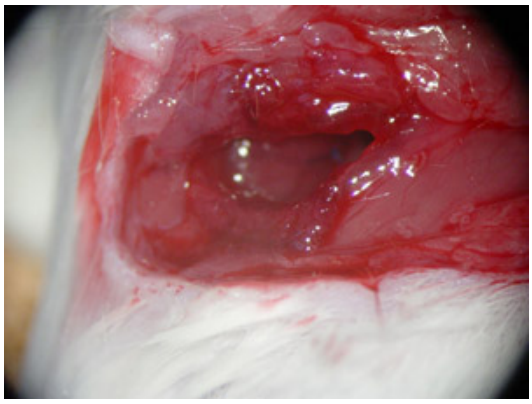

Figure 2: The heart is exposed through a left sided thoracotomy, the LAD is ligated.

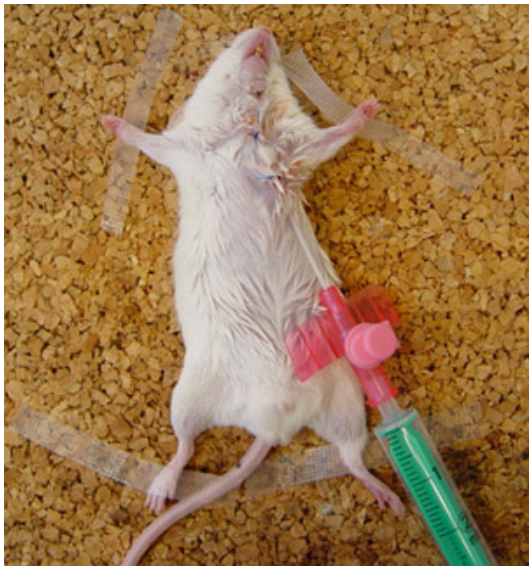

Figure 3: After the thorax is closed, a pleura drain is performed. The mouse is still under anesthesia.

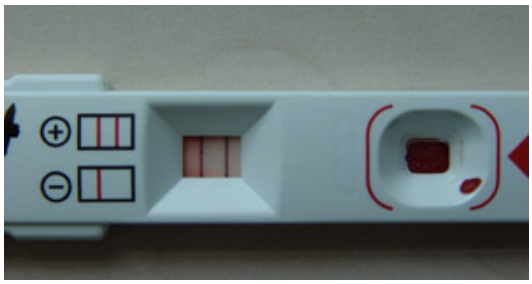

Figure 4: Two lines appear after a few minutes when the troponin test is positive.

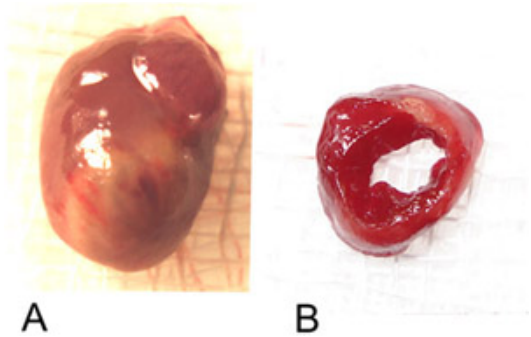

Figure 5: Shows the explanted heart.
5A: Macroscopic view of the heart with the ischemic area with a faded whitish color.
5B: Cross section of the infarcted heart, the ischemic area shows in the upper right corner.

Discussion

In summary, this video visualizes the LAD-ligation model step-by-step and identifies LAD-ligations in mice as easily feasible surgical procedures.

Disclosures

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Statement

All animals received human care in compliance with the Principles of Laboratory Animal Care, experiments on animals were performed in accordance with the guidelines and regulations set forth by the National Society for Medical research and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (National Institutes of Health publication 85-23, revised 1985).

All animals were obtained from Charles River Laboratories (Sulzfeld, Germany) and were maintained in the animal care facilities of the University Hospital Hamburg Eppendorf. The animals received standard chow and water ad libitum.

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