Video Article

Veno-Venous Extracorporeal Membrane Oxygenation in a Mouse

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Abstract

The use of extracorporeal membrane oxygenation (ECMO) has increased substantially in recent years. ECMO has become a reliable and effective therapy for acute as well as end-stage lung diseases. With the increase in clinical demand and prolonged use of ECMO, procedural optimization and prevention of multi-organ damage are of critical importance. The aim of this protocol is to present a detailed technique of veno-venous ECMO in a non-intubated, spontaneously breathing mouse. This protocol demonstrates the technical design of the ECMO and surgical steps. This murine ECMO model will facilitate the study of pathophysiology related to ECMO (*e.g.,* inflammation,bleeding and thromboembolic events). Due to the abundance of genetically modified mice, the molecular mechanisms involved in ECMO-related complications can also be dissected.

Video Link

The video component of this article can be found at https://www.jove.com/video/58146/

Introduction

Extracorporeal membrane oxygenation (ECMO) is a temporary life support system that takes over functions of the lungs and heart to allow adequate gas exchange and perfusion. Hill et al[1] described the first use of ECMO in patients in 1972; however, it only became widely used after its successful application during the H1N1 influenza pandemic in 2009[2]. Today, ECMO is routinely used as a lifesaving procedure in end-stage heart and lung diseases[3]. Veno-venous ECMO is increasingly employed as an alternative to invasive mechanical ventilation in awake, non-intubated, spontaneously breathing patients with refractory respiratory failure[4].

Despite its widespread adoption, diverse complications have been reported for ECMO[5,6,7]. Complications that can be experienced by patients on ECMO include bleeding, thrombosis, sepsis, thrombocytopenia, device-related malfunctions, and air embolism. Moreover, a systemic inflammatory response syndrome (SIRS) resulting in multi-organ damage is well-described both clinically and in experimental studies[8,9]. Neurological complications such as brain infarction are also frequently reported in patients undergoing long-term ECMO therapy. To confuse matters, it is often difficult to distinguish whether complications are caused by ECMO itself or arise from the underlying disorders accompanying acute and end-stage diseases.

To specifically study the effects of ECMO on a healthy organism, a reliable experimental animal model must be established. There are very few reports on performance of ECMO on small animals and are all limited to rats. To date, no mouse model of ECMO has been described in the literature. Due to the availability of a large number of genetically modified mouse strains, establishment of a mouse ECMO model would allow further investigation of the molecular mechanisms involved in ECMO-related complications[10,11].

Based on our previously described murine model of cardiopulmonary bypass (CPB)[12], we have developed a stable method of veno-venous ECMO in non-intubated, spontaneously breathing mice. The ECMO circuit (**Figure 1**), containing outflow and inflow cannulas, a peristaltic pump, oxygenator, and air-trapping reservoir, is similar to our previously described model of murine CPB[12] with the exception of having a smaller priming volume (0.5 mL). This protocol demonstrates the detailed techniques, physiological monitoring, and blood gas analysis involved in a successful ECMO procedure.

Protocol

Experiments were performed on male C57BL/6 mice, aged 12 weeks. This study was conducted in compliance with guidelines of the German Animal Law under Protocol TSA 16/2250.

1. Materials Preparation

NOTE: All steps are performed under clean, non-sterile conditions. Sterile conditions would be required if animal is to be survived postoperatively.

1. Introduce 3 fenestrations into a 2-Fr polyurethane tube using a surgical blade under a microscope with 16X magnification.
   NOTE: All fenestrations must be located in the distal third of the cannula to ensure optimal blood drainage.
2. Prepare the priming solution (**Materials Table**). Include 30 IU/mL heparin and 2.5% v/v of an 8.4% solution of $NaHCO_3$. Refrigerate this solution at 4 °C until it is ready to use. Prime the circuit with 500 uL of priming solution.
3. Place the outflow cannula into the priming solution and fill the ECMO machine by switching on the peristaltic pump. Continue to circulate the priming solution through the machine for the next 30 min at a flow rate of 1 mL/min.
4. Give 0.5 L/min of 100% oxygen to the oxygenator.

2. Anesthesia

1. Place the animal in an induction chamber filled with a 2.5% v/v isoflurane/oxygen mixture. Provide 0.5 L/min of 100% oxygen to the vaporizer. Before surgery, check that full anesthesia is achieved by testing pedal withdrawal and pain reflexes. Apply eye gel to prevent drying damage.
2. Use a warming pad to maintain the body temperature at 37 °C.
3. Perform inhalation mask anesthesia using an isoflurane vaporizer and inject 5 mg/kg carprofen subcutaneously.
4. Regularly observe spontaneous breathing and adjust the concentration of isoflurane so that it is between 1.3 and 2.5%.

3. Surgery

1. Expose the left jugular vein by using a lateral skin incision of 4 mm with the help of fine scissors on the left side of the neck. Together with sharp and blunt preparation using micro-forceps and cotton swabs, use bipolar coagulation of the small vessels.
2. Once the left jugular vein is exposed, ligate the distal part using an 8-0 silk suture with the help of micro-forceps.
3. Place a slip knot at the proximal end of the vein. Incise the anterior wall of the vein using micro-scissors.
4. To achieve full heparinization, inject 2.5 IU/g heparin into the jugular vein via a 26 G braunula.
5. Raise the head side of the animal pad by 30° to avoid excessive blood loss from the vein during insertion of the cannula.
6. Insert a 2-Fr polyurethane (PU) cannula into the proximal part of the jugular vein, rotating it slightly while pushing it to a depth of 4 cm; while doing so, the iliac bifurcation of inferior vena cava (IVC) will be reached.
7. Secure the cannula with 8-0 silk knots using microforceps.
8. Expose the right jugular vein using the steps described in 3.1, 3.2, and 3.3.
9. Cannulate the right jugular vein with a 1-Fr PU cannula and gently move it 5 mm towards the direction of right atrium.
10. Repeat step 3.7.
11. Catheterize the left femoral artery with another 1-Fr PU cannula and use it for invasive pressure monitoring as well as blood sampling for blood gas analysis (BGA).
12. Insert electrocardiogram (ECG) needles connected to a data acquisition device subcutaneously into both forelimbs and into the left thoracic wall.
13. Insert a rectal thermometer connected to a data acquisition device.

4. Veno-Venous Extracorporeal Membrane Oxygenation and Blood Gas Analysis

NOTE: For a schematic of the complete ECMO circuit, see **Figure 1**.

1. Initiate ECMO on the animal by turning on the pump with an initial flow rate of 0.1 mL/min. Adjust the flow rate of the pump within the next 2 min to 3-5 mL/min.
2. In case of air suction in the outflow cannula via the cannulation site, reduce the flow and add 0.1 mL of priming solution to the circuit via an air-trapping reservoir.
3. Under stable flow, continue to monitor in real-time mode all vital parameters via the data acquisition device.
4. Constantly observe backflow from the venous drainage and monitor the level of the blood in the air-trapper reservoir.
5. Collect any blood leaking from wounds into a 1 cc syringe with the tip of a 24 G branula andreturn it to the ECMO circuit via the air-trapping reservoir.
6. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood at the following time points and from the following locations:
   1. 10 min after the initiation of ECMO, collect blood from the IVC via an extra tube built in before the oxygenator, via similar extra tube after oxygenator (control), and directly from the femoral artery.
   2. 30 min after the initiation of ECMO, collect blood from the femoral artery.
7. Give an extra 0.1 mL of priming solution to compensate for intravasal liquid loss every 45 min via the air-trapper or femoral artery catheter or by sucking the air bubbles through the blood draining cannula.
8. For BGA, use a blood sampling cartridge to collect approximately 75 µL of arterial blood:
   1. 1 h after the initiation of ECMO from the femoral artery.
   2. 2 h after the initiation of ECMO, collect blood from the IVC via an extra tube built in before the oxygenator, via similar extra tube after oxygenator (control), and directly from the femoral artery.
9. After 2 h, reduce the flow rate on the pump gradually (over the course of 5 min), thereby stopping ECMO.
10. Continue to record vital parameters for another 10 min.

11. Finish the experiment by exsanguinating the animal and harvesting the blood and organs.

Representative Results

This protocol describes the method of veno-venous ECMO in a mouse. This model is reliable and reproducible, and compared to our previously described model of CPB with respiratory and circulatory arrest[12,13], it is less technically demanding to establish.

ECMO flow in the venous system was maintained between 1.5 and 5 mL/min. The mean arterial pressure was kept between 70 and 85 mmHg by adding extra priming solution into the ECMO circuit. Usually, the adding of 0.1 mL of priming solution to the circuit during ECMO allows substitution of blood volume. All volume substituting or buffering solutions were given via the femoral artery or air-trapping reservoir.

Physiological parameters were recorded every 10 min and data from a representative ECMO experiment are presented in **Figure 2**. BGA data from a successful ECMO are shown in **Table 1**.

Hematological parameters showed relevant hemodilution during ECMO; however, no blood transfusion was necessary to compensate for moderate anemia (**Table 1**). Oxygenation parameters from BGA demonstrated proper performance of the oxygenator at an oxygen/air mixture at FiO_2 1.0 (**Table 1**).

Metabolic changes during ECMO showed respiratory alkalosis at the start and moderate acidosis at the end of the experiment (**Table 1**). No extra buffering of the blood was performed.

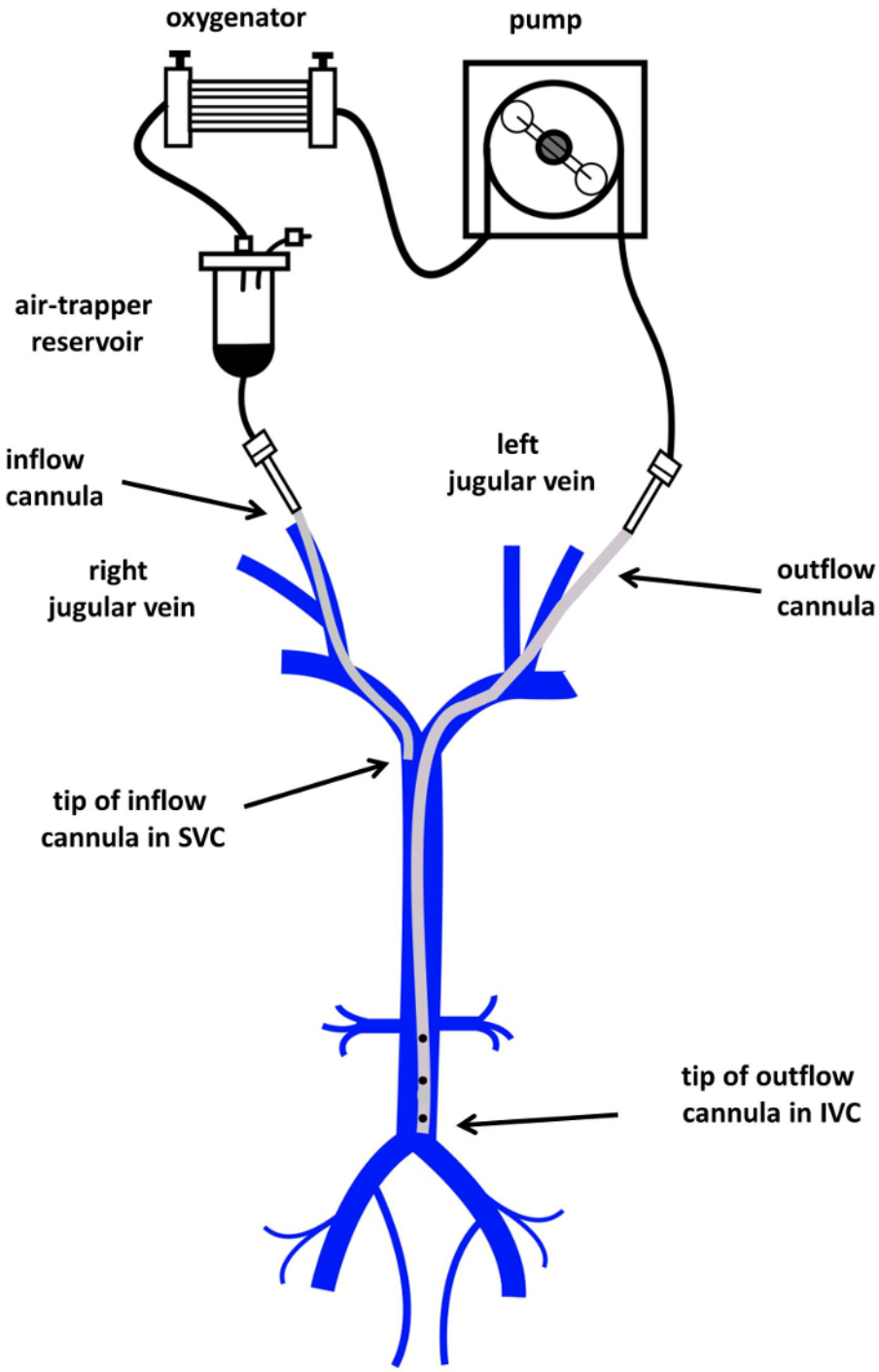

Figure 1: ECMO layout in a mouse. Blood is drained from the inferior vena cava (IVC) via the left jugular vein and oxygenated blood is pumped into the superior vena cava (SVC) via the right jugular vein. Please click here to view a larger version of this figure.

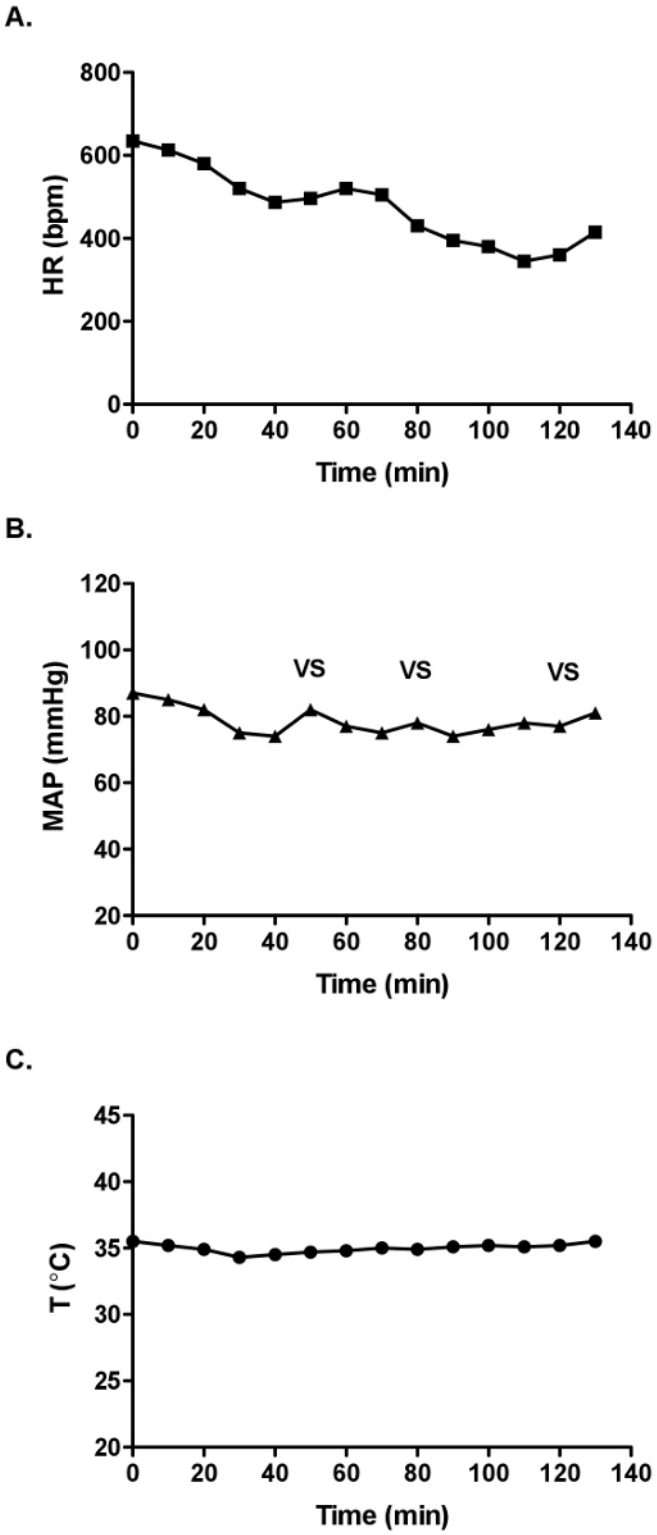

Figure 2: Physiological parameters measured during 2 h of ECMO. A = heart rate, B = mean arterial pressure (VS = volume substitution), and C = rectal temperature. Please click here to view a larger version of this figure.

	10 min			30 min	1 h	2 h		
Parameters	O	FA	IVC	FA	FA	O	FA	IVC
pH	7.67	7.51	7.31	7.57	7.5	7.6	7.57	7.34
pCO2 (mmHg)	24.5	24	52	26	25	22	26	51.1
pO2 (mmHg)	707	656	135	643	621	638	573	101
HCO_3 (mmol/L)	28.3	25.3	26	24	23	27	23	25
sO2 (%)	100	100	99	100	100	100	100	98
HCT (%)	24	23	23	20	18	17	17	16
Hb (g/dl)	8.8	8.6	8.5	8	7.8	7.6	7.2	7
Lac (mmol/L)	1.9	1.7	1.8	2.1	2.4	3.2	3.1	3.3

Table 1: BGA results over the course of the experiment. O = oxygenator, FA = femoral artery, and IVC = inferior vena cava.

Discussion

Previously, we described a successful model of CPB in a mouse[12,13]. To implement such a model for acute or end-stage lung disorders we developed an easy-to-use veno-venous ECMO circuit for mice. Different to the CPB model, veno-venous ECMO does not require complicated surgical procedures such as sternotomy and clamping of the aorta, thus reducing the risk of wound bleeding in a fully heparinized animal. To avoid embolization of the oxygenator with blood clots, 2.5 IU of heparin/kg is given to each animal. This dose was based on previous measurements of the activated clotting time (ACT) that showed full anticoagulation of the blood (ACT > 800 sec). Due to the absence of heparin coating in the micro-oxygenator, our anticoagulation protocol was kept similar to our CPB procedure.

In comparison to the CPB circuit, we could reduce the overall priming volume to 0.5 mL by reducing the volume of the air-trapper and micro-oxygenator. Moreover, a slower flow was necessary to keep adequate oxygenation of the animal. Intravasal loss of the blood volume resulted in a gradual drop of mean arterial pressure. Adding an extra 0.1 mL of priming volume to the animal led to an increase in blood pressure over 20 mmHg, but a small linear reduction in arterial pressure over the next 30 min was always present. Volume substitution was called for if air was sucked through the drainage cannula or there was a drop in blood pressure below 75 mmHg.

The most difficult challenge in the surgical procedure for mouse ECMO model is the placement of the cannula via the left jugular vein into the IVC. To establish this method, different types of cannulas were tested, and a laparotomy was performed in mouse cadavers to perfect the positioning of the cannula tip into the IVC just before the iliac bifurcation. Sometimes, in bigger animals, placement of the cannula can lead to dislocation of the cannula into the right kidney vein. Nevertheless, the whole blood from all segments of the IVC could be well-drained due to side fenestrations of the cannula.

In preliminary trials, we performed cannulation via the femoral vein. Unfortunately, only a 1-Fr cannula can be placed into the femoral vein, which results in inadequate blood flow (≤ 1 mL/min). 1-Fr catheters pushed into the IVC all displayed insufficient backflow. To achieve substantial backflow, both femoral veins would need to be cannulated; therefore, we abandoned this procedure and achieved adequate draining via a 2-Fr cannula placed in the IVC via the jugular vein. Blood loss during placement of the cannula into jugular vein is very typical. Therefore, before placement, the head end of the animal pad is raised 30-40°, so backflow from the vein is significantly reduced.

A gradual reduction in hemoglobin and hematocrit is explained by hemolysis and repetitive blood samplings taken to demonstrate the performance of the device. For survival experiments, to avoid blood transfusions, blood sampling should be extremely limited or even avoided. Moreover, at the end of the experiment, the blood from the ECMO circuit should be returned into the animal. However, survivability of the model has to be studied in a separate project using a less invasive protocol.

Blood flow during our ECMO runs was between 3 and 5 mL/min. Normal mouse cardiac output is reported to be between 6 and 9 mL/min; therefore, on average, we were able to achieve an ECMO flow of 54% of the mouse's cardiac output. Usually, veno-venous ECMO requires lower blood flow compared to veno-arterial ECMO, as overperfusion of the right atrium can lead to right ventricular overload and consequently, heart failure. Clinically, to achieve adequate oxygenation, a veno-venous ECMO flow of 50-75% of cardiac output is enough for sufficient oxygenation in ventilated or spontaneously breathing patients. Unnecessarily increasing the ECMO flow may lead to more damage caused by SIRS and hemolysis and useless recirculation of the major part of the venous blood between the IVC and SVC. Moreover, we observed that by increasing of the flow in the veno-venous ECMO, excessive negative pressure leads to air suction at the site of cannulation. Our animals received 100% oxygen under isoflurane anesthesia, and with the help of veno-venous ECMO, were hyper-oxygenated. In our model we have tried to reproduce conditions of "awake ECMO"[4] having less damage to lungs.

The molecular mechanisms involved in ECMO-related complications can now be investigated due to the plethora of genetically modified mouse strains available. There are also more than eighty strains of mice with lung disorders that may simulate ECMO in the context of these underlying diseases. Therefore, we believe that our veno-venous ECMO mouse model may be implemented in multiple synergistic projects.

Disclosures

The authors have nothing to disclose.

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