Video Article

Mouse Complete Stasis Model of Inferior Vena Cava Thrombosis

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Abstract

Venous thromboembolism (VTE) includes both deep vein thrombosis (DVT) and pulmonary embolism (PE). In the United States (U.S.), the high morbidity and mortality rates make VTE a serious health concern [1-2]. After heart disease and stroke, VTE is the third most common vascular disease [3]. In the U.S. alone, there is an estimated 900,000 people affected each year, with 300,000 deaths occurring annually [3]. A reliable in vivo animal model to study the mechanisms of this disease is necessary.

The advantages of using the mouse complete stasis model of inferior vena cava thrombosis are several. The mouse model allows for the administration of very small volumes of limited availability test agents, reducing costs dramatically. Most promising is the potential for mice with gene knockouts that allow specific inflammatory and coagulation factor functions to be delineated. Current molecular assays allow for the quantitation of vein wall, thrombus, whole blood, and plasma for assays. However, a major concern involving this model is the operative size constraints and the friability of the vessels. Also, due to the small IVC sample weight (mean 0.005 grams) it is necessary to increase animal numbers for accurate statistical analysis for tissue, thrombus, and blood assays such as real-time polymerase chain reaction (RT-PCR), western blot, enzyme-linked immunosorbent (ELISA), zymography, vein wall and thrombus cellular analysis, and whole blood and plasma assays [4-8].

The major disadvantage with the stasis model is that the lack of blood flow inhibits the maximal effect of administered systemic therapeutic agents on the thrombus and vein wall.

Video Link

The video component of this article can be found at https://www.jove.com/video/2738/

Protocol

1. Mouse Anesthesia Procedures

1. Animals are removed from their cage and placed in an anesthetic pre-operative induction chamber for gas induction at 5% isoflurane and 100% oxygen, at a flow rate of 0.5 liters per minute (vaporizer controlled).
2. Mice are sedated in the anesthetic induction chamber, removed from chamber and the ventral abdomen is shaved with electric clippers, or maintained within the chamber if a hair depilatory is used (1min).
3. While still under sedation, they are weighed, the eyes are lubricated with sterile opthalmic ointment and then placed in a dorsal recumbancy on a warm water circulating heating device under general anesthesia, with a nose cone, using isoflurane and oxygen. At this point the oxygen rate is reduced to 0.2 liters per minute.
4. The abdomen is sprayed with chlorhexidine solution (chlorhexidine diacetate is rapidly bacterialcidal and persistent) and wiped with sterile gauze three times or until the surgical site is clean.

2. Mouse Micro-surgical and Recovery Procedures

1. A ventral midline incision (2cm) is made with iris scissors through the skin and abdominal wall exposing the abdomen. A sterile saline soaked 2x2in gauze is used to reflect the intestines to the animal's left side. A mouse restrainer is put in place allowing visualization of the inferior vena cava (IVC).
2. At this point, a maintenance level of 2% isoflurance and 100% oxygen rate of 0.2 liters/min is used for the remainder of the surgical procedure.
3. Blunt dissection is facilitated using a sterile applicator swab and extra delicate iris half curved tissue forceps. Care must be taken in handling mouse tissues for they are extremely fragile. Upon entering the abdominal cavity, use a sterile saline soaked 4x4in gauze to reflect and exteriorize the intestines onto a sterile drape, thus allowing visualization of the IVC.
4. All IVC back branches, from the renal veins to the iliac bifurcation, are cauterized while side branches are ligated with 7-0 non-reactive prolene sutures.
5. The IVC is separated from the aorta, just inferior to the renal veins. 7-0 prolene is used to ligate the IVC.

6. The laparotomy site is closed in a two-layer fashion. Our laboratory uses 5-0 vicryl, a synthetic suture, in a continuous pattern to close the abdominal wall. Adhesive skin glue is used to close the skin. There are no external sutures for removal.
7. Mice are then recovered in an individual mouse cage, observed post-operatively (45 minutes to 2 hours) under a heating lamp (minimum distance- 24 inches away from cage), then returned to their original housing units. Pain medication and anti-inflammatory agents can be used if it does not interfere with study objective. Scientific justification to withhold pain medications, veterinary, and ICUCA approval are needed. Our laboratory has found that Buprenorphine, 0.05-0.1 mg/kg SQ is an excellent pre- and post-operative analgesic for mice. In addition, Lidocaine at 4mg/kg (0.4 ml/kg of a 1% solution) can be infused in the incision site when systemic analgesics may confound experimental results.
8. Sham animals from each experimental group undergo the above dissection used for the surgical approach, except the IVC or any draining branches are not ligated or cauterized.
9. Euthanasia is performed in accordance with the recommendations set forth by the American Veterinary Medical Association Guidelines on Euthanasia for rodents.

Discussion

The Mouse Complete Stasis Model of Inferior Vena Cava Thrombosis can be performed on multiple strains of mice of varying ages. The model is most suited to mice of at least 20 grams in weight. This animal model of venous thrombosis allows investigators to study inflammatory interactions between the vein wall and the occlusive venous thrombus. From our experience, we have found that 10 or more animals per group are needed to obtain statistical significance between the groups for thrombus weights, vein wall inflammatory cell counts, vein wall protein, vein wall gene expression, and whole blood or plasma analysis for activity assays.

Disclosures

No conflicts of interest declared.

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