Video Article

Murine Isolated Heart Model of Myocardial Stunning Associated with Cardioplegic Arrest

Brenda Cordeiro[1], Richard Clements[1,2]

[1]Cardiothoracic Surgery Research Center, Brown University

[2]Rhode Island Hospital and Alpert Medical School, Brown University

Correspondence to: Richard Clements at richard_clements@brown.edu

URL: https://www.jove.com/video/52433
DOI: doi:10.3791/52433

Keywords: Medicine, Issue 102, Ischemia, Langendorff, cardioplegia, myocardial stunning, cardiac function, isolated hearts

Date Published: 8/6/2015

Citation: Cordeiro, B., Clements, R. Murine Isolated Heart Model of Myocardial Stunning Associated with Cardioplegic Arrest. *J. Vis. Exp.* (102), e52433, doi:10.3791/52433 (2015).

Abstract

The following protocol is of use to evaluate impaired cardiac function or myocardial stunning following moderate ischemic insults. The technique is useful for modeling ischemic injury associated with numerous clinically relevant phenomenon including cardiac surgery with cardioplegic arrest and cardiopulmonary bypass, off-pump CABG, transplant, angina, brief ischemia, *etc*. The protocol presents a general method to model hypothermic hyperkalemic cardioplegic arrest and reperfusion in rodent hearts focusing on measurement of myocardial contractile function. In brief, a mouse heart is perfused in langendorff mode, instrumented with an intraventricular balloon, and baseline cardiac functional parameters are recorded. Following stabilization, the heart is then subject to brief infusion of a cardioprotective hypothermic cardioplegia solution to initiate diastolic arrest. Cardioplegia is delivered intermittently over 2 hr. The heart is then reperfused and warmed to normothermic temperatures and recovery of myocardial function is monitored. Use of this protocol results in reliable depressed cardiac contractile function free from gross myocardial tissue damage in rodents.

Video Link

The video component of this article can be found at https://www.jove.com/video/52433/

Introduction

Myocardial stunning is defined as reversible reduced contractile activity despite restoration of adequate blood flow following a brief period of ischemia or prolonged periods of ischemic insults with cardioprotection[1,2,3,4,5]. The method presented is specifically used to model clinically relevant ischemic insults which can result in reversible impairments in contractile function (*i.e.,* ischemic insults associated with cardiac surgery utilizing cardioplegic arrest, brief periods of ischemia, angina, *etc.*). In contrast to severe ischemia studies (myocardial infarction, necrosis) this protocol was developed to evaluate myocardial functional recovery and cardioprotection without tissue injury, remodeling, and cell death. The majority of the paper discusses a standard cardioplegic arrest protocol with elements similar to a cardiac surgery using hypothermia and intermittent cardioplegia delivery.

Myocardial protection during the majority of cardiac surgeries relies on cardioplegia and cardiopulmonary bypass. Although cardioplegia (CP) solutions and strategies vary widely (blood, crystalloid, cold, warm *etc.*) the most common elements are 1) hyperkalemia and/or other strategies to arrest the heart in diastole, thereby limiting energy utilization resulting from myocardial contraction, and 2) hypothermia to slow metabolism and help maintain ATP and other energy reserves while arrested. Current cardioplegia solutions provide protection to the heart against ischemic insults that would otherwise prove lethal. However, cardioprotective strategies during surgical ischemic insults are not perfect, and the resultant mild ischemic injury can result in reversible cardiac contractile dysfunction despite adequate blood flow (myocardial stunning), acidosis, cardiomyocyte damage, and vascular effects including reduced coronary perfusion and vasospasm.

This protocol differs from standard isolated heart ischemia models evaluating myocardial infarction and severe ischemia in that it evaluates milder ischemic insults which can result in impaired cardiac function following brief ischemia or ischemic insults associated with cardioplegic arrest. (for review on Langendorff perfusion techniques and I/R studies see[6–8]). For general guidelines and a thorough analysis of experimental parameters associated with mouse isolated perfused hearts see Sutherland e*t al.* 2003[9] The technique presented here details the necessary equipment, reagents, steps, strategies and tips to reliably induce stunning in mouse hearts. Minor modifications are necessary to apply the technique to rats.

Briefly isolated mouse hearts are Langendorff perfused for approximately 30 min with physiologic Krebs-Henseleit buffer (KHB), followed by cold protected cardiac arrest via delivery of a hyperkalemic hypothermic cardioplegia solution. Following arrest, cardiac functional recovery is monitored during rewarming and reperfusion of the heart with KHB. Changes in the degree of recovery of cardiac contractile function can be evaluated to assess cardioprotective agents and different cardioprotection strategies.

Copyright © 2015 Journal of Visualized Experiments

Protocol

NOTE: All Procedures were approved by the Lifespan Institutional Animal Care and Use Committee and all animals and procedures according to the National research Council Guide for Care and Use of Laboratory Animals[10].

1. Balloon Fabrication and Left Ventricular Pressure Monitoring Circuit

1. Construct LV balloons according to Miller *et al.*[11] In a beaker with a stir bar, mix 9.5 ml of distilled water, 14.2 ml of light corn syrup and 33.8 g of sucrose, and heat on a hot plate, mix until the sugar is dissolved. Continue heating solution until it reaches approximately 150 ºC.
   NOTE: Numerous methods exist to construct LV balloons including modification of condom tips and cling film construction[9,12]. We find the above method to be relatively easy to construct leak-free balloons, however an advantage of cling film balloons includes previous characterization for appropriate frequency-response relationships[9].
2. Break dry spaghetti strands into pieces about 5 cm in length and dip one side of each piece about 1 cm deep into the sugar solution and slowly remove.
3. Place the dry end of the pasta strand into a polystyrene foam block and suspend so the sugar mix drips down and forms a teardrop shape mold. Leave O/N in a desiccator so the mold hardens.
4. The following day, dip the molds into silicone dispersion gel. Place pasta strands back into polystyrene foam block and place in a a 37 ºC oven for 2 hr or until dry. Repeat this step once more so two layers of silicone are applied.
5. Once dry, place in water for several hours to assist in removal of balloon from the mold. Store balloons in 0.02% sodium azide solution.
6. Use a 23 G blood collection set to produce a customized balloon cannula by cutting a needle to create a blunt tip and placing notches in the needle.
7. Connect the tubing to a pressure transducer and flush with water while keeping tubing and cannula completely submerged to prevent air from entering the system. Place a balloon that has been filled with water onto the cannula and use 2-0 silk sutures to tie onto cannula. Test balloon by ability to maintain pressure (~100 mmHg for at least 1 hr)

2. Preparation of the Isolated Heart Perfusion System

1. Initially, wash and warm the system. Turn on a 37 ºC warm water bath circulator that has been connected to the Langendorff apparatus, and fill buffer reservoir with distilled water. Connect a new glass fiber filter to tubing flowing into apparatus and turn on pump to flush out the system. During prep time, monitor the temperature of water expelled from the Langendorff block using a temperature probe, to ensure that water bath is set accurately. Also, turn on a refrigerated circulator pump and set to 20 ºC that will be used during cardioplegic arrest of the heart.
2. In the meantime prepare the following solutions. Prepare 1 L of cardioplegia solution (110 mM NaCl, 16 mM KCl, 16 mM $MgCl_2$, 1.5 mM $CaCl_2$, 10 mM $NaHCO_3$) and filter. Also, prepare 2 L of KHB (118 mM NaCl, 4.8 mM KCl, 1.2 mM KH_2PO_4, 1.7 mM $MgSO_4$, 2 mM Na-pyruvate, 6 mM dextrose, 24.9 mM $NaHCO_3$ (pre-aerated with CO_2),1.4 mM $CaCl_2$ (added last). Once dissolved, remove precipitates by filtering into a flask using a 5 µm glass fritted funnel. Place some into a small dish on ice to be used during surgery and isolation.
3. Oxygenate the perfusate (KHB) with 95% O_2/5% CO_2 for at least 30 min prior to use. Perfuse Langendorff system with KHB to remove any remaining water from system and buffer reservoir. For mice, set the initial pump speed to ~2.0 ml/min. Place 2-0 and 4-0 silk sutures near perfusion cannula. Run the pump until the tubing and apparatus are filled with perfusate and ensure the bubble strup is filled with perfusate.
   NOTE: For long periods of perfusion and stable function it is highly recommended to install an in-line glass fiber filter (~1 µm) in the perfusion circuit to collect any precipitates that could form clots in the heart

3. Mouse Surgery

3.1) Mouse Anesthesia and Handling

1. Prepare syringe with anesthetic dose of 80 mg/kg ketamine and 5 mg/ml xylazine mixture, and add sterile 0.9% saline to bring up volume to 0.2 ml. Inject Heparin I.P. (50 µl of 1,000 U/ml solution).
2. Place mouse back into transport container (~10-20 min) and wait for it to lose consciousness as heparin takes effect. Periodically do a toe pinch to monitor pain reflex.

3.2) Remove the Heart

1. Once mouse is fully unconscious and does not respond to toe pinch, secure it to an appropriate operating table using pins or 25 G syringe tips through the extremities. Perform a thoracotomy to expose the heart. Cut a small hole just below the sternum and extend the cut to the sides of the mouse avoiding the diaphragm.
2. Quickly cut the diaphragm and then rapidly cut up the sides of the rib cage. Flip back the rib cage like a clam shell to expose the thoracic cavity. Gently take hold of the heart, place scissors underneath and remove the heart.
   NOTE: It is essential to quickly remove the heart once it is exposed in the thoracic cavity and the lungs are non-functional.

3.3) Clean the Heart

1. Place the heart into the dish containing ice cold KHB and trim off any large pieces of lung tissue attached. Use forceps to pick up the heart and locate the aorta. Gently squeeze the heart and look for any blood that appears, as this should be the open end of the aorta. Use fine tip tweezers to hold the heart by the open end of the aorta.

Copyright © 2015 Journal of Visualized Experiments

4. Mounting the Heart, Starting Perfusion, and Placing the Balloon

4.1) Mounting and Cannulating the Heart

1. Before transporting the heart to the Langendorff apparatus, turn on the pump ensuring that the perfusion pressure is low (~20 mmHg). Hold the heart by the aorta just under the cannula. Using another set of fine tip tweezers, carefully open the aorta and slide heart up onto the cannula. Hold it in place with one set of tweezers and then use a soft edged clip to temporarily secure it to cannula.
   NOTE: Quickly mounting the heart to the perfusion cannula is critical for a good preparation. With experience, the procedures from opening the thoracic cavity until mounting the heart should take between 1 and 2 min.
2. Tie and initiate perfusion as follows, using 4-0 silk suture secure aorta to the cannula directly below the clip. Ensure the tie is also around the metal aortic cannula, so the aorta does not get tied off when tightening the knot. Once one tie is firmly placed, remove the clip.
3. Use additional silk sutures to fasten the aorta securely to the cannula making sure to tie off below any vessel branches that may be coming off the aorta. These can often be spotted by perfusate leaking or shooting out from the aorta.
   NOTE: During the initial stages of perfusion the blood should be washing from the heart and the entire heart should appear a soft pink color. Dark discolorations that do not wash out indicate ischemic areas likely due to air emboli or clots and the heart should not be used.

4.2) Establishing Perfusion and LVP Measurement

1. Slowly increase the perfusion pressure and increase pump speed until the perfusion pressure reaches 70 mmHg.
   NOTE: Standard perfusion pressures for mouse perfusion can be 70-90 mmHg, but must be kept constant from animal to animal within an experiment.
2. Clean the remaining tissue (lung, thyroid *etc.*) that may still be attached to the heart. Use scissors to cut off the left atria in order to create an opening into the left ventricle.
3. Place the balloon onto the holder and deflate. Position the balloon cannula near the aortic cannula, directly over the opening into the left ventricle. Carefully insert balloon downward into the left ventricle while holding the heart in place so the aorta does not tear.
4. Once it is in place slowly start to inflate the balloon until the LVEDP reaches ~8 mmHg. Place the temperature probe against the bottom of the heart so it measures the temperature of the effluent.

4.3) Basal Measurement

1. Seal the heart in a water jacketed perfusion chamber. During this time continuously perfuse the heart with KHB, and ensure that the temperature continues to rise. Monitor the temperature and adjust the water bath accordingly until it reaches approximately ~37 ºC.
   NOTE: Monitoring temperature in the initial phases of perfusion is critical as coronary flows and subsequently temperature can vary from heart to heart. Temperature of the effluent perfusate is monitored for desired myocardial temperature via a temperature probe placed at the apex of the heart. In addition, in comparison to other Langendorff perfusion protocols, the heart is not submerged in CP or KHB during perfusion or arrest, this is mainly done to quickly adjust temperature as well as provide efficient CP delivery without diffusion.
2. Begin to continuously record functional measurement using a data acquisition system attached to the appropriate sensors, including perfusion pressure, left ventricular pressure (pressure transducer attached to LVP balloon), temperature, and electrophysiological parameters (EKG, MAP if so equipped) (attachment will be according to manufacturers and specific to the individual sensors and data acquisition system). Once the effluent KHB reaches ~37 ºC for at least 15 min and cardiac functional parameters are stable, note the time for baseline measurements.
   NOTE: Inclusion/exclusion criteria are applied to each heart. A LVDP of <60 mmHg at baseline indicates a heart that should be removed from analysis. In addition, coronary flow >4.5 ml/min, or inability to maintain perfusion pressure at baseline likely indicates a leaking or torn aorta. Also any hearts that have clear ischemic areas which can be confirmed visually (*i.e.,* clot) or exhibit ischemia-associated reductions in cardiac function (*i.e.,* increasing LVeDP, extremely arrhythmic) are removed from analysis.
3. Measure coronary flow via collection of the coronary effluent in a graduated cylinder for 1 min. Close the chamber and allow temperature and function to return to baseline values. Alternatively measure the coronary flow continuously in the perfusion line via a flow probe placed after the pressure relief circuit.

5. Initiating Cardioplegic Arrest

1. Place 100 ml of cold cardioplegia buffer into another reservoir and. transfer the KHB reservoir cannula to the cardioplegia reservoir.
2. Detach the water jacketed housing and the perfusion warming circuit from the warm water bath and connect to the refrigerated circulator using tubing quick disconnects. Use separate heating and cooling circulators to allow rapid temperature changes of the heart and perfusate.
3. Follow the air bubble that gets introduced into the tubing during the switch, and once it reaches near the isolated heart start the timing for cardioplegia. Deliver cardioplegia for 2 min. After the initial dose stop the pump. Ensure to arrest the heart in diastole and be close to the desired CP temperature.
   NOTE: A sufficient bubble trap is necessary to avoid air emboli in the heart. Alternatively a delivery system for both reservoirs switched by a stopcock can be easily constructed, however we find following the small air bubble that is introduced when swapping the cannula between reservoirs, an easy method of timing CP delivery given potentially different pump speeds between experiments (*i.e.,* due to mouse/rat, perfusion pressure, filter resistance, *etc.*)
4. Keep the heart in cardioplegia for 2 hr at ~20 ºC. Every half hour during CP turn the pump back on for 1 min so another dose is administered.
   NOTE: Intermittent doses of CP every 30 min results in functional impairment of the heart without necrosis of the tissue. Longer periods between doses of CP (*i.e.,* >45 min) can result in necrosis and ischemic contracture and would be more appropriate for a model of necrotic injury associated with unprotected ischemia.

Copyright © 2015 Journal of Visualized Experiments

6. Reperfusion

1. At the end of CP, transfer the reservoir cannula back to the oxygenated KHB. Connect the temperature control circuits to the heating circulator and turn the pump on to start reperfusion. At this point the cardioplegia washes out, observe the temperature rise and the heart starts beating again after 2-4 min of reperfusion. When the heart starts the heart beat is slow and often arrhythmic.
   NOTE: There usually is not a large rise in diastolic LVP (*i.e.,* ≤10 mmHg rise) which is often characteristic in pure ischemia models. Arrhythmias can persist well into reperfusion and occasionally the heart will need to be defibrillated. This can be achieved with the use of a stimulator set to higher voltages (~10-50 V) and electrodes placed at the base and apex of the heart.
2. Allow the heart to reperfuse for 30 min, and during this time period take coronary flow measurements as necessary. Measure coronary flow by collecting the effluent with a graduated cylinder for 30-60 sec.

7. Collecting the Tissue

1. Deflate the balloon and remove it from the left ventricle. Take the heart off the cannula and weigh it. In initial experiments, at least one transverse slice of the heart should be taken and TTC stained to evaluate necrosis to ensure that the heart is free of any necrotic injury.
2. Collect heart slices for microscopy studies, wet/dry weights, *etc.* The remainder or whole heart should be rapidly frozen by placing into liquid nitrogen. Store frozen hearts in a -80 ºC freezer.
   NOTE: For measurement of high energy phosphate compounds (*i.e.,* ATP, creatine phosphate), the hearts should be frozen immediately.

Representative Results

Figure 1 presents typical results from one mouse experiment. LVP (red line), dP/dt (green line), and temperature (purple line) were continusouly recorded over ~3 hr. Letters indicate a - baseline measurement, b, c, d, e - delivery of cardioplegia solution, f - start of reperfusion, g, h, i, j - measurement of cornary flow during reperfusion. Note, depressed LVDP and dP/dt upon reperfusion compared to baseline. **Figure 1B** includes the data from A recorded over ~2 sec. Note, the decrease in LVP and dP/dt similar to A, slight reductions in HR and slight increases in LVEDP. Usually after 30 min of reperfusion there is an ~40% decrease in cardiac function as evidenced by the left ventricular developed pressure LVDP depicted in **Figure 1C**.

Copyright © 2015 Journal of Visualized Experiments

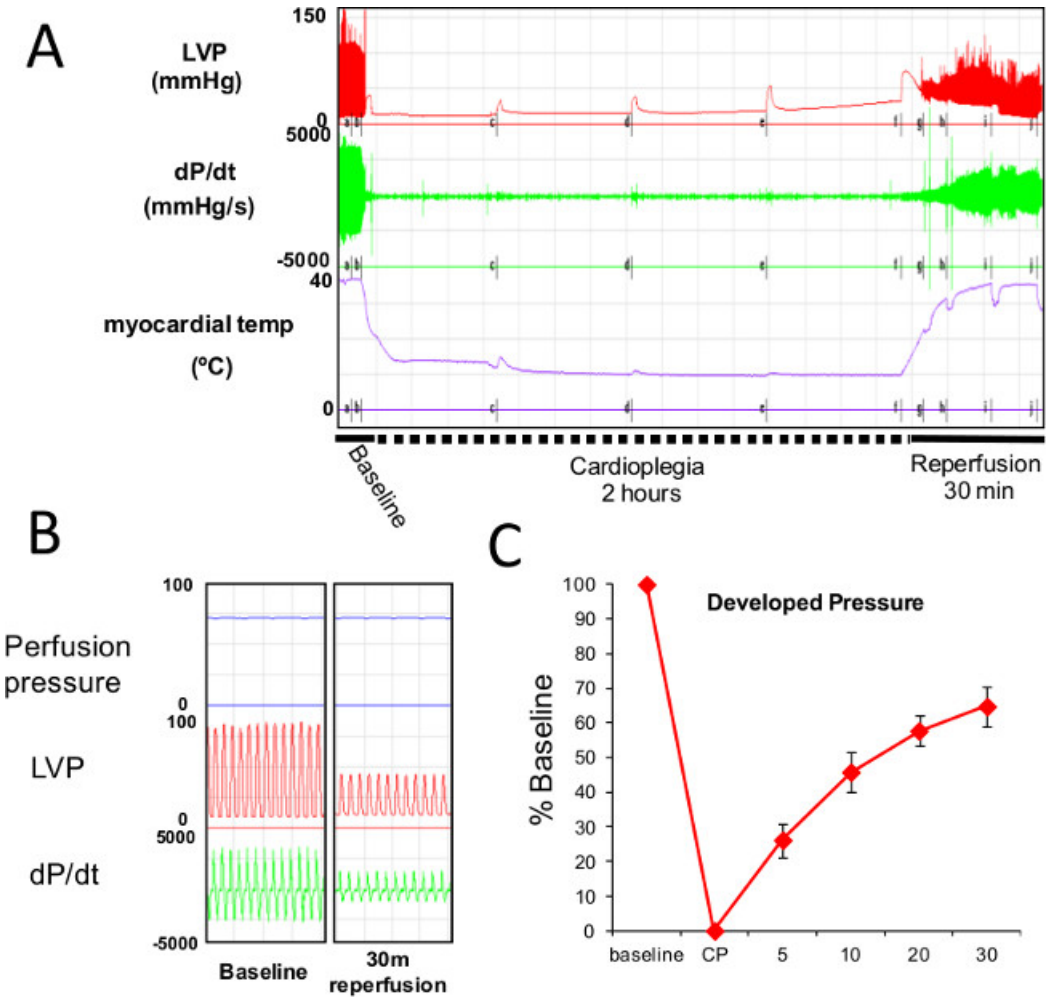

Figure 1. Representative results of a whole experiment. (**A**) recording left ventricular pressure (top), first derivative of LV pressure, and temperature. (**B**) Short interval recordings to see trace detail and (**C**) Quantitation of left ventricular developed pressure (LVDP) for multiple experiments (n = 6). Please click here to view a larger version of this figure.

Discussion

The preceding protocol details methods to evaluate myocardial stunning secondary to global ischemia associated with cardioplegic arrest. In our hands this protocol produces an approximate ~40% reduction in cardiac function (LVDP, +/- dP/dt) with minimal changes in heart rate at the 30 min post-reperfusion time point. As the heart is reperfused and rewarmed all parameters of cardiac function are reduced at initial time points with a greatly reduced heart rate before stabilizing between 20 and 30 min. Coronary flow is generally greatly increased during early reperfusion due to hyperemia, and then falls to ~20-30% less than control levels following 30 min reperfusion.

It is important to remember that myocardial stunning by definition should leave the heart free of cell death and necrosis which is characteristic of pure ischemia/MI models (*i.e.,* >20 min no flow regional ischemia). Initial studies should evaluate tissue histology to ensure a lack of necrotic injury. In addition, although myocardial stunning by definition should result in normal function following prolonged reperfusion (hr to days), It is likely this protocol will not demonstrate fully recovered function due to the *ex vivo* Langendorff perfusion which is associated with reductons in cardiac function in control perfused hearts over time. Nevertheless changes in the acute recovery of cardiac function in the absence of cell death/ necrosis can be used as indices of the severity of stunning The major difference in this protocol vs. classic no-flow ischemia protocols is the use of a cardioprotection strategy, in this case being hyperkalemic cardioplegia. Hyperkalemic cardioplegia solutions provide protection from necrotic injury and cell death by causing diastolic cardiac arrest. Diastolic arrest of the heart promotes preservation of energy reserves. In addition, most clinically used cardioprotection protocols use hypothermia to further limit myocardial injury by reducing metabolic demand. Other factors that can be modulated during the above protocol include different formulations of cardioplegia solutions (hyperpolarizing formulations, Mg++, K+ levels, *etc.*), strategies (warm vs. cold, 'hot shot', *etc.*), and various drugs (kinase inhibitors, ion channel modulators, cardioprotective agents, *etc.*).

Since the heart is in a relatively well protected state, to get reproducible functional impairments in this model requires necessarily longer ischemia times (*i.e.,* greater than 2 hr). We have found that rodent hearts are relatively resistant to injury in this model, especially when compared to larger animals (pigs, humans) which reliably display stunned myocardium during much shorter periods (*i.e.,* 30 min). We have also found that intermittent delivery is necessary to protect the heart from severe ischemic damage as a 45-60 min interval following CP delivery can result in gross diastolic dysfunction, ischemic contracture, and tissue injury upon reperfusion. Other readily adaptable components of the protocol

Copyright © 2015 Journal of Visualized Experiments

can include investigations related to CP constituents and the role of hypo/normothermic arrest as well as different arrest strategies not reliant on K+ (Na channel blockers, hyperpolarizing agents)[3].

There are also a number of important limitations to this technique when being used to model stunning associated with clinical cardiac surgery. First, the gross majority of clinical CP solutions are mixed with blood (~4 blood:1 CP ratio). This is generally not feasible in mice due to the perfusion circuitry volume as well as the need for treated tubing and fiber oxygenators. Often for larger animals (guinea pigs / rabbits) donor animals would also be required. In addition, as in all isolated organ models, the influence of peripheral factors (*i.e.,* inflammatory signals, blood reperfusion, *etc.*) is completely ignored. Nevertheless it is a practical, efficient, and economical model for preliminary studies to test pharmacological additives and different cardioprotection strategies.

The protocol is essentially the same in rat hearts, with the exception of a larger balloon and aortic cannula[13,14]. In addition, the rat heart preparation requires significantly greater flow rates (12-20 ml/min). Due to its size, the rat heart preparation is considerably easier to learn and reproducibly perform. To assess other types of injury resulting in myocardial stunning the protocol can be easily modified. To emulate reversible ischemic injury, simply stop the perfusion pump for brief periods. In rats ~20 min global ischemia will result in reduced contractile function without gross effects on cell death and infarction.

Disclosures

The authors have nothing to disclose.

References

1. Kloner, R. a, Jennings, R. B. Consequences of Brief Ischemia: Stunning, Preconditioning, and Their Clinical Implications: Part 1. *Circulation.* **104**, (24), 2981-2989 (2001).
2. Mentzer, R. M. Myocardial protection in heart surgery. *J Cardiovasc Pharmacol Ther.* **16**, (3-4), 290-297 (2011).
3. Chambers, D. J., Fallouh, H. B. Cardioplegia and cardiac surgery: pharmacological arrest and cardioprotection during global ischemia and reperfusion. *Pharmacol Ther.* **127**, (1), 41-52 (2010).
4. Bolli, R., Marbán, E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev.* **79**, (2), 609-634 (1999).
5. Kloner, R. a, Bolli, R., Marban, E., Reinlib, L., Braunwald, E. Medical and Cellular Implications of Stunning, Hibernation, and Preconditioning An NHLBI Workshop. *Circulation.* **97**, (18), 1848-1867 (1998).
6. Mersmann, J., Latsch, K., Habeck, K., Zacharowski, K. Measure for measure-determination of infarct size in murine models of myocardial ischemia and reperfusion: a systematic review. *Shock (Augusta, Ga).* **35**, (5), 449-455 (2011).
7. Bell, R. M., Mocanu, M. M., Yellon, D. M. Retrograde heart perfusion: the Langendorff technique of isolated heart perfusion). *J Mol Cell Cardiol.* **50**, (6), 940-950 (2011).
8. Skrzypiec-Spring, M., Grotthus, B., Szelag, A., Schulz, R. Isolated heart perfusion according to Langendorff---still viable in the new millennium. *J Pharmacol Toxicol Methods.* **55**, (2), 113-126 (2007).
9. Sutherland, F. J., Shattock, M. J., Baker, K. E., Hearse, D. J. Mouse isolated perfused heart characteristics and cautions. *Clin Exp Pharmacol Physiol.* **30**, (11), 867-878 (2003).
10. *Guide for the Care and Use of Laboratory AnimalsSource*. National Research Council Available from: http://grants.nih.gov/grants/olaw/Guide-for-the-care-and-use-of-laboratory-animals.pdf (2011).
11. Miller, A., Wright, G. L. Fabrication of Murine Ventricular Balloons for the Langendorff Heart Preparation. *J Biotecnol Biomater.* **1**, (101), 1-4 (2011).
12. Curtis, M. J. Characterisation, utilisation and clinical relevance of isolated perfused heart models of ischaemia-induced ventricular fibrillation. *Cardiovasc Res.* **39**, (1), 194-215 (1998).
13. Clements, R. T., Feng, J., Cordeiro, B., Bianchi, C., Sellke, F. W. p38 MAPK-dependent small HSP27 and αB-crystallin phosphorylation in regulation of myocardial function following cardioplegic arrest. *American journal of physiology. Heart and circulatory physiology.* **300**, (5), H1669-H1677 (2011).
14. Clements, R. T., Cordeiro, B., Feng, J., Bianchi, C., Sellke, F. W. Rottlerin increases cardiac contractile performance and coronary perfusion through BKCa++ channel activation after cold cardioplegic arrest in isolated hearts. *Circulation.* **124**, (11 Suppl), S55-S61 (2011).

Copyright © 2015 Journal of Visualized Experiments