Video Article

Normothermic Cardiac Arrest and Cardiopulmonary Resuscitation: A Mouse Model of Ischemia-Reperfusion Injury

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Abstract

Acute Kidney Injury (AKI) is a common, highly lethal, complication of critical illness which has a high mortality[1-4] and which is most frequently caused by whole-body hypoperfusion.[5,6] Successful reproduction of whole-body hypoperfusion in rodent models has been fraught with difficulty.[7-9,9,10] Models which employ focal ischemia have repeatedly demonstrated results which do not translate to the clinical setting, and larger animal models which allow for whole body hypoperfusion lack access to the full toolset of genetic manipulation possible in the mouse.[11,12] However, in recent years a mouse model of cardiac arrest and cardiopulmonary resuscitation has emerged which can be adapted to model AKI.[13] This model reliably reproduces physiologic, functional, anatomic, and histologic outcomes seen in clinical AKI, is rapidly repeatable, and offers all of the significant advantages of a murine surgical model, including access to genetic manipulative techniques, low cost relative to large animals, and ease of use. Our group has developed extensive experience with use of this model to assess a number of organ-specific outcomes in AKI.[14,15]

Video Link

The video component of this article can be found at http://www.jove.com/video/3116/

Protocol

All procedures described are conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research and all animal protocols were approved by the Oregon Health & Science University Institutional Animal Care and Use Committee.

1. Surgical Preparation

1. Weigh the mouse. The procedure described is performed on C57BL/6 mice weighing between 20 and 25 g. Anesthesia is induced in an induction box using 3-4% isoflurane, and subsequently maintained using 1.5-2.5% isoflurane in air/oxygen mixture.
2. Lubricate the eyes and position the animal supine on a heating pad. Immobilize 4 extremities using tape. The hind-paws may be taped in a neutral position, however, the forepaws should be secured as near to the chest wall as possible to allow full chest wall excursion during chest compressions.
3. Lubricate and place a rectal temperature probe. Temperature is controlled using a heating pad and lamp connected to an electronic temperature controller (Digi-Sense, Cole Parmer, Vernon Hills IL), which is set to maintain 37.0°C. Because it is possible that a temperature gradient within the animal could develop during the no-flow state, it is important that temperature be measured and controlled near the organ of interest.
4. Intubate the trachea using a 2.5 cm 22 ga teflon catheter (Insyte-W, BD, Franklin NJ) and the cut distal end of an angled introducer (Frova Introducer, Cook Medical, Bloomington IN). Other methods of tracheal intubation are acceptable, however, use of the angled introducer allows proper positioning of the neck in a slightly extended position, which optimizes surgical exposure for placement of the intravenous catheter. The endotracheal catheter hub is secured with a loop of suture to the incisor and maintained with slight tension to immobilize the head during chest compressions.
5. Mechanically ventilate the mouse with a rodent ventilator set to 140 µL, 150 breaths/minute. This is not adjusted for weight as this protocol is always performed in 20-25 g animals. Do not over-deepen anesthesia. Do not set the ventilator to apply end expiratory pressure as this impairs resuscitation.
6. Using sterile technique and an operating microscope, place a pre-flushed PE-10 catheter in the jugular vein. Either side may be used, but use of the side closest to the operator reduces the chance of catheter dislodgement during chest compressions or other manipulations. 0.5% bupivicaine, 0.1 mL is infiltrated into wound edges to control postoperative pain.
7. Secure the PE-10 catheter into the skin closure with cyanoacrylate surgical adhesive.

8. Place subcutaneous EKG electrodes and connect to monitoring device. Careful attention to the signal path and maximization of the signal itself is critical to the success of resuscitation. Ensure that all wires are secured to the operating surface, minimize signal crossings, and minimize insulators (like air in hollow needles or stranded wire) within the signal path. Solid needles connected to nonstranded lead wire may be purchased or made in the lab. Once connected, optimize the EKG signal on the monitor.

2. Cardiac Arrest

1. Ensure that the mouse is normothermic, defined as rectal temperature 36.5°C - 37.5°C. Administer 40 µL of room temperature 0.5 M potassium chloride intravenously and observe isoelectric tracing on EKG. Dose adjustment for weight is not necessary within the range 20-25 g. Start arrest timer.
2. Disconnect the ventilator. Discontinue the anesthetic vapor. Turn off the heating pad and any other equipment which produces electronic noise that may interfere with EKG monitoring. Place an insulating blanket over the mouse.
3. Record temperature every minute during cardiac arrest. If necessary, a heating lamp may be used to bring core temperature up to the normothermic range. During cardiac arrest, prepare supplies and equipment (for example, the epinephrine syringe) for resuscitation. A checklist can be helpful to ensure uninterrupted resuscitation, which is essential for survival.
4. After 7 minutes, 30 seconds of cardiac arrest, reconnect the ventilator and increase the rate to 180 breaths/min, keeping the tidal volume at the pre-arrest setting.
5. At 8 minutes, initiate chest compressions at 300 BPM. Motion artifact on the EKG can be used to judge CPR rate. Chest compressions should be delivered with the index finger, 5 mm above the xiphoid process and slightly to the left of midline. The chest should be compressed 1/3-1/2 of the anteroposterior distance and full recoil must be allowed between compressions. Finger positioning and optimal compression pressure are absolutely critical. Failure to achieve survival in this model is almost always due to suboptimal CPR.
6. Infuse 0.5 mL of epinephrine, diluted to 15 µg/mL, in the first 30 seconds of CPR. Total epinephrine dose is 8-12 µg. Carefully observe the EKG for return of spontaneous circulation (ROSC). Narrow complex QRS complexes are seen between compression artifacts. ROSC usually occurs between 90 seconds and 2 minutes after starting CPR. Resuscitation is abandoned if ROSC does not occur by 3 minutes. Frequent premature ventricular contractions and changes in EKG axis are observed in the first 2 minutes after ROSC, and almost always resolve into steady sinus tachycardia at 2 minutes.
7. Record total time of resuscitation and epinephrine dose. Record temperature every minute for 10 minutes after ROSC.
8. EKG leads can be removed when spontaneous respiration begins, usually within 12-15 minutes after ROSC.
9. Extubate the trachea when spontaneous respiratory rate is >60/min.
10. Place the mouse in a recovery cage on a temperature controlled surface set to 37°C for the first 2 hours postprocedure, ensuring easy access to food and water. The cage may then be moved to standard postoperative housing conditions when the mouse has completely recovered from anesthesia and is active.

3. Exsanguination/Perfusion-Fixation and Kidney Harvest

1. 24h after CA/CPR, induce anesthesia with isoflurane 3-4% and secure the animal in the supine position on a surgical surface within a fume hood which is appropriate for use with formalin..
2. Deepen the anesthetic by gradually increasing the anesthetic vapor concentration to 5% and ensure the mouse is deeply anesthetized as evidenced by cessation of spontaneous respiration.
3. Perform a clamshell thoracotomy and exsanguinate the mouse via apical puncture of the cardiac left ventricle according to standard techniques.
4. Via the same needle, administer 0.9% saline by slow infusion. To acclerate perfusion/exsanguination, nick the right atrial appendage with scissors. Perform a laparotomy and observe the kidneys. When they are blanched, saline may be changed to 4% formalin for fixation. Kidneys are then embedded in paraffin and stained with Fluoro-jade B for quantification of tubular epithelial necrosis.

4. Representative Results:

When CA is induced, mean arterial pressure (MAP) and regional renal blood flow (RRBCF) drop to near zero and remain stable until resuscitation begins (**Figure 1**). 24h after CA/CPR, serum indices of renal function (blood urea nitrogen, BUN and creatinine) are significantly elevated relative to sham-operated animals. AST/ALT are also elevated, offering evidence of profound whole-body ischemia (**Figure 2**). Neutrophil-gelatinase associated lipocalin (NGAL), a sensitive indicator of renal ischemic injury is massively upregulated 24h after CA/CPR (**Figure 3**). Finally, photomicrographs demonstrate the patchy, medullary necrosis typical of ischemic renal injury with thinning of tubular epithelium and luminal filling on hematoxylin and eosin stain (**Figure 4, panel a**) and extensive cell death in medullary tubular epithelium when stained with fluoro-jade B (**Figure 4, panel b**).

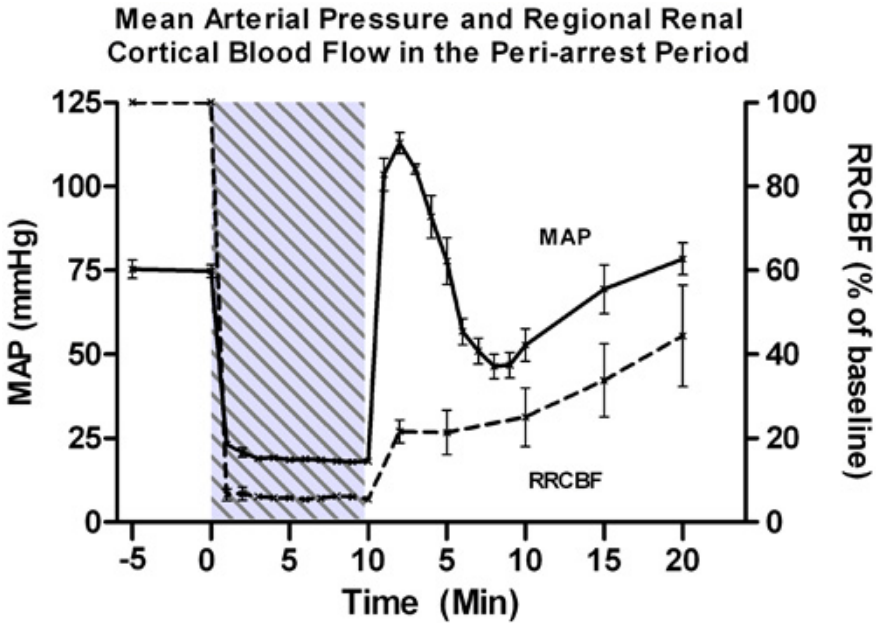

Figure 1. Cardiac arrest induces instant loss of perfusion pressure, represented here as mean arterial pressure (MAP) measured in the femoral artery, resulting in near-complete cessation of regional renal cortical blood flow (RRCBF) throughout the period of cardiac arrest (shaded area). Resuscitation with chest compressions and epinephrine returns MAP to normal and RRCBF steadily rises in the post resuscitation period. *Reprinted with permission from*[14].

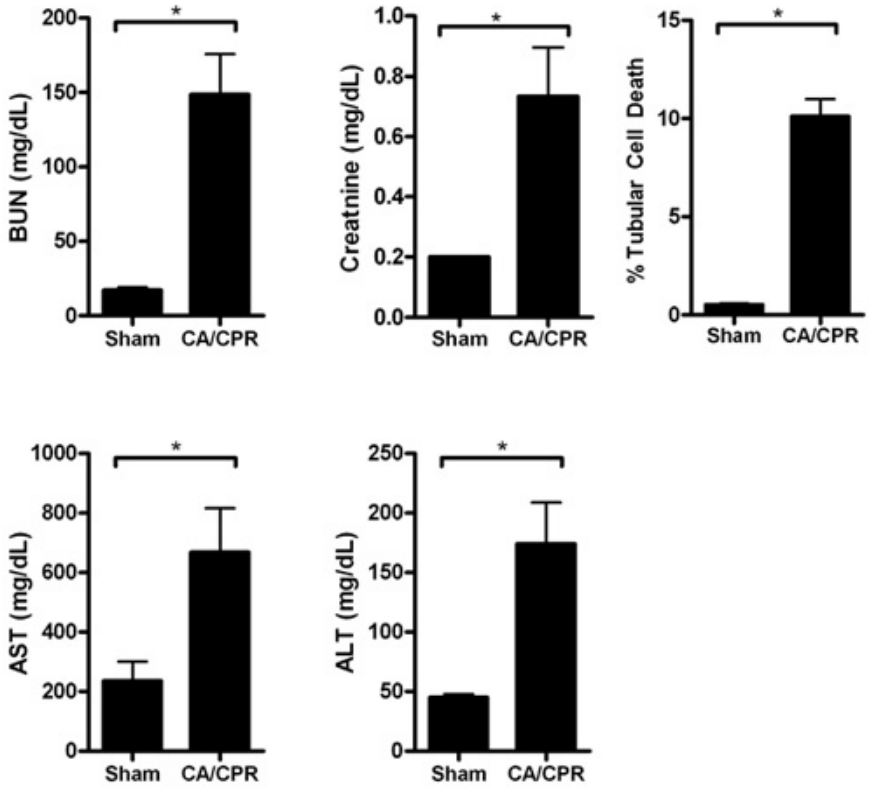

Figure 2. 24h postprocedure, blood urea nitrogen (BUN), serum creatinine, and the extent of tubular cell death are all significantly elevated in animals having undergone CA/CPR as compared with animals treated with a sham procedure. CA/CPR induces a pan-organismal ischemic insult, here evidenced by massive elevation of liver-function enzymes alanine amino transferase (ALT) and aspartate aminotransferase (AST) in CA/CPR mice as compared with sham-treated animals.

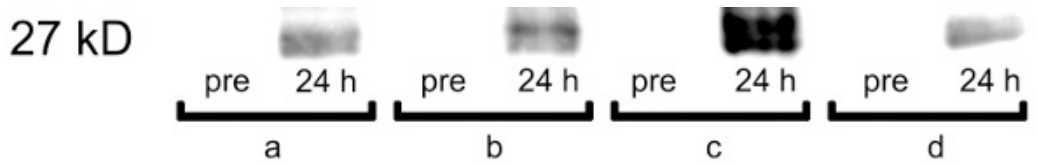

Figure 3. Western blot performed using polyclonal antibody to neutrophil gelatinase-associated lipocalin (NGAL), a sensitive indicator of renal ischemic injury. Urine samples were obtained immediately prior to ("pre") and 24h after ("24h") CA/CPR in 4 animals (labeled a, b, c, and d above). NGAL is massively upregulated in mouse urine after CA/CPR. *Reprinted with permission from*[14].

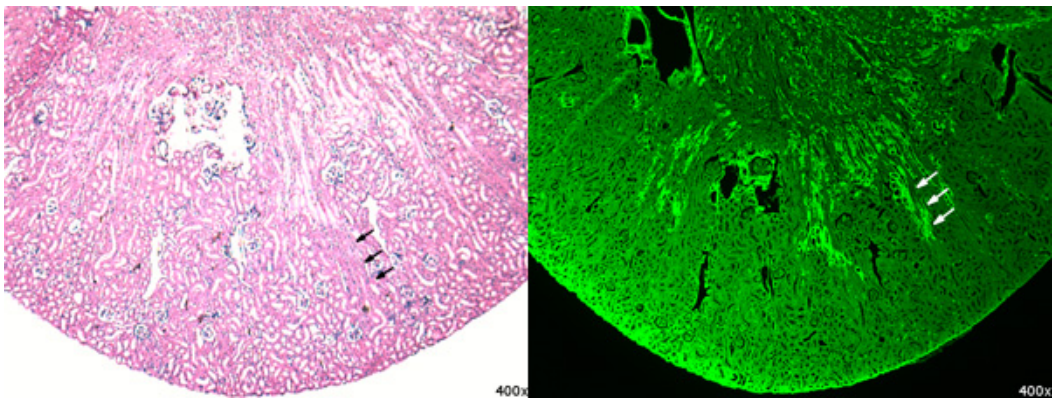

Figure 4. A) Hematoxylin and eosin stain of a short axis hilar section of renal tissue 24h after CA/CPR. There is patchy but clear damage to medullary and corticomedullary tubules with tubular plugging. Arrows point to damaged tubules with swollen, pyknotic nuclei at the corticomedullary junction. B) Flouro-jade B stain of the same region in the same animal, 24h after CA/CPR. Fluoro-jade B stains necrotic cells bright green, showing patchy corticomedullary tubular necrosis. Arrows point to brightly stained damaged tubules at the corticomedullary junction. These findings are substantially similar to renal biopsy findings from humans who develop AKI, and unlike those produced by other animal models of AKI.

Discussion

The normothermic model of cardiac arrest and cardiopulmonary resuscitation in the mouse offers multiple avenues of evaluation in a model which replicates the pathophysiology and morphology of the most common clinical cause of AKI, whole-body hypoperfusion. Hypothesis testing may be aided by access to a panoply of genetic manipulation techniques and the well-understood and characterized anatomy and physiology of the laboratory mouse.

As described here, survival in experienced hands is 80%. Although the surgical preparation and post-arrest care are straightforward, the actual resuscitation of an arrested mouse is a challenging surgical skill which requires significant practice. In our experience, successful resuscitations become the norm after about the first 30-50 attempts at resuscitating individual animals by an inexperienced surgeon, and consistent injury and resuscitation take additional practice. In particular, during the early phase of learning the technique (i.e., for the first 20 animals), meaningful survival is unlikely, and any survivors should be euthanized immediately after resuscitation to prevent unecessary distress. After early training, when survivors demonstrate vigorous cardiac recovery in the minutes following resuscitation, it is appropriate to continue surviving animals through the 15 minute post-ROSC mark. Animals that demonstrate vigorous spontaneous respiratory efforts by that point will likely survive 24h with appropriate recovery. This practice phase should be specifically addressed in IACUC protocols. The most difficult skill to learn is the chest compressions themselves as it is difficult to deliver compressions at the needed rate without increasing pressure to injurious levels. Mechanical resuscitators have been devised and used in our lab and others[16] but to date no mechanical resuscitator for the mouse has produced acceptable survival.

There are some limitations to this model. First, no mouse model can fully model human physiology, and results must be interpreted with respect for interspecies differences. In particular, the mouse heart is robust; we achieve 80% survival at 8 minutes of cardiac arrest, but in clinical studies human survival is less than 50% even with resuscitation beginning 2-3 minutes after arrest.[17] The small size of the mouse renders procedures technically exacting, and there is a significant learning curve, particularly for chest compressions. Second, in this protocol, drug doses and ventilator settings are not indexed to animal weight. This is because we use only animals in the 20-25 g weight range in order to minimize the effect of equipment size. For example, we use a 22 gauge catheter to intubate the trachea; the seal obtained is not the same in a 30 g mouse as it is in a 25 g mouse and the increased gas leak may be physiologically significant. However, the use of animals in a defined weight range does not faithfully reproduce the variety of body classes that are subject to clinical ischemia-reperfusion injury. Third, the no-flow state may not faithfully replicate results from low-flow states. In particular, temperature gradients may develop within the mouse during no-flow. To minimize the effect (if any) of gradients on results, temperature is measured near the organ of interest. Finally, because of early data from our lab which confirmed significant metabolic acidosis in the postarrest period, we hyperventilate during resuscitation. As recent data indicate that hyperventilation during CPR is deleterious,[18,19] it is possible that this practice reduces survival in the model. Many alternatives models for whole-

body ischemia reperfusion injury exist. For example, CA/CPR has been described in dogs,[20] rabbits,[21] lambs,[22] pigs,[23] rats,[16] and arctic ground squirrels.[24]

We have shown that postoperative renal function may be evaluated by a number of chemical, immunological, or histological techniques at 24 hours. Animals can be survived for longer than 24h, however, and the transient nature of AKI makes this model an attractive one to use for investigation of mechanisms of recovery and/or permanent injury. The injury itself is titratable by altering the parameters of time and temperature during CA. Longer arrests and maintenance of higher temperatures produce greater injury (but adversely effect survival). As we have shown with the included liver function data, other organ systems are also affected and may be investigated using adaptations of this model, however, significant effort may be required to optimize experimental conditions for the organ system of interest.

Disclosures

No conflicts of interest declared.

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