**TITLE:**

Method of direct segmental intra-hepatic delivery using a rat liver hilar clamp model

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**KEYWORDS:**

Hilar clamp, ischemia-reperfusion injury, liver transplantation, polyethylene glycol-superoxide dismutase (PEG-SOD), rat, direct cannulation, hepatic delivery

**SHORT ABSTRACT:**

A unique rat liver hilar clamp model was developed for studying the impact of pharmacologic molecules in ameliorating ischemia-reperfusion injury. This model includes direct cannulation of the portal supply to the ischemic liver segment via a branch of the portal vein, allowing for direct hepatic delivery.

**LONG ABSTRACT:**

Major hepatic surgery with inflow occlusion, and liver transplantation, necessitates a period of warm ischemia, and a period of reperfusion leading to ischemia/reperfusion (I/R) injury with myriad negative consequences. Potential I/R injury in marginal organs destined for liver transplantation contributes to the current donor shortage secondary to a decreased organ utilization rate. A significant need exists to explore hepatic I/R injury in order to mediate its impact on graft function in transplantation. Rat liver hilar clamp models are used to investigate the impact of different molecules on hepatic I/R injury. Depending on the model, these molecules have been delivered using inhalation, epidural infusion, intraperitoneal injection, intravenous administration or injection into the peripheral superior mesenteric vein. A rat liver hilar clamp model has been developed for use in studying the impact of pharmacologic molecules in ameliorating I/R injury. The described model for rat liver hilar clamp includes direct cannulation of the portal supply to the ischemic hepatic segment via a side branch of the portal vein, allowing for direct segmental hepatic delivery. Our approach is to induce ischemia in the left lateral and median lobes for 60 min, during which time the substance under study is infused. In this case, pegylated-superoxide dismutase (PEG-SOD), a free radical scavenger, is infused directly into the ischemic segment. This series of experiments demonstrates that infusion of PEG-SOD is protective against hepatic I/R injury. Advantages of this approach include direct injection of the molecule into the ischemic segment with consequent decrease in volume of distribution and reduction in systemic side effects.

**INTRODUCTION:**

Major hepatic surgery with inflow occlusion, and liver transplantation, necessitate a period of warm ischemia, and a period of reperfusion leading to ischemia/reperfusion (I/R) injury 1. The consequences of I/R injury in the liver have been detailed extensively 1-3. Consequences of I/R injury detailed in the literature include: generation of reactive oxygen species, initiation of the inflammatory cascade including activation of neutrophils, Kupffer cells, and endothelial cells, activation of the heme oxygenase system and activation of toll-like receptors, an imbalance between endothelin and nitric oxide, activation of nuclear factor-**κ**B, and promotion of proinflammatory cytokine and adhesion molecule synthesis 1-3. These proinflammatory events may lead to apoptosis, necrosis, organ dysfunction and eventual organ failure 3.

I/R injury in organs destined for liver transplantation can lead to early graft loss and contributes to the current donor shortage as marginal organs are more susceptible to injury 3. There are currently 15,226 potential recipients on the waiting list for liver transplantation in the United States 4 and only 5,950 liver transplants were performed in 2015 5. Due to this extreme limitation in organ availability, research exploring hepatic I/R injury is needed in order to optimize graft function and organ utilization.

Animal models used to study hepatic I/R injury include rat hilar clamp models and rat liver transplantation models. There are a variety of rat hilar clamp models currently in use. The most common is one in which the portal vein, hepatic artery and bile duct supplying the left lateral and median lobes are clamped using microsurgical clips 6-12 for 30 to 60 min 6,7,10,13,14, and then a period of reperfusion from 60 min to 24 hr 7,9,10,13,14 is allowed. The left lateral and median lobes of the rat liver comprise about 70% of the hepatic parenchyma 9. Some protocols designed to study ischemic preconditioning include intermittent clamping of the hilar vessels or the hind-limb prior to a longer period of ischemia induced by clamping the hilar vessels 9,13. There are also several modifications described in the literature. The first is to clamp the portal vein and hepatic artery supplying the left lateral and median lobes, but exclude the bile duct 15. A second modification is to induce total hepatic ischemia by clamping the portal vein, hepatic artery and bile duct prior to their division 16-20. A third modification includes clamping of the hilar vessels to the right lobe for 30 to 60 min 8. An additional modification involves clamping the vascular bundle in one hind limb in order to induce injury in the liver 13,21. Various approaches to the hilar clamp procedure are illustrated in Figure 1A-D.

Rat liver hilar clamp models have been used to study the impact of different molecules and compounds on hepatic I/R. Depending on the model used these molecules have been delivered using inhalation 11, epidural infusion 12, intraperitoneal injection 17,18,21,22, intravenous administration 10,14,15,19,23,24 or injection into the peripheral superior mesenteric vein 8.

The model for rat liver hilar clamp detailed in this report includes direct cannulation of the portal supply to the ischemic segment via a side branch of the portal vein (Figure 2), which allows for direct segmental hepatic delivery of the pharmacological substance under study. Our approach is to induce ischemia in the left lateral and median lobes for 60 min, during which time an infusion of the substance under study, in this case, pegylated-superoxide dismutase, a free radical scavenger25, is infused directly into the ischemic segment. Blood samples are taken prior to induction of ischemia and at 120 min post-reperfusion. At this point, the rat is sacrificed and samples are taken from the left and median lobes. Additionally, samples are taken from the right lobe to serve as an internal control.

There are numerous advantages to this approach. First and foremost, when the pharmacologic substance under study can be directly injected into the ischemic segment the volume of distribution is quite low in comparison to the volume of distribution of injection into the systemic circulation or the peritoneal cavity. Additionally, this approach reduces, although does not eliminate, the possibility of systemic side effects.

**PROTOCOL:**

All procedures were performed according to the guidelines of the Institutional Animal Care and the National Research Council’s Guide for the Humane Care and Use of Laboratory Animals (IACUC) and has undergone approval by the Ohio State University IACUC committee.

1. **Initial Set-up**

1.1 Set-up the surgical microscope and the operating theater (Figures 3, 4).Turn on all equipment including that for maintaining anesthesia and monitoring vital signs. Turn on the electrosurgical unit and warming pad. Position the infusion pump near the operating table.

* + 1. Draw up 10 mL of liquid isoflurane for inhalation (molecular weight 184.5) in the anesthesia syringe and place it in the anesthesia unit.
    2. Set-up a 200 mL container of liquid nitrogen near the operating table and another near the centrifuge where blood samples will be processed.
    3. Position the surgical instruments, 4-0 and 7-0 braided silk suture, sterile cotton swabs, 4x4 non-woven sponges, 5 mL syringes, and 27 gauge insulin syringes near the operating table.
  1. Prepare the isoflurane chamber and ensure that sufficient isoflurane is instilled in the anesthesia induction delivery system.

1. **Induction of Anesthesia**
   1. Before handling the rat put on the following personal protective equipment (PPE): surgical mask, surgical gloves, and disposable gown.
   2. Weigh the rat and record the weight.

Note: Sprague Dawley rats should be used.

* 1. Place the rat in the anesthesia chamber and turn on the isoflurane and the oxygen. Induce anesthesia using the isoflurane chamber**.**
  2. Clip the animal’s abdominal hair using an electric hair clipper to allow for cleaner exposure (Figure 5).
  3. Place the animal back in the isoflurane chamber for an additional one minute. Perform a toe pinch to verify depth of anesthesia.

1. **Procedure**
   1. Position the rat with the animal’s nose in the nose cone and four extremities immobilized with restraints or tapeon the warming pad.
   2. Continue anesthesia using the anesthesia delivery system, nose cone and isoflurane with anesthesia at 3.6% for animals weighing between 200 and 250 g and 4% for animals weighing greater than 250 g. Confirm depth of anesthesia by performing a toe pinch and a skin pinch.
   3. Make a midline abdominal incision from pubis to xiphoid through the skin using sharp scissors (Figure 6).
   4. Make an incision in the peritoneum along the linea alba from pubis to xiphoid and enter the abdomen taking care not to damage the bladder or bowel. As the liver also sticks to the peritoneum anteriorly near the xiphoid process, ensure that it releases prior to incising the abdominal wall in this area.
   5. Make a transverse incision through the skin and the peritoneum at the level of the inferior border of the right lobe of the liver.
   6. Turn the anesthesia down to 1.6% for animals weighing between 200 and 250 g and 2% for animals weighing greater than 250 g.
   7. Retract the xiphoid process using a curved mosquito clamp.
   8. Place rib retractors pulling the ribs as far apart as possible from the midline (Figure 7). Cut the falciform, phrenic and gastric ligaments. Flip the liver up using moistened sterile cotton swabs.
   9. Cut additional ligaments as necessary to gain access to the porta. Perform visceral rotation with saline moistened gauze (Figure 7).
   10. Remove the loose connective tissue overlying the portal hilum using sharp or blunt dissection. Remove the loose connective tissue overlying the length of the portal vein.
   11. Use forceps to push through the loose connective tissue posterior to the left portal vein, artery and bile duct making a window and place 4-0 Potts suture but do not cinch down (Figure 8).
   12. Clear off the loose connective tissue overlying the posterior branch to the portal vein that comes in at approximately the level of the right kidney. This vein will be used for cannulation.
   13. Draw 0.5 mL of blood out of the inferior vena cava (IVC) with an insulin syringe (Figure 9). Place the 0.5 mL of blood in a small vial, centrifuge at 135 x g for 12 min. Attempt to draw off serum.
       1. If a distinct line cannot be appreciated between red blood cells and serum, try to centrifuge for an additional 2-3 min at 135 x g. Draw off serum and place in a vial for alanine-aminotransferase (ALT). Snap freeze this specimen by placing it directly into liquid nitrogen.
   14. Cut two pieces of 7-0 suture and place near the vein that will be used for cannulation. Place the first 7-0 loop around this vein as far medial as possible. Tie this loop and use it to retract using a curved mosquito clamp (Figure 10). Place a second 7-0 loop on the vein that will be used for cannulation near its intersection with the portal vein and place one tie, but do not cinch down.
   15. Prepare the infusion pump with a 5 mL syringe with 3 mL of reagent. Prime the tubing.
   16. Clamp distal portal vein using a microsurgical clamp.

Note: This will reduce bleeding when the vein is incised for cannulation.

* 1. Cut a 0.5 mm hole in the vein in between the 7-0 stay suture and its intersection with the portal vein using small microsurgical scissors. Use 27-0 catheter to cannulate the left portal venous system (Figures 11, 12).Insert the catheter past the bifurcation of the left and right portal veins.
  2. Check placement of the cannula by infusing 1 mL of normal saline and watch for the left lateral and median lobes of the liver to blanch. Manually confirm that catheter is past the take-off of the right portal vein, but not beyond the take-off of the portal vein feeding the median lobe.
  3. Cinch down the Potts suture and start ischemia time. Tighten 7-0 suture around vein and 27-0 catheter to hold it in place and remove the clamp from the distal portal vein.

3.20 Start the infusion of polytheylene glycol-superoxide dismutase (PEG-SOD, 0.00067 g/mL) using the infusion pump. Start the infusion as close as possible to the start of the ischemic time.

1. **Monitoring**
   1. Continue to monitor the animal’s vital signs throughout the infusion. Deliver 2 mL of 0.9% normal saline or 2 mL of PEG-SOD (0.00067 g/mL) dissolved in 0.9% normal saline over a period of 15 min.
2. **Reperfusion**
   1. Allow one hour to pass from the beginning of the ischemic time. This is 1-hour of warm ischemia time.
   2. Remove the Potts suture. Remove the 27-0 catheter. Cinch down the 7-0 suture around the vein. Note the time. This marks the time of reperfusion.
3. **Continued Sampling**
   1. Draw 0.5 mL of blood out of the IVC at 120 min post-reperfusion. Draw the blood slowly to avoid lysing red blood cells. Slowly drip the blood into a vial. Ensure that bleeding from the IVC is controlled after each blood draw.
      1. If there is continued bleeding apply gentle pressure with a sterile cotton swab or a small 1 cm by 1 cm section cut from gauze.
   2. Centrifuge at 135 x g for 12 min. If sufficient separation is not achieved, try an additional 2-3 min at 135 x g.
   3. Place half of the serum in a vial for later processing for ALT. Snap freeze these specimens.
4. **Euthanasia**
   1. While the rat is still under anesthesia cut the IVC and superior vana cava (SVC) and monitor until blood flow, respiration and heart beat cease.
   2. Incise the diaphragm and perform a brief hepatectomy by incising the diaphragm in a circle and incising additional connective tissue that remains connecting the liver to the peritoneal cavity. Remove the liver from the peritoneal cavity.
   3. Take four samples from the left and median lobes of the liver and four samples from the right lobe of the liver. The samples should be as large as possible and their size will be limited only by the amount of available liver tissue. Place these in small, labeled vials, and snap freeze in the liquid nitrogen. Use these for later processing for tissue adenosine triphosphate (ADP), malondialdehye (MDA) and glutathione (GSH).
5. **Post-experiment Analysis**
   1. Determine glutathione (GSH), malondialdehyde (MDA) and alanine aminotransferase (ALT) activities in liver tissue and serum samples using diagnostic kits according to the manufacturer’s instructions.
   2. Homogenize the liver tissue with lysis buffer and quantify using a Bradford assay. Analyze tissue lysate by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblot using antibodies against cleaved capase-3 and actin. Quantify western blots performed with publically available software.

**REPRESENTATIVE RESULTS:**

This experiment was performed with 2 groups of n=3 rats each. Three rat livers were injected with 2 mL of normal saline (NS) with the infusion pump over a period of 15 min. Three rat livers were injected with 2 mL of normal saline (NS) mixed with pegylated-superoxide dismutase (PEG-SOD, 0.00067 g/mL) with the infusion pump over a period of 15 min. As described in the above protocol, blood samples were taken pre-hilar clamp and at 120-min post-reperfusion. Additionally, after completion of 120-min of reperfusion four liver tissue samples were taken from the left and median lobes and four liver samples were taken from the right lobe of the rat liver.

Serum Alanine Aminotransferase (ALT) was measured pre-hilar clamp and at 120-min post-reperfusion in control (NS) and experimental (PEG-SOD) animals. There was a significant difference between the ALT level of control (NS) animals pre-hilar clamp and at 120-min post-reperfusion. There was a significant difference between ALT level of control (NS) and experimental animals (PEG-SOD) at 120-min (Figure 13A). Tissue malonaldehyde (MDA) was measured for control (NS) and experimental (PEG-SOD) animals in both right and left lobes of the liver. Tissue MDA in right lobe (non-hilar clamp) with control injection (NS) and experimental injection (PEG-SOD) demonstrate no significant difference. Left lobe (post-hilar clamp and reperfusion) tissue MDA with control injection (NS) is significantly different than right lobe (non-hilar clamp) p<0.001. Left lobe (post-hilar clamp and reperfusion) has significantly different levels of tissue MDA with control injection (NS) versus experimental injection (PEG-SOD) p<0.005 (Figure 13B).Tissue glutathione (GSH) was measured and tissue glutathione in right lobe (non-hilar clamp) with control injection (NS) and experimental injection (PEG-SOD) demonstrate no significant difference. Left lobe (post-hilar clamp and reperfusion) tissue GSH with control injection (NS) is significantly different than right lobe (non-hilar clamp) with control injection (NS) p<0.05. Left lobe (post-hilar clamp and reperfusion) has significantly different levels of tissue glutathione with control injection (NS) versus experimental injection (PEG-SOD) p<0.005 (Figure 13C). Western blot was performed comparing right and left lobe of control animals and demonstrates increased cleaved caspase-3 in the left lobe after hilar clamp and reperfusion (Figure 13D). A second western blot was performed comparing the left lobes of animals treated with control and with PEG-SOD (Figure 13E). This demonstrates decreased cleaved caspase-3 in the liver tissue of animals treated with PEG-SOD. Densitometry was also performed demonstrating that the level of cleaved caspase-3 in liver tissue is significantly increased in the left versus right lobe of control animals (Figure 13F). In comparing the left lobe liver tissue of experimental animals, infused with PEG-SOD, and left lobe liver tissue of control animals, infused with Normal Saline, densitometry demonstrates significantly decreased cleaved caspase-3 in animals treated with PEG-SOD in comparison to animals treated with control (Figure 13G).

**Figure 1 – Anatomical Illustrations.** 1A. Anatomical illustration of the rat liver. 1B. Anatomical illustration of the rat liver. The portal pedicle to the left and median lobes of the liver is clamped. The left and median lobes are ischemic. 1C. Anatomical illustration of the rat liver. The portal pedicle to the left lobe is clamped. The left lobe is ischemic. 1D.Anatomical illustration of the rat liver. The portal pedicle to the right lobe is clamped and the right lobe is ischemic.

**Figure 2 – Anatomical Illustrations.** Anatomical illustration of the rat liver with portal vein cannulated via a side branch. The portal pedicle to the left and median lobes of the liver is surrounded by a suture and a microvessel clamp has been used to tighten around the vascular bundle. The left and median lobes are ischemic.

**Figure 3 – Instrument Set-up.** Here is the instrument set-up.

**Figure 4 – Operating Room Set-up.** Here is the operating room set-up.

**Figure 5 –Trimming of Abdominal Hair.** Here shows the trimming of the abdominal hair.

**Figure 6 – Immobilization and Skin Incision.** Here shows the immobilization of the rat and the skin incision.

**Figure 7 – Rib Retractor Placement and Evisceration.** Here shows the rib retractor placement and evisceration.

**Figure 8 – Placement of Suture.** Here shows the placement of the suture.

**Figure 9 – Blood Draw from the Inferior Vena Cava.** Here is blood draw from the inferior vena cava.

**Figure 10 – Vein Branch Tied Off and Retracted.** Here is vein branch tied off and retracted.

**Figure 11 – Process of Cannulation.** Here is the process of cannulation.

**Figure 12 – Cannulation.** Here is the cannulation.

**Figure 13: Representative Results: Direct Segmental Intrahepatic Delivery of Pegylated-Superoxide Dismutase Using a Rat Hilar Clamp Model.** NS = normal saline. PEG-SOD = pegylated-superoxide dismutase, ALT = alanine aminotransferase, MDA = malondialdehyde.A. Serum Alanine Aminotransferase (ALT, mU/mL) compared between pre-hilar clamp and 120-min post-reperfusion. There is a significant difference between control (NS) pre-hilar clamp and control (NS) at 120-min post-reperfusion (p < 0.001). There is also a significant difference between control (NS) and experimental groups (PEG-SOD) at 120-min post-reperfusion (p<0.05). A student’s T-test was used. Error bars represent standard deviation. B. Tissue malondialdehyde in right lobe (non-hilar clamp) with control injection (NS) and experimental injection (PEG-SOD) demonstrate no significant difference. Left lobe (post-hilar clamp and reperfusion) tissue malondialdehyde with control injection (NS) is significantly different than right lobe (non-hilar clamp) p<0.001. Left lobe (post-hilar clamp and reperfusion) has significantly different levels of tissue malonaldehyde with control injection (NS) versus experimental injection (PEG-SOD) p<0.005. A student’s T-test was used. Error bars represent standard deviation. C. Tissue glutathione in right lobe (non-hilar clamp) with control injection (NS) and experimental injection (PEG-SOD) demonstrate no significant difference. Left lobe (post-hilar clamp and reperfusion) tissue glutathione with control injection (NS) is significantly different than right lobe (non-hilar clamp) with control injection (NS) p<0.05. Left lobe (post-hilar clamp and reperfusion) has significantly different levels of tissue glutathione with control injection (NS) versus experimental injection (PEG-SOD) p<0.005. A student’s T-test was used. Error bars represent standard deviation. D. Western blot demonstrating increased cleaved caspase-3 in liver tissue of the left lobe (post-hilar clamp and reperfusion) versus the right lobe (non-hilar clamp) of control animals (Normal Saline). E. Western blot demonstrating decreased cleaved caspase-3 in liver tissue of animals treated with PEG-SOD in comparison to animals treated with control (Normal Saline). F. Level of cleaved caspase-3 in liver tissue is significantly increased in post-hilar clamp and reperfusion animals (p<0.05). A student’s T-test was used. Error bars represent standard deviation. G. In comparing left lobe liver tissue of experimental animals (infused with PEG-SOD) and left lobe liver tissue of control animals (infused with Normal Saline), there is significantly decreased cleaved caspase-3 in animals treated with PEG-SOD in comparison to animals treated with control (Normal Saline). A student’s T-test was used. Error bars represent standard deviation.

**DISCUSSION:**

This series of experiments demonstrated that injection of PEG-SOD into the left and median lobes led to significant decreases in the release of ALT, lipid peroxidation of cell membranes (MDA), and maintenance of glutathione (GSH) when compared with controls (Normal Saline). Liver tissue transaminases including Alanine Aminotransferase (ALT) are established markers of hepatocellular injury. The decrease in ALT when the left lobe is injected with PEG-SOD suggests a protective effect of PEG-SOD. Increased tissue MDA indicates increased lipid peroxidation and is considered a marker of oxidative stress and tissue injury. Overproduction of reactive oxygen species causes an increase in production of MDA 26. The significant reduction in tissue MDA in the animal’s left and median lobes when injected with PEG-SOD demonstrates a protective effect of PEG-SOD. This is consistent with the current understanding that PEG-SOD protects cells from damage caused by partially reduced reactive oxygen species 27. Additionally, in the presence of reactive oxygen species, glutathione disulfide is reduced to glutathione (GSH) 28. The maintenance in GSH in the left and median lobes of the liver injected with PEG-SOD further reinforces the protective effect of PEG-SOD. Additionally it is demonstrated that there is increased cleaved caspase-3, a product of apoptosis, in tissue exposed to ischemia-reperfusion injury. The decrease in cleaved caspase-3 in the left lobe when treated with PEG-SOD suggests that PEG-SOD leads to a decrease in apoptosis.

Superoxide dismutase (SOD) is a critical enzyme in the detoxication of reactive oxygen species. The enzyme catalyzes the conversion of two superoxide anions into hydrogen peroxide and water. The enzyme catalase then converts hydrogen peroxide to water and oxygen, completing the process.25 The half-life of native SOD limited its use in experimental models until the development of conjugated polyethylene glycol-superoxide dismutase (PEG-SOD). Conjugation of SOD to polyethylene glycol increases its half-life from 6 min to 14 hr. Nguyen et al. demonstrated its ability to mitigate lipid peroxidation in hepatic ischemia in a rat model, using systemic delivery.29

There are a variety of potential modifications of the technique detailed here and some have previously been described in the literature. Depending on the model used molecules have been delivered using inhalation 11, epidural infusion 12, intraperitoneal injection 17,18,21,22, intravenous administration 10,14,15,19,23,24 or injection into the peripheral superior mesenteric vein 8.

There are several critical steps in this protocol. The most important is the cannulation of the portal vein. Care must be taken that the hole cut in the vein is not too large. The tissue is very elastic and the hole will enlarge on its own. We recommend starting by cutting a hole that is 0.5 mm with the microsurgical scissors. The cannula can be fed through the hole using an instrument, which allows for greater agility than if trying to perform this portion of the procedure by hand. Additionally, while initially feeding the cannula, it should be aimed directly towards the bifurcation of the left and right portal veins to avoid poking a hole through the back wall of the vein. When the cannula tip reaches the bifurcation it can then be fed into the left vein specifically. Once the cannula is fed into the left portal vein, which supplies both the left and median lobes, its position can be confirmed manually by feeling it inside the vein. Its position can also be confirmed by injecting a small amount of cold saline and seeing the blanching effect on the supplied segments of the liver.

The liver hilar clamp model in the rat provides a reproducible and stable platform for demonstrating hepatic ischemic-reperfusion injury. Variable hilar clamp models have been used by researchers to study the protective effects of anti-oxidants and other small molecules. 6-14. Points of variation include which vessels are clamped, which segment are made ischemic, whether or not the bile duct is included and the length of the period of reperfusion. 6-21.Additionally, when this model is used to study the impact of administration of a molecule the route of administration is also heterogeneous 11,12 8,10,14,15,17-19,21-24. There are several advantages to the described approach. First, direct cannulation of the portal supply to the ischemic segment allows for direct segmental hepatic delivery of the pharmacological substance under study. This allows utilization of the other lobes of the livers as an internal control. Second, segmental hepatic cannulation allows for a reduced volume of distribution for the molecule being studied. This approach thereby reduces the risk of systemic side effects as the substance is injected directly into the liver segment of interest. Direct cannulation of the hepatic segment allows for the substance to be delivered pre-ischemia, intra-ischemia or post-ischemia. This allows for study of the molecule’s effect at any point in the ischemia-reperfusion injury cycle. With increased length of ischemic time and increased level of injury additional opportunity to study liver regeneration would be available.

There are also some limitations of this approach. The first is start-up cost. The purchase of a surgical microscope could be a significant start-up cost for a lab that does not already possess one. This technique may be difficult or impossible without a microscope. The second is learning curve time. Although this procedure is relatively simple it does require some practice and it is likely that a novice will require a significant number of procedures to become an expert.

In summary, this model allows for a reproducible, simple, and cost-effective platform to study hepatic ischemia-reperfusion injury. Although in the protocol described here polyethylene glycol-superoxide dismutase, a free radical scavenger,25 was infused, this model could be used to infuse a variety of different pharmacologic substances in order to evaluate their impact on I/R injury in the liver.

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**DISCLOSURES:**

All authors report they have no disclosures.

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