Video Article
Use of a Hanging-weight System for Liver Ischemia in Mice

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Abstract

Acute liver injury due to ischemia can occur during several clinical procedures e.g. liver transplantation, hepatic tumor resection or trauma repair and can result in liver failure which has a high mortality rate[1-2]. Therefore murine studies of hepatic ischemia have become an important field of research by providing the opportunity to utilize pharmacological and genetic studies[3-9]. Specifically, conditional mice with tissue specific deletion of a gene (cre, flox system) provide insights into the role of proteins in particular tissues[10-13]. Because of the technical difficulty associated with manually clamping the portal triad in mice, we performed a systematic evaluation using a hanging-weight system for portal triad occlusion which has been previously described[3]. By using a hanging-weight system we place a suture around the left branch of the portal triad without causing any damage to the hepatic lobes, since also the finest clamps available can cause hepatic tissue damage because of the close location of liver tissue to the vessels. Furthermore, the right branch of the hepatic triad is still perfused thus no intestinal congestion occurs with this technique as blood flow to the right hepatic lobes is preserved. Furthermore, the portal triad is only manipulated once throughout the entire surgical procedure. As a result, procedures like pre-conditioning, with short times of ischemia and reperfusion, can be easily performed. Systematic evaluation of this model by performing different ischemia and reperfusion times revealed a close correlation of hepatic ischemia time with liver damage as measured by alanine (ALT) and aspartate (AST) aminotransferase serum levels[3,9]. Taken together, these studies confirm highly reproducible liver injury when using the hanging-weight system for hepatic ischemia and intermittent reperfusion. Thus, this technique might be useful for other investigators interested in liver ischemia studies in mice. Therefore the video clip provides a detailed step-by-step description of this technique.

Video Link

The video component of this article can be found at https://www.jove.com/video/2550/

Protocol

General remarks. All operations should be performed under an upright dissecting microscope (Leica) and by using a surgical coagulator. The mice in the experimental and sham groups should be matched in age and weight to ensure comparability of the results. Temperature, blood pressure, anesthesia and fluid administration should be stable and monitored during the entire experiment.

1. Anesthesia and Surgery

1. C57BL/6 mice should be within an age range of 10 to 16 weeks and they should be gender-matched in the respective groups.
2. Anesthetize mice with sodium pentobarbital (70 mg/kg body weight i.p.) and maintain anesthesia with approx. 10 mg/kg/h sodium pentobarbital. Overdosing can significantly lower blood pressure and thus can alter the results.
3. Place mice on a temperature-controlled heated table (RT, Effenberg, Munich, Germany) with a rectal thermometer probe attached to a thermal feedback controller to maintain body temperature at 37 °C.
4. Place mice in a supine position on the surgery table, with the upper and lower extremities attached to the table with removable tape.
5. In case of survival experiments proper aseptic techniques including clipping of hair, disinfection of skin with iodophors (e.g. Betadine), sterile handling of autoclaved instruments, and using masks a sterile gloves for the surgeon are required.

2. Portal Triad Occlusion

1. Cover the incision area with mineral oil to prevent inhalation of mouse hair by cutting the skin and thus preventing the risk of mouse hair allergies. Perform midline laparotomy and incision of the linea alba up to the upper end so that you can easily expose the liver. If possible perform the incision with a coagulation electrode (Erbe, ICC50, Tuebingen, Germany) to prevent bleeding.
2. Place the heating table with the mouse in a position that the tail is directed to the surgeon. That position provides you with the best setting to localize the triad and to place the suture.
3. Displace the intestine on the right side by using a wet cotton tip swab to expose the portal triad.

4. Slightly shift the median and left lobe towards the diaphragm and the right lobe towards the intestine. That will give you a clear view of the portal triad above the bifurcation of right, median/left lobes.
5. Carefully inspect the portal triad and hold the right lobe gently down with bland forceps. That will give you a nice view on the left portal triad.
6. Once visually identified place the needle with the suture (7/0 nylon suture; Ethicon, Norderstedt, Germany) via a needle holder under the left portal triad, including the hepatic artery, hepatic vein, and bile duct from the left to the right side of the mouse.
7. The stitch has to be closely under the triad. A deeper stitch can injure the vena vaca inferior which is located under the triad. Therefore hold the right liver lobes down with the forceps to identify the vena cava before placing the stitch.
8. Take the tip of the needle on the right side with a forceps and pull it gently out of the liver area without injuring the liver.
9. Attach to each end of the suture an Eppendorf tube (approximately 3 g, filled with water) and place both suture ends over suture holders. Place the suture holder distally of the stitch so that the suture does not interfere with the right triad.
10. By applying the weights the triad will be immediately occluded, causing interruption of blood supply to the left and median lobes of the liver. Successful occlusion can be easily confirmed by visual inspection of pale blanching of the ischemic lobes. In contrast, the change of color immediately disappears when the hanging weights are released from the poles and the liver is reperfused. This partial hepatic ischemia model avoids mesenteric congestion by preserving blood flow to the right liver lobes.
11. Replace the intestine into the abdominal cavity. Keep the liver and intestine warm with a wet swab soaked with water at 37.0 °C. In addition to keep body temperature stable cover the mice with commercially available food wrap after finishing surgery.
12. Perform ischemia as required per experimental protocol. After a defined ischemia time release the weights and reperfusion of the median and left liver lobes will start immediately. This technique allows producing reversible ischemia in mice. If no further ischemia times will be performed carefully remove the suture.
13. Add approximately 400 µl sodium chloride into the abdominal cavity 15 minutes before and directly after reperfusion. Continue to substitute fluid every 30 minutes in an amount of 100 µl to compensate fluid loss during the reperfusion time.
14. For survival experiments the surgical wound is closed using continuous suture of the muscle wall and skin with a 4/0 suture.

3. Changes in Liver Perfusion

The following outcome parameters are recommended to determine liver injury following hepatic ischemia:

Determine liver enzymes (e.g. ALT, AST) and liver histology (H&E staining) following liver ischemia and reperfusion[3,9].
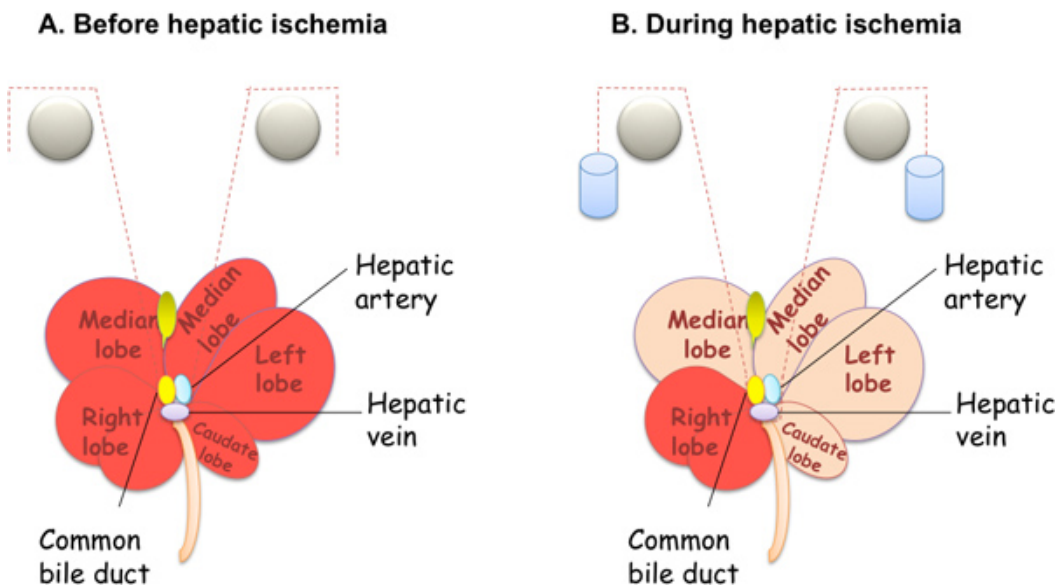

Figure 1. Model of a hanging-weight system for liver ischemia: A hanging weight system has been established to induce liver ischemia of the left and median lobe[3]. (A) Therefore the caudate and right lobe were gently separated from the left lobe. The right lobe was then slightly shifted to clearly view the portal triad above the bifurcation of right, median and left lobes. An 8.0 nylon suture was placed under the left hepatic triad. (B) At the end of each suture a small weight (3 g Eppendorf tubes filled with water) was attached and applied. Hepatic ischemia is visible by a color change of the left and median lobe from red to pale. This experimental design allows precise occlusion and reperfusion of the left hepatic triad by applying and releasing the weight load.
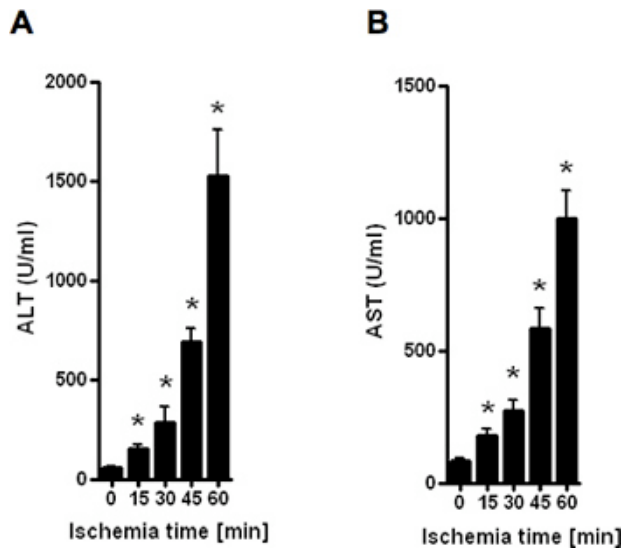
Figure 2. Effect of different liver ischemia times on alanine (ALT) and aspartate (AST) aminotransferase in mice. Partial portal triad ischemia was induced as indicated (0 - 60 min ischemia). After 2 hr of reperfusion, ALT (A) and AST (B) were measured. Results are expressed as means ± SE of 6 - 7 mice/group. *P < 0.05 compared with 0 min ischemia (sham control).

Discussion

The present study describes a technique of performing ischemia in a murine model by using a hanging-weight system for occlusion of the left hepatic portal triad. Although it is an easy method to learn the following pitfalls should be taken into account. A very common mistake in the beginning is a deep stitch which includes the portal triad and the vena cava. The breathing and heart rate will slow down within 15 minutes due to the increased resistance and the decrease in returning blood to the heart resulting in an early death of the mice. The best way to prevent the including of the vena cava is to localize the vena cava before the stitch by holding the right lobes down with a forceps. Another important issue is that the weights are not to heavy which can result in too much stress on the left and right portal trial which also stops blood flow to the right lobes. Therefore the amount of water in the Eppendorf vials should be adjusted to the mouse weight and the suture holders should be placed distally from the stitch location. On the opposite if the weights are to low blood pressure is not completely stopped. Both mistakes are easily detectable by an additional color change to pale of the right lobe or insufficient color change of the median and left lobes, respectively. Therefore the hepatic color change following applying the weights should be controlled every 10 minutes. Another common surgical complication is the injuring of the liver with the needle. Therefore the right lobes should be held down with a bland forceps so that the tip of the needle can be easily seen and taken with a needle holder. If these pitfalls in the beginning are taken into account the hanging-weight system for hepatic ischemia provides a highly reproducible injury due to ischemia by minimizing the variability of liver damage associated with clamping of the portal triad. By using a hanging-weight system, the portal triad is only manipulated once throughout the entire surgical procedure, causing significantly less damage to the hepatic lobes. In addition, no hepatic or intestinal congestion occurs with this technique. More tissue trauma occurs by removing and replacing vessel clamps, especially with reapplication of the clamp during multiple ischemia cycles like during ischemic preconditioning. The use of hanging weights that are in a remote location from the liver tissue, according to our observations, provides the advantage of reliable occlusion while preventing tissue trauma due to manipulation of the liver lobes by reapplication of a clamp. Taken together, the present study provides feasibily of the hanging-weight system for portal triad occlusion during ischemia, minimizing the variability and limitations associated with clamping. Thus this technique might be of interest for other investigators who consider studying hepatic ischemia.

Disclosures

No conflicts of interest declared.

Acknowledgements

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