OPEN ACCESS

GAVIN PUBLISHERS

Journal of Orthopedics and Muscular System Research

Video Article

Smith PN, et al. J Orthop Muscular Syst Res: JOMSR-104.
DOI: 10.29011/ JOMSR-104. 100004

Surgical Technique for Establishment of a Multiple-organ Ischemia and Reperfusion (I/R) Injury Model in Rat

P.N. Smith[1,2], Y.L. Yang[1,3], C. Song[1], S. Weiss[4], J. Dai[1], R.W. Li[1,4*]

[1]Trauma and Orthopaedic Research Laboratory, Department of Surgery, The Medical School, The Australian National University, Canberra, Australia

[2]Clinical Orthopaedic Surgery, The Canberra Hospital, Yamba Drive, Garran, Australia

[3]Department of Orthopedics, Shandong Provincial Trauma and Orthopedics Hospital Affiliated to Shandong University, China

[4]Department of Immunology and Infectious Disease, John Curtin School of Medical Research, The Australian National University, Acton, Australia

[*]**Corresponding author:** Rachel W. Li, Trauma & Orthopaedic Research Laboratory, The Medical School, The Australian National University, Building 131, Garran Road, Acton, ACT 2601, Australia. Tel: +61261254577; Email: rachel.li@anu.edu.au

Citation: Smith PN, Yang YL, Song C, Weiss S, Dai J, et al. (2018) Surgical Technique for Establishment of a Multiple-organ Ischemia and Reperfusion (I/R) Injury Model in Rat. J Orthop Muscular Syst Res: JOMSR-104. DOI: 10.29011/ JOMSR-104. 100004

Received Date:17 September, 2018; **Accepted Date:** 18 September, 2018; **Published Date:** 25 September, 2018

Abstract

Animal models of ischemia/reperfusion (I/R) injury have been widely applied to the study of myocardial, liver, renal and skeletal muscle ischemia, I/R injury and drug efficacy. However, these require large numbers of experimental animals and human resources in performing the surgical procedures. Animal models described for experimental research regarding multiple-organ I/R injury are relatively sparse. Therefore, there is a clear potential to evaluate the drugs for protection against I/R injury of multiple organs in an established multiple organ I/R injury animal model. Here we provide useful information for medical researchers who are learning the surgical process of establishing I/R injury animal models. We demonstrate a novel multiple-organ procedure for both skeletal muscle I/R injury and kidney I/R injury in a rat model. The surgical process is described from an anesthetized animal to the completion of reperfusion, including detailed descriptions of each step throughout the procedure. It should also be noted that this video guide is focused towards procedures conducted in rodent models. Modifications of the described procedures are applicable to other animal models.

Keywords: I/R-Injury; Ischemia; Kidney; Rat; Reperfusion; Skeletal Muscle; Surgery

Introduction

Ischemia/reperfusion injury (I/R) results from a temporary impairment of (oxygenated) blood supply to a tissue (ischemia) followed by a resumption of the supply (reperfusion) [1,2]. Tourniquet application, vascular injury, bone fracture, and skeletal muscle crush injury are among the list of causative factors of I/R in skeletal muscle [3,4]. I/R injury of the skeletal muscle occurs also during multiple trauma situations affecting blood supply to the muscle and major organs, such as combined pelvic and limb fractures. Other surgical procedures, such as abdominal aortic surgery, extremity revascularization, transplantation, and free flap transfer, unavoidably cause skeletal muscle I/R injury, which potentially leads to severe postoperative complications [5-7].

Animal models of I/R injury have been widely applied to the study of myocardial, liver, renal and skeletal muscle ischemia and drug efficacy [3,8,9]. However, these require large numbers of experimental animals and human resources in performing the surgical procedures. There is a paucity of information regarding animal models of multiple-organ ischemia and reperfusion injury. In relation to causality, there are clearly similarities to the mechanisms of I/R injury known in a number of organ systems [10]. Therefore, there is a clear potential to evaluate the drugs for protection [9] against I/R injury of multiple organs in an established multiple organ I/R injury animal model. While several techniques and modifications of skeletal muscle I/R injury in rats

have been reported [1,11,12], the skeletal muscle and kidney I/R injury and the introduction of anti-ischemic drug testing in our surgical procedure is a major advancement because it mimics the surgical situation of multiple trauma. The multiple-organ I/R injury rat model of the described procedures are also applicable to other animal models.

Protocol

Animals, Materials and General Procedures

1. Male Wistar rats (300-350g) were purchased from the Animal Resource Centre (ARC) Western Australia, Australia.
2. All animals were housed for one week and fasted for 13 hours before surgery.
3. Each animal had general anesthesia induced with isoflurane USP, 0.5 - 1% in oxygen and maintained with pentobarbital sodium injection, 50 mg/kg body weight, intraperitoneally.
4. Under anesthesia, all animals were placed in a supine position on a warmed mat in order to maintain body temperature.
5. This study was performed after receiving approval from the Australian National University Animal Experimental Ethics Committee (Reference # F.MS.20.09).

Isolation Procedures of Femoral Artery

1. Each rat is placed in a supine position with the limbs appropriately secured to the operating table.
2. The lower abdomen and groin are shaved and sterilized with povidone iodine solution, and then the left inguinal skin flap is excised.
3. Once the skin flap is excised, a fat pad immediately overlying the femoral triangle is visible and the fat pad is then elevated as shown. The fat pad is dissected free distally and then proximally.
4. The femoral vessels are immediately visible beneath the fat pad. The inferior epigastric artery is seen passing to the fat pad arising from the femoral vessels.
5. Gentle dissection of the fat pad further proximally will further mobilize the fat pad as a separate structure and expose the femoral vessels up to the level of the inguinal ligament.
6. Once the fat pad has been fully exposed, the proximal extent of the fat pad can be crushed off with artery forceps and sectioned as shown. The fat pad can then be tucked free out of the way under the skin flap.
7. Femoral vessels are then well exposed and meticulously dissected using blunt dissection. Tissue overlaying the femoral artery is freed and the vessels mobilized.
8. In this example, 2-0 vicryl suture material is looped around the femoral artery. The suture is passed twice around the artery and left loosely applied.
9. The suture, which will later be tightened to occlude the artery and create ischemia in the distal tissue, is looped around the vessel rather than tied-off to facilitate release of the occlusion during the reperfusion steps.

Isolation Procedures of Renal Artery

1. Once the inguinal dissection has been completed and the femoral artery is isolated, an abdominal skin flap is created in an L-shaped fashion as shown.
2. Once the skin has been incised, the abdominal wall muscle is grasped and once again incised in the same line as the skin, distal to proximal, up to the costal margin.
3. Once the costal margin had been reached, the abdominal musculature is excised in a transverse fashion in order to create the L-shaped flap.
4. Following completion of muscle dissection, clips are placed to retract the L-shaped incision of the abdominal cavity, and the intestines are retracted to expose the left kidney.
5. One can see the renal vascular pedicle, the aorta and the inferior vena cava. The renal vascular pedicle is then gently dissected from the posterior abdominal wall.
6. Once again, an appropriate suture, in this example 2-0 vicryl, is gently placed around the renal vascular pedicle. The suture is looped twice and left loose at this point in time pending injection of the test drug into the inferior vena cava.

Heparinization and Drug Delivery Procedures

1. Once the renal vascular pedicle has been dissected and looped, the next step is to gently retract the intestines and once more expose the inferior vena cava.
2. In this experiment we use heparin as an anti-coagulant to prevent coagulation of the blood which will become stagnant in the left renal artery and in the left femoral artery after occlusion (during ischemia). It is important for this blood not to clot as that will prevent reperfusion of the tissue after the prescribed period of ischemia.
3. We inject heparin (200 IU) under direct vision into the inferior vena cava as shown, using a 25gauge needle and a 0.5 ml syringe.
4. The test drug, which in this experiment is a preventive agent for I/R injury, is also carefully administrated under direct vision through the inferior vena cava. Only one test drug is administered per animal.

5. Vehicle for the test drug is administered instead of the test drug in control animals.

Procedures of Creating Femoral and Renal Ischemic Model

1. Once the heparin and test drug have been injected into the vena cava, the drug is then given time to circulate. The next step is to occlude the femoral and renal arteries.
2. The pre-placed suture loops are tightened around the respective vessels and then under a small amount of tension clipped back onto the skin of the animal. In this study we clipped both sutures together onto the skin but multiple sutures can be clipped onto the skin separately.
3. This step occludes each artery both by the tightening of the sutures and by kinking the artery around each suture when tensioning and clipping it back onto the skin.
4. Ischemia is characterized by the absence of an arterial pulse and loss of colour and pressure in each artery distal to the occlusion.
5. Once that is completed, the abdominal musculature and skin flap are closed. The L-shaped flap is closed initially at its apex and from this the flap is closed proximal to distal in a continuous suture.
6. A secure and water tight closure is thus achieved. The horizontal part of the L-shaped incision is left open to enable adding sterile saline to the abdominal cavity thus preventing dehydration of the animals during the course of the experiment.
7. Following adding the saline to the abdominal cavity, the horizontal part of the L-shaped incision is also closed. Once again a water tight closure is aimed to prevent fluid loss.
8. The animal is then maintained under general anesthesia (for 3 hours in this experiment) until the next stage of the experiment.

Reperfusion Procedures

1. After completion of the ischemia time period, the suture line is then re-opened and the previously looped and occluded femoral and renal vessels are re-exposed.
2. The tightened suture loops are unclipped from the skin and gently released from around the femoral and renal arteries. Great care must be exercised not to damage the very small arterial structures, particularly if the sutures became dry during the ischemia period and adhered to the vessels.
3. Reperfusion is confirmed by visual observation of the re-establishment of an arterial pulse, and color and pressure in each artery distal to the site of the occlusion.
4. Once again, the animal's abdominal cavity is closed and reperfusion of the left kidney and left lower limb is allowed - in these experiments for another 45 minutes.
5. Rats are sacrificed by cervical dislocation while under general anesthesia.
6. Tissues, including the right control kidney and tibialis anterior muscle, and the left test kidney and tibialis anterior, are removed by standard dissection procedures. The collected tissue sections are immediately prepared for analyses of tissue damage as per the experimental protocol.

Representative Results

A successful rat multiple-organ I/R injury surgery showed pathological features of tissue damage with detectable apoptosis states. These disease states were significantly different from those of control animals. Apoptosis induced nuclear DNA fragmentation detected via a fluorescence assay is shown in Figure 1.

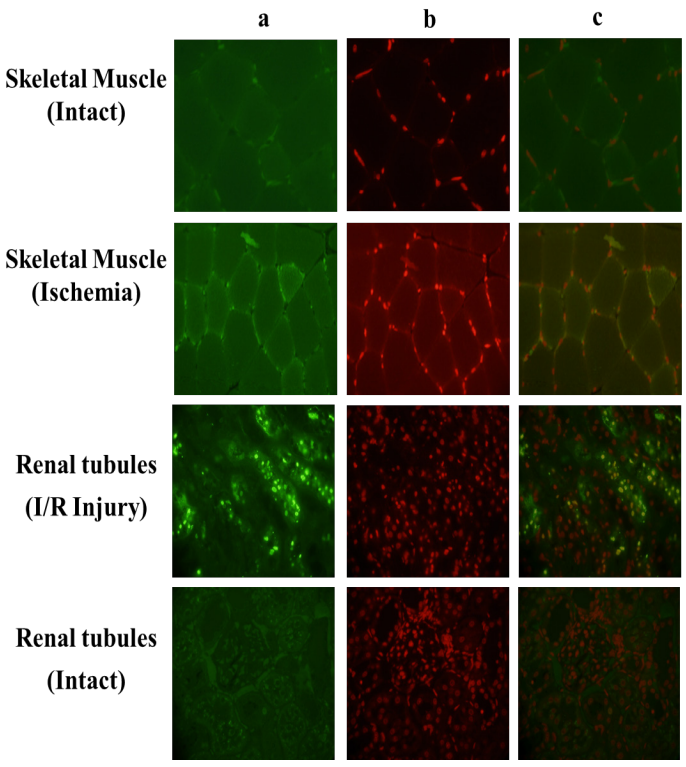

Figure 1: Representative results of apoptosis in skeletal muscle cells and renal tubules. Apoptosis-induced nuclear DNA fragmentation was detected via a fluorescence assay. a) DNA fragments detected using Fluorescence Isothiocyanate (FITC, green) tissue sampled from an intact rat. b) DNA was detected using propidium iodide (PI, red), and c) merged picture of a) and b). The DNA fragmentation was detected under fluorescent microscopy equipped with FITC and PI filters. These pictures were taken at a magnification of 40X.

Discussion

I/R injury is a major cause of tissue damage and cell death across the world [13]. Considerable investigation is underway to find therapies for treating I/R injury in many different tissues. To facilitate these investigations, this project developed a novel multiple-organ procedure for both skeletal muscle I/R injury and kidney I/R injury in a rat model, which is simple to perform, reproducible in the extent of I/R created in each tissue, and independent of animal and surgical variability. It is noted that the technique shown can also be used for studies which are only concerned with ischemia (no reperfusion) by eliminating steps 1-4 of Section 6.

The method described here also enables control tissue samples (tissue not subjected to I/R) to be obtained from the same animal by using tissues from the animal's alternate side. In this study, we created I/R in the left kidney and the left tibialis anterior skeletal muscle, and used the right kidney and right tibialis anterior skeletal muscle as the control tissue in each animal. In this manner, the extent of I/R could be normalized for each animal. For the purposes of this study, kidney and skeletal muscle I/R was created by 3 hours of occlusion of both the renal artery and the femoral artery, followed by 45 minutes of reperfusion. Biological assays, such as DNA fragmentation and glutathione assay were performed on the tissue samples and results will be reported separately.

The principle benefit of this surgical procedure is the reproducibility of the extent of I/R damage created - both between animals in a study, and between surgeons (our unpublished data shows low variability between researchers and animals). This low variability enables easy detection of the effects of an investigative I/R therapy.

Acknowledgments

This work was funded by the Canberra Hospital Private Practice Fund. Animal facilities funded by ACT Health Research Laboratory. The authors wish to thank Professor Chris Parish of the John Curtin School of Medical Research at The Australian National University for his support in biological assay facilities.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Skjeldal S, Grogaard B, Reikeras O, Muller C, Torvik A, et al. (1991) Model for skeletal muscle ischemia in rat hindlimb: evaluation of reperfusion and necrosis. Eur Surg Res 23: 355-65.
2. Lejay A, Meyer A, Schlagowski AI, Charles AL, Singh F, et al. (2014) Mitochondria: Mitochondrial participation in ischemia-reperfusion injury in skeletal muscle. Int J Biochem Cell Biol 50: 101-105.
3. Blaisdell FW (2002) The pathophysiology of skeletal muscle ischemia and the reperfusion syndrome: a review. Cardiovasc Surg 10: 620-630.
4. Dillon JP, Laing AJ, Chandler JR, Wang JH, McGuinness A, et al. (2006) Pravastatin attenuates tourniquet-induced skeletal muscle ischemia reperfusion injury. Acta Orthop 77: p. 27-32.
5. Kirisci M, Oktar GL, Ozogul C, Oyar EO, Akyol SN, et al. (2013) Effects of adrenomedullin and vascular endothelial growth factor on ischemia/reperfusion injury in skeletal muscle in rats. J Surg Res 185: 56-63.
6. Avci G, Kadioglu H, Sehirli AO, Bozkurt S, Guclu O, et al. (2012) Curcumin protects against ischemia/reperfusion injury in rat skeletal muscle. J Surg Res 172: p. e39-e46.
7. Beyersdorf F, Unger A, Wildhirt A, Kretzer U, Deutschlander N, et al. (1991) Studies of reperfusion injury in skeletal muscle: preserved cellular viability after extended periods of warm ischemia. J Cardiovasc Surg (Torino) 32: 664-676.
8. Weiss S, Benoist D, White E, Teng W, Saint DA (2010) Riluzole protects against cardiac ischaemia and reperfusion damage via block of the persistent sodium current. Br J Pharmacol 160: 1072-1082.
9. Wu H, Zhou J, Ou W, Li Y, Liu M, et al. (2017) TAK1 as the mediator in the protective effect of propofol on renal interstitial fibrosis induced by ischemia/reperfusion injury. Eur J Pharmacol 811: 134-140.
10. Fan C, Zwacka RM, Engelhardt JF (1999) Therapeutic approaches for ischemia/reperfusion injury in the liver. J Mol Med (Berl) 77: 577-592.
11. Zhang ZY, Liu XH, Guo XS, Liu FY (2007) Calreticulin is involved in ischemic postconditioning-induced attenuation of ischemia/reperfusion injury in rat skeletal muscle. Sheng Li Xue Bao 59: 643-650.
12. Smith A, Hayes G, Romaschin A, Walker P (1990) The role of extracellular calcium in ischemia/reperfusion injury in skeletal muscle. J Surg Res 49: 153-156.
13. Messner F, Grahammer J, Hautz T, Brandacher G, Schneeberger S (2016) Ischemia/reperfusion injury in vascularized tissue allotransplantation: tissue damage and clinical relevance. Curr Opin Organ Transplant 21: 503-509.