A novel rat lung transplantation model using venoarterial extracorporeal membrane oxygenation support

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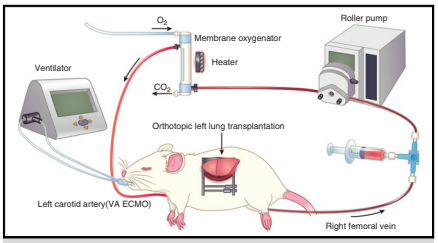
ABSTRACT

Objectives: Extracorporeal membrane oxygenation (ECMO) has become an important life support technique during lung transplantation. We aimed to develop a rat model for lung transplantation using venoarterial (VA) ECMO support.

Methods: Adult male Sprague-Dawley rats weighing 400 to 450 g were used in this study. ECMO circuits were created by obtaining venous access from the femoral vein with subsequent extracorporeal oxygen exchange, which was then returned to the circulatory system through the left carotid artery (ie, VA-ECMO). Simultaneously, the donor lungs were retrieved and immersed in cold, low-potassium dextran lung preservation solution. Orthotopic left lung transplantation supported by VA-ECMO was performed. Thereafter, a respiratory failure rat model was constructed using ventilation with a hypoxic and hypercapnic gas mixture, consisting of 6% oxygen, 8% carbon dioxide, and 86% nitrogen, before lung transplantation. Similarly, left lung transplantation supported by VA-ECMO was performed in rats with respiratory failure. Arterial blood gas levels were measured at designated time points throughout the experiment.

Results: We found that VA-ECMO provided sufficient oxygenation and carbon dioxide removal to allow for smooth left lung transplantation in healthy rats and those with respiratory failure.

Conclusions: We established a rat model for lung transplantation using VA-ECMO. Left lung transplantation using VA-ECMO support is also feasible and safe in rat models of respiratory failure. These models provide efficient and economical models for translational medicine for lung transplantation using ECMO. Moreover, it will be invaluable to evaluate the physiological and pathophysiological roles of ECMO during lung transplantation. (JTCVS Techniques 2024;27:211-6)



A novel rat lung transplantation model using VA-ECMO support.

CENTRAL MESSAGE

A hypercapnic and hypoxemic respiratory failure rat model was successfully constructed and VA-ECMO-supported left lung transplantation was performed.

PERSPECTIVE

These models provide efficient and economical models for translational medicine for lung transplantation using ECMO.

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Video clip is available online.

Extracorporeal membrane oxygenation (ECMO) has become a primary therapeutic option for acute hypoxemic respiratory failure, particularly in patients with COVID-19.[1] Similarly, intraoperative ECMO support has become an increasingly important life support treatment for lung transplantation.[2] Previous studies have used large animals as ECMO translational medicine research models,[3] which may increase experimental costs. Although the use of ECMO has been reported in rats,[4,5] there are no rodent models for lung transplantation using ECMO support. In this study, we first established a rat model for lung transplantation using venoarterial ECMO (VA-ECMO). Furthermore, to the best of our knowledge,

Abbreviations and Acronyms

ABG = arterial blood gas
ECMO = extracorporeal membrane oxygenation
VA-ECMO = venoarterial extracorporeal membrane oxygenation

rats with respiratory failure lung transplant models were also constructed for the first time in this study.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats weighing 400 to 450 g (n = 40) were used in this study. Rats were kept in cages at temperatures of 20 to 22°C with a light/dark cycle of 12/12 hours and had free access to food and water. Animal experiments followed a protocol approved by the Animal Ethics Committee (Ethics approval No.: 2022-31), Wuxi People's Hospital Affiliated to Nanjing Medical University, China.

Anesthesia and ECMO Setup

Sprague-Dawley rats were anesthetized with 5% isoflurane and maintained with 3% isoflurane. After oral tracheal intubation with a 14 G catheter, the rat was placed in the supine position and mechanically ventilated with a tidal volume of 8 mL/kg.

The first step involved vascular puncture and catheterization. The right femoral vein and artery were separated and a 16 G catheter with side holes was inserted into the femoral vein under the guidance of an ultrasliding guidewire. A 24 G catheter was inserted in the right femoral artery for arterial blood sampling for blood gas monitoring. Likewise, we catheterized the left carotid artery with the help of a guidewire and a 20 G catheter.

The ECMO circuit consisted of a micromembrane oxygenator (Micro-1; Micro-1 Oxygenator), roller pump (Masterflex L/S-Easy-load II; Barnant Co), and pipeline. A 3-way valve with a syringe was connected to the tube to control tube pressure and rehydration. Following heparin saline flushing, the entire system was filled with 8 mL heparinized blood from a donor rat. ECMO support was initiated via venous drainage from the right femoral vein with extracorporeal oxygen exchange, which was then returned to the left carotid artery (ie, VA-ECMO).

Donor Lung Procurement

Donor rats were anesthetized with 5% isoflurane and placed in the supine position. After hair removal and disinfection, a median thoracoabdominal incision was made. Heparin 500 IU/kg was administered via the inferior vena cava, and 8 to 10 mL heparinized blood was collected. After the pulmonary artery was perfused with cold low potassium dextran lung preservation solution (4 °C, 20 mL), the donor heart-lung blocks were procured and immersed in cold perfusate. After the left lung was isolated, the cuff technique was used. The donor pulmonary artery, trachea, and pulmonary vein were passed through polyethylene tubes (16 G, 14 G, and 14 G, respectively) and the pipe walls were everted over cuffs and ligated with 7-0 silk thread. The entire process was performed on ice.

Orthotopic Left Lung Transplant Procedure

The recipient rat, supported by VA-ECMO, was placed in the right lateral decubitus position. After thoracotomy, the left lung was gently pulled and pinned. After clamping the hilum with arteriole clips, the hilar structures were separated, and small horizontal incisions were made in the distal portions of the trachea and vessels. Finally, the donor pulmonary vein, trachea, and pulmonary artery were inserted into the recipient incisions separately and successively and tied with silk thread. The arteriole clips were then released and the explanted lung was subsequently ventilated and perfused. The ECMO flow was set at 12 mL/minute and the oxygen flow rate (100% oxygen) of the membrane oxygenator was 40 mL/minute. Arterial blood gases were measured during the lung transplant procedure.[6-8]

Respiratory Failure Rat Models

To construct a respiratory failure rat model,[9] the ventilator air supply was switched from 21% oxygen to a certified gas mixture of 8% carbon dioxide, 6% oxygen, and 86% nitrogen before the lung transplant procedure. Arterial blood gases (ABGs) were measured after 10 minutes of ventilation. The lung transplant procedure was initiated with VA-ECMO support. Once the model was successfully established and confirmed by ABG results, the ECMO was started on time. The ECMO flow was set at 17 mL/minute for control at 40 mL/kg/minute and the oxygen flow rate (100% oxygen) of the membrane oxygenator was 60 mL/minute. The ABG levels were measured during the surgical procedure.

Statistics

Descriptive statistics were used for statistical analysis. Data are presented as mean ± SD. Statistical analyses were performed using GraphPad Prism 7.0 (GraphPad Inc).

RESULTS

Rat Left Lung Transplant With VA-ECMO Support

As depicted in Figure 1, *A*, Figure 2, *A*, and Video 1, VA-ECMO was successfully performed. ABG analysis showed that the P_{O_2} in the postoxygenator was significantly higher than that in the preoxygenator (Figure 1, *B*) ($P < .0001$). The P_{O_2} established during ECMO-support alone could reach that established by ventilation alone, and was even higher using ventilation (21% oxygen) and VA-ECMO together (Figure 1, *C*) ($P < .001$). The intraoperative P_{O_2} during single right lung ventilation after 10 minutes was lower than that with VA-ECMO support (Figure 2, *B*) ($P < .001$). All left-lung transplant surgeries were successful.

Left Lung Transplant With ECMO Support in Rats With Respiratory Failure

A rat model for respiratory failure was successfully established using 8% carbon dioxide, 6% oxygen, and 86% nitrogen mixed gas ventilation. ABG results were consistent with hypercapnic and hypoxemic respiratory failure (Figure 3, *A*). All rats with respiratory failure tolerated left lung transplantation when supported by high-flow VA-ECMO (blood flow was 17 mL/minute for control at 40 mL/kg/minute). ABG analysis showed that the P_{O_2} and P_{CO_2}) were maintained at physiological levels during left lung transplantation (Figure 3, *B* and *C*) ($P < .001$ and $P < .0001$, respectively).

DISCUSSION

Lung transplantation is the only effective method for the treatment of many end-stage pulmonary diseases. Multiple

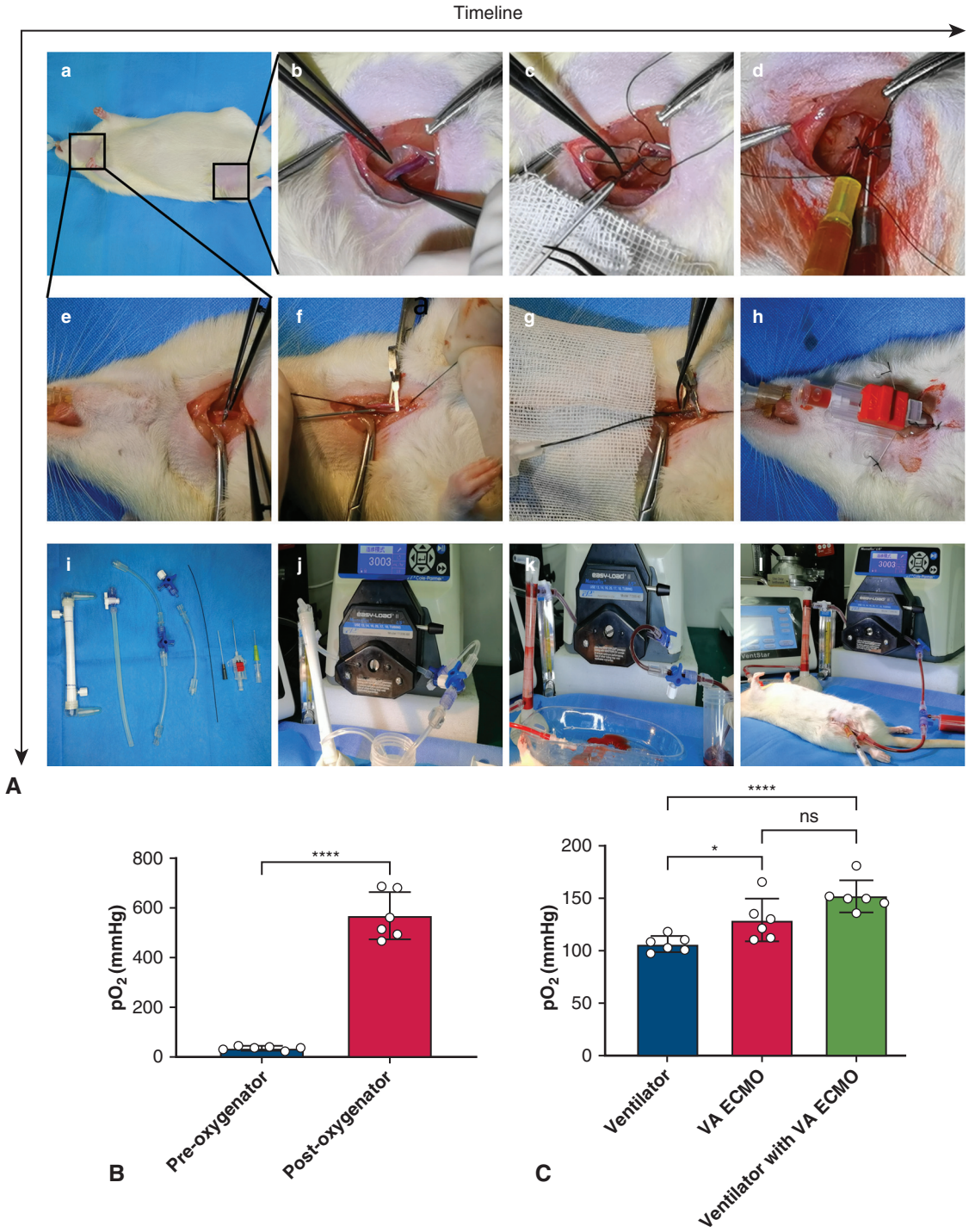


FIGURE 1. A, Establishment of venoarterial extracorporeal membrane oxygenation (*VA-ECMO*) in rats (n = 6). A through D, The right femoral vein and artery puncture. E through H, The left carotid artery puncture. I through L, VA-ECMO set up and initiation. B, Arterial Po_2 of preoxygenator and postoxygenator. C, Arterial Po_2 of ventilator alone (21% oxygen), VA-ECMO alone, ventilator (21% oxygen) with VA-ECMO. The data shown are mean ± SD with individual values presented as a dot plot. Statistical significance was analyzed by Student *t* test. Nonsignificant $P > .05$. *$P < .05$. ****$P < .0001$.

studies have shown that intraoperative ECMO for lung transplantation results in improved survival and lower primary graft dysfunction rates.[10-12] When the physiological demands on ventilatory support exceeds the capability of mechanical ventilation, ECMO may become necessary.[13,14] The orthotopic left lung transplantation model in rats has

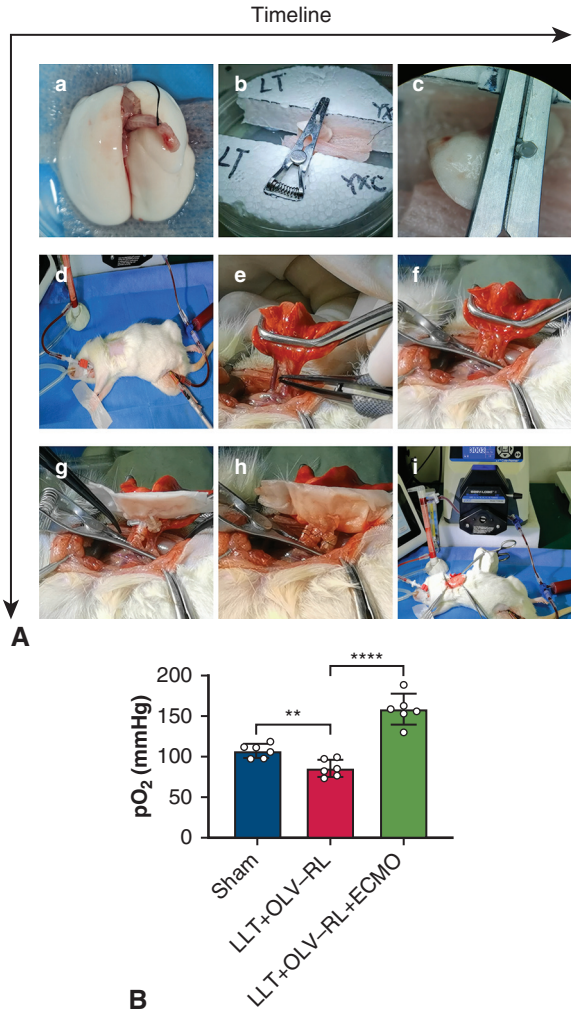
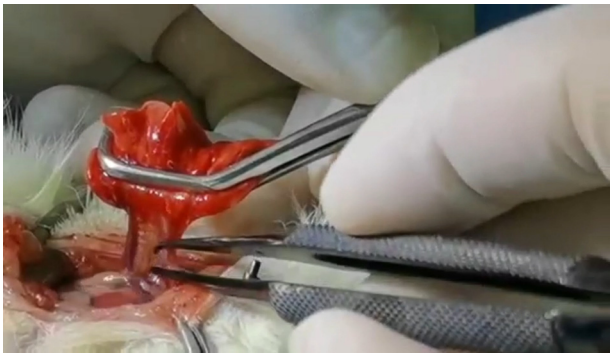


FIGURE 2. A, Left lung transplant (*LLT*) with venoarterial extracorporeal membrane oxygenation (*VA-ECMO*) support. A through C, Donor lung retrieval. D through I, Left lung transplant procedure. B, Arterial Po_2 of sham group (n = 6, 21% oxygen), LLT supported by single right lung ventilation (OLV-RL) group (n = 6, 21% oxygen), LLT supported by ventilator (21% oxygen) and VA-ECMO group (n = 6). The data shown are mean ± SD with individual values presented as a dot plot. Statistical significance was analyzed by Student *t* test. $^{**}P < .01$. $^{****}P < .0001$.

become an important tool in translational medicine for lung transplantation,[8] but there are no rat models for intraoperative ECMO during lung transplantation.

In this study, we established a rat model for lung transplantation using VA-ECMO. This study showed that VA-ECMO is sufficient for oxygenation in lung transplant rat models, which provide major benefit in clinical lung transplantation where most models utilized are in large animals.[3] Obviously, the ease and cost are beneficial using small animal models. Besides, using this model, a variety of genetic variants can be employed to further advance the field of both ECMO and lung transplant.



VIDEO 1. A novel rat lung transplantation model using V-A ECMO support. Video available at: https://www.jtcvs.org/article/S2666-2507(24)00276-1/fulltext.

During model building, it is necessary to introduce a guidewire, which could reduce the risk of vessel wall rupture. To control the tube pressure and ensure proper rehydration, a 3-way valve with a syringe was efficient for the ECMO circuit. To avoid the effects of hemodilution, tubes were filled with heparinized blood from donor rats.

Intraoperative ECMO is commonly used in patients with hemodynamic instability or in those who cannot tolerate single-lung ventilation.[2] Although rat lung transplantation models are common at present, lung transplantation models for respiratory failure in rats are still unreported. The main reason for this is that rats with respiratory failure cannot tolerate single-lung ventilation. This limits further research in translational medicine.

In the present study, we constructed a hypercapnic and hypoxemic respiratory failure rat model and successfully performed VA-ECMO-supported left lung transplantation. This shows that VA-ECMO allows smooth orthotopic left single lung transplantation in rats with respiratory failure.

Although our rat model of respiratory failure was not established by common methods, such as lipopolysaccharide injection, acid aspiration, hemorrhagic shock, or injurious mechanical ventilation,[3,9,15,16] we succeeded in developing a simple respiratory failure rat model that satisfied the criteria for severe respiratory failure.[9] This model is reasonable for simulating clinical situations during lung transplantations. The model required a higher flow rate of ECMO support; therefore, we chose VA-ECMO for intraoperative support.

This study had some limitations. The complexity of the model dictates the number of animals included in each group, which may lead to biased results in the statistical analysis. Owing to the small size of rats, fine surgical skills were required, which could be improved with repeated training over a period of time. Another limitation is the lack of baseline hemodynamic studies and investigation of pathophysiological parameters,[17-20] this may result in some information loss of blood circulation. However, this

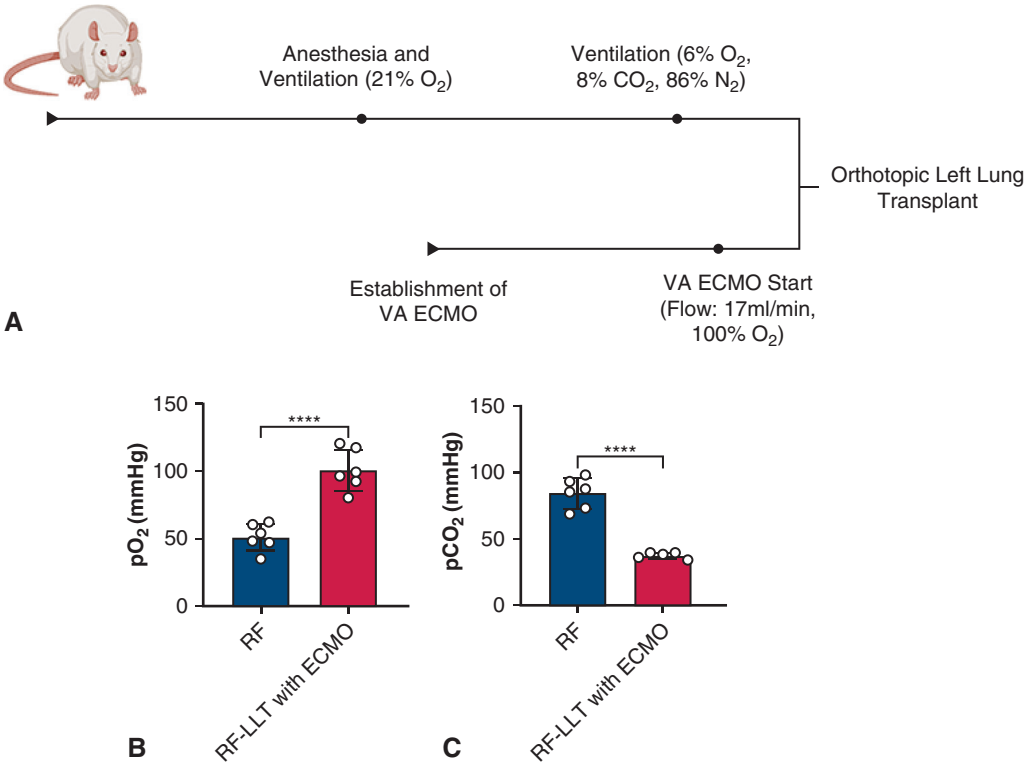


FIGURE 3. A, Left lung transplantation (*LLT*) in rats with respiratory failure supported by venoarterial extracorporeal membrane oxygenation (*VA-ECMO*) (blood flow was 17 mL/minute for control at 40 mL/kg/minute). B, Arterial Po_2 of respiratory failure (*RF*) group (100% oxygen) and RF-LLT with VA-ECMO. C, Arterial partial pressure of carbon dioxide (Pco_2) of RF group (100% oxygen) and RF-LLT with VA-ECMO group (n = 6). The data shown are mean ± SD with individual values presented as a dot plot. Statistical significance was analyzed by Student *t* test. ****$P < .0001$.

would not affect the validity of the results and conclusions from our study. In this study, we constructed a rat model of acute respiratory failure; however, there is still a need for chronic respiratory failure rat models to validate the feasibility of ECMO as bridging therapy to lung transplantation.[13]

CONCLUSIONS

In this study, we established a rat model of lung transplantation using VA-ECMO. Left lung transplantation with VA-ECMO support is also feasible and safe in respiratory failure rat models. These models provide efficient and economical models for translational medicine for lung transplantation using ECMO. Moreover, the evaluation of the physiological and pathophysiological roles of ECMO during lung transplantation is needed.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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Key Words: extracorporeal membrane oxygenation, lung transplantation, animal model, respiratory failure