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Title: A Large Animal Model for Acute Kidney Injury by Temporary Bilateral Renal Artery Occlusion

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interview Statements are read by JoVE's voiceover talent.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 18
Number of Shots: 41

Introduction

1. Introductory Interview Statements

NOTE to VO: Please record all introduction and conclusion statements.

REQUIRED:

1.1. This study describes a reproducible large animal model of renal ischemia reperfusion injury, which can be used to study the pathophysiology of acute kidney injury and explore potential therapeutic modalities.

1.1.1. LAB MEDIA: Figure 1.

1.2. This method is highly reproducible and minimizes the impact of the inflammatory response by limiting the amount of insult to the body compared to an open approach.

1.2.1. *2.4.1, 2.4.2*

OPTIONAL:

1.3. This preclinical model mimics clinical scenarios such as kidney transplantation, renal hypoperfusion following cardiogenic shock, transcatheter procedures resulting in renal ischemia and cardiovascular procedures with prolonged cardiocirculatory arrest times.

1.3.1. *2.2.1, or 3.2.2, 3.2.3*

Introduction of Demonstrator on Camera

1.4. Demonstrating the procedure will be Ilias Doulamis MD, PhD, Mossab Saeed MD and Rio Nomoto MD. Research Fellows at the Cardiac Surgery Department.

1.4.1. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.5. All in vivo studies were conducted in accordance with the National Institutes of Health's guidelines on animal care and use and was approved by the Boston Children's Hospital's Animal Care and Use Committee.

Protocol

2. Surgical preparation and vascular access

2.1. To begin, drape the animal in a sterile fashion **[1]**. Disinfect the right lateral area of the neck by applying betadine and then 95% ethanol 3 times **[2].**

2.1.1. Talent draping the animal

2.1.2. Talent applying betadine and ethanol to the animal

2.2. Perform a cut-down for the catheterization of the right carotid artery and the right jugular vein **[1]**. Retract the sternocleidomastoid muscle laterally and dissect it down to the right carotid artery and the right jugular vein **[2]**.

2.2.1. Talent performing a cut-down

2.2.2. Talent moving the muscle laterally and dissecting it

2.3. Insert a 5F angiography sheath in both the artery and the vein **[1]**. Secure it with a silk 2-0 suture **[2]**.

2.3.1. Talent inserting the sheath

2.3.2. Talent suturing the cut

2.4. Insert a 5F angiography sheath using the Seldinger technique into the left femoral artery by puncturing the artery with a hollow needle **[1]**. Insert a soft tip guidewire through the lumen and advance it into the femoral artery **[2]**.

2.4.1. Talent puncturing the artery

2.4.2. Talent inserting guidewire into the artery *Videographer: This step is important!*

2.5. Hold the guidewire secure with the hand while removing the needle **[1]**. Pass the angiography sheath over the guidewire into the femoral artery **[2]** and withdraw the guidewire by using ultrasound guidance if necessary **[3]**.

2.5.1. Talent removing the needle

2.5.2. Talent passing the sheath into artery

2.5.3. Talent withdrawing the guidewire

3. Induction of renal ischemia-reperfusion injury

3.1. Intravenously administer 200 IU per kilogram sodium heparin to achieve systemic anticoagulation **[1-TXT]**. To perform angiography, inject an iodinated contrast agent under fluoroscopy to identify the renal arteries **[2]**.

3.1.1. Talent performing intravenous administration **TEXT: Target activated clotting time > 300 s.** *Videographer: This step is important!*

3.1.2. Talent injecting contrasting agent and visualizing renal arteries on monitor *Videographer: This step is important!*

3.2. Identify the renal arteries and manually advance the guidewire in the guiding catheter **[1]**. Position the 5F JL4 guiding catheter in the left renal artery through the right carotid artery **[2]**. Position the second 5F JL4 guiding catheter in the right renal artery through the left femoral artery **[3]**.

3.2.1. Talent advancing the guide wire

3.2.2. Talent positioning the guiding catheter *Videographer: This step is difficult!*

3.2.3. Talent positioning the second catheter *Videographer: This step is difficult!*

3.3. Use the guidewires to direct a 5F percutaneous transluminal angioplasty, or PTA, dilatation catheter in each renal artery **[1]**. Position each balloon catheter in place and connect a pressure line to each catheter **[2]**.

3.3.1. Talent directing the catheters *Videographer: This step is important!*

3.3.2. Talent connecting pressure line to the catheter *Videographer: This step is important!*

3.4. Check the presence of arterial pulse waveforms in the pressure monitor to ensure the correct positioning of the catheter **[1]**. Inflate each balloon and aim for a pressure of approximately 2.5 atmospheric pressure inside the balloon **[2]**.

3.4.1. Arterial pulse waveforms in the pressure monitor *Videographer: This step is important!*

3.4.2. Talent inflating the balloon *Videographer: This step is important!*

3.5. To confirm the cessation of blood flow to the kidneys, observe the flattening of the pulse waveform at the tip of the balloon catheter **[1]**. Inject iodinated contrast medium in a 1 to 1 dilution with saline and check for any opacification of the renal vessels **[2]**.

3.5.1. Flattening of the pulse on pressure monitor *Videographer: This step is important!*

3.5.2. Visualization of renal vessels on monitor *Videographer: This step is important!*

3.6. After 60 minutes of occlusion, carefully deflate and remove the balloon catheters from the renal arteries **[1]**. Perform an angiography using a 1 to 1 diluted contrast medium to confirm renal artery patency and the establishment of renal reperfusion **[2]**. Remove the 5F angiography sheath from the left femoral artery **[3]**.

3.6.1. Talent removing the catheters

3.6.2. Visualization of renal artery on monitor *Videographer: This step is important!*

3.6.3. Talent removing the sheath from artery *Videographer: This step is important!*

3.7. Apply firm pressure at the site of catheterization for 30 minutes **[1]**. Reverse the effect of heparin by the administration of 3 milligrams per kilogram of protamine until normalization of active clotting time **[2]**.

3.7.1. Talent applying the pressure at the site of catheterization

3.7.2. Talent administrating protamine

3.8. To sample urine during the post-operative period, secure a tube to the Foley catheter with a silk 2-0 suture using an interrupted stitch on the skin **[1]**. For blood sampling, leave the angiography sheaths in the right carotid artery and the right jugular vein in place and secure them with a silk 2-0 suture **[2]**.

3.8.1. Talent securing tube to Foley catheter

3.8.2. Talent securing sheath using suture

3.9. Close the neck incision with a silk 2-0 suture using a continuous stitch in 2 layers **[1]**. Administer 3 milligrams per kilogram of bupivacaine at the incision site to minimize pain **[2]**. Continue to hydrate the animal with 0.9% sodium chloride for a total of 2 hours following the end of ischemia **[3-TXT]**.

3.9.1. Talent closing the neck incision

3.9.2. Talent administering bupivacaine

3.9.3. Talent hydrating the animal with NaCl **TEXT: NaCl at 5 mL/kg/h**

3.10. Place a fentanyl patch of 25 to 50 micrograms per hour on the back of the animal to minimize post-operative pain **[1]**. Administer an intramuscular injection of buprenorphine at 0.005 to 0.1 milligrams per kilogram **[2]** and monitor the animal on mechanical ventilation until it awakens **[3]**.

3.10.1. Place a fentanyl patch on the back of the animal

3.10.2. Talent administering the injection of buprenorphine to minimize the pain

3.10.3. Animal on mechanical ventilation

~~4.~~ Post-reperfusion

4.1. Collect the final blood and urine samples and calculate the urine output **[1]**. Perform a 15-centimeter midline laparotomy incision using a size 10 blade from the xiphoid down to the mid pelvis **[2]**.

4.1.1. Talent collecting final blood and urine samples

4.1.2. Talent performing the incision

4.2. Use a straight lateral retractor to retract the abdominal skin **[1]**. Dissect the lateral peritoneal attachments of the abdominal wall to expose the right and left retroperitoneum **[2]**.

4.2.1. Talent retracting the abdominal skin

4.2.2. Talent dissecting the lateral peritoneal attachments

4.3. Identify and bluntly dissect both renal arteries and veins **[1]**. Ligate both renal arteries and veins with a 2-0 silk suture and perform bilateral nephrectomies to collect whole tissue specimens for histological and metabolic analysis **[2]**.

4.3.1. Talent dissecting renal arteries and veins

4.3.2. Talent ligating the renal artery with suture

Results

5. Results: Assessment of renal function before and after renal ischemia-reperfusion injury

5.1. Renal function was assessed by determining the urine output, eGFR, plasma creatine, and BUN. Following 60 minutes of bilateral renal artery occlusion, urine output was significantly decreased and remained this way at 6- and 24-hours following reperfusion **[1-TXT]**.

5.1.1. LAB MEDIA: Figure 2A **TEXT: eGFR: Estimated glomerular filtration rate, BUN: blood urine nitrogen**

5.2. Similarly, a significant decrease was observed in eGFR, which dropped from baseline as compared to the end of ischemia at 2 hours, 6 hours, and 24 hours of reperfusion **[1]**.

5.2.1. LAB MEDIA: Figure 2B

5.3. Plasma creatinine was significantly increased at 2, 6, and 24 hours of reperfusion compared to baseline **[1]**. BUN was increased as compared to baseline at 6 and 24 hours of reperfusion **[2]**.

5.3.1. LAB MEDIA: Figure 2C

5.3.2. LAB MEDIA: Figure 2D

5.4. Evident necrotic and hemorrhagic areas were unevenly distributed in both kidneys at the end of the 60 minutes of bilateral renal ischemia and the 24 hours of reperfusion **[1]**. Masson's Trichrome staining revealed confluent coagulative necrosis, which was located at the proximal tubules of the renal cortex. **[2]**.

5.4.1. LAB MEDIA: Figure 3A

5.4.2. LAB MEDIA: Figure 3B

5.5. Plastic embedded sections were also assessed **[1]**. All Masson's Trichrome slides were evaluated for cell necrosis, loss of brush border, cast formation, and tubule dilatation. Acute tubular necrosis or ATN scoring showed significant injury in the renal cortex and medulla **[2]**.

5.5.1. LAB MEDIA: Figure 3C

5.5.2. LAB MEDIA: Figure 3

Conclusion

6. Conclusion Interview Statements

NOTE to VO: Please record all introduction and conclusion statements.

6.1. When performing this protocol, check the positioning of the balloon so it is on the proximal renal artery and totally occludes flow to the kidney. Make sure it is not kinking in the aorta.

6.1.1. *3.4.2*

6.2. Following this procedure, endovascular catheter flow probes can be inserted in the renal arteries to assess renal blood flow or renal biopsies can be performed to assess renal tissue injury.

6.2.1. *3.4.1, 3.5.2, 4.3*