Anterior hilum anastomosis versus posterior hilum anastomosis in a mouse lung transplantation model

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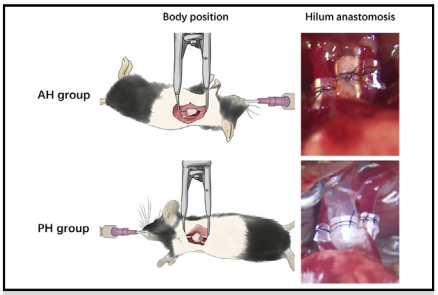
ABSTRACT

Objectives: The mouse orthotopic lung transplantation (LTx) model is of enormous research value in lung transplantation. This study compares 2 anastomotic methods (anterior and posterior hilum anastomosis) of mouse LTx in term of difficulty, operation time, and postoperative effects.

Methods: Twenty mice received LTx with slipknots for anterior hilum anastomosis (AH group), and 28 received LTx with a microvessel clip for posterior hilum anastomosis (PH group), all by a single surgeon. The operation time was recorded and the grafts were evaluated 24 hours after surgery.

Results: The success rates in the recipient animals were 85% (17/20) in AH group and 89% (25/28) in PH group ($P > .05$). The recipient operation time and back table time in AH group were longer than those in PH group (52.8 ± 5.0 vs 47.3 ± 5.7 minutes, 27.8 ± 3.9 vs 25.3 ± 2.8 minutes, $P < .05$), but the warm ischemia time did not differ significantly (13.1 ± 2.1 vs 12.2 ± 2.6 minutes, $P = .258$), meaning that the time discrepancies predominantly originated from the hilum treatment. In AH group, 2 cases failed due to pulmonary venous thrombosis and atelectasis respectively at 24 hours after LTx, but none failed in PH group. No significant difference was observed in the postoperative performance of the successful recipients (thoracic radiographs, macroscopic appearance, oxygenation index, pulmonary compliance, pathologic changes) between the 2 groups.

Conclusions: Compared with anterior hilar anastomosis, posterior hilum anastomosis with a microvessel clip is less complicated and less time-consuming in the management of hilar structures and causes fewer postoperative complications. (JTCVS Techniques 2022;14:159-65)



Schematics of anterior and posterior hilum anastomosis in mouse lung transplantation.

CENTRAL MESSAGE

Posterior hilum anastomosis in mouse lung transplantation is less complicated and less time-consuming in the management of hilar structures and causes fewer postoperative complications.

PERSPECTIVE

Mouse orthotopic lung transplantation is technically challenging, and thus selecting appropriate surgical techniques is of vital importance. Compared with anterior hilar anastomosis, posterior hilum anastomosis is less complicated and less time-consuming in the management of hilar structures and causes fewer postoperative complications.

See Commentary on page 166.

Video clip is available online.

Lung transplantation (LTx) is an important therapy for selected patients with end-stage lung failure.[1] Although the outcomes of LTx are improving year by year, limitations of this procedure, such as primary graft dysfunction, acute rejection, and chronic lung allograft dysfunction, hinder its further application.[2] The clinical progression of LTx relies on the observations made in animal models. Larger animal models (eg, dogs, pigs, and monkeys) offer advantages

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Abbreviations and Acronyms
AH = anterior hilum
Br = bronchus
F_{IO_2} = inspired oxygen fraction
H&E = hematoxylin and eosin
LPD = low-potassium dextran
LTx = lung transplantation
PA = pulmonary artery
PH = posterior hilum
PV = pulmonary vein

in developing surgical techniques,[3] but the scarcity of knockout and transgenic animals limit their usage as LTx models. Ideally, the availability of genetic modifications in mice enables a wide range of studies on mouse models. However, orthotopic pulmonary transplantation in mice remains difficult because it requires masterly microsurgical skills.

In 2007, Okazaki and colleagues[4] reported the first successful orthotopic vascularized aerated left lung transplantation using cuff techniques in a mouse model. In this surgical procedure, anterior hilum (AH) anastomosis was adopted and the framework of mouse LTx was established. Based on this framework, the surgical techniques were improved in the subsequent studies.[5-9] In 2012, Suzuki and colleagues[10] developed a new mouse LTx model using posterior hilum (PH) anastomosis, reporting a shorter warm ischemia time. In this study, we compared the 2 anastomotic methods of mouse LTx regarding their difficulty, operation time, and postoperative effects to provide guidance for subsequent learners.

METHODS

Animals

Male inbred C57BL/6J mice were purchased from Changzhou Cavans Animal Experiment Co, Ltd Ten- to twelve-week-old animals weighing 25 to 28 g were used as both donors and recipients. The LTx using anastomosis in the AH (AH group, n = 20) or the PH (PH group, n = 28) were performed on the animals. The animals were maintained in accordance with the ethical guidelines of the International Council for Laboratory Animal Science and the Policy of Animal Care and Use Committee of Nanjing Medical University. All samples collected after LTx in this study were used for another animal experiment of ischemia-reperfusion injury.

Surgical Techniques

The transplantations were performed under clean conditions by a single surgeon (Z.J.). He completed 2 to 3 mouse lung transplantations using AH and PH approach in alternation each day. An Olympus operating microscope with 10 to 40× magnification was used for both donor and recipient operations. Induction of anesthesia was initiated with intraperitoneal injection of pentobarbital (0.1 mg/g). The animals were intubated orotracheally with a 20-G angiocatheter and connected to a ventilator (VentStar Small Animal Ventilator; RWD Life Science) for respiratory support, using 100% oxygen at a tidal volume of 0.5 mL (2% of the body weight) and a respiratory rate of 100 to 120/min. Anesthesia was maintained with inhaled 1% to 2% isoflurane. No antibiotics were given to both donor and recipient mice.

Donor Procedure

Donor operation was performed in the same way in both groups, as previously reported.[8,10] The cuff for the bronchus (Br) was fashioned from an 18-G Teflon catheter (instead of a 20G one) to reduce air flow resistance.[6] The 22-G cuff was used for pulmonary vein (PV) and 24-G cuff for pulmonary artery (PA). The cuffs were inserted into the distal ends of PA, PV, and Br, and secured with a 9-0 suture. The lung was stored in low-potassium dextran (LPD) solution at 4 °C until transplantation.

Recipient Procedure

Body position. The left chest wall was shaved and prepped with 70% alcohol. The recipient of AH group was placed in the right-half supine position (Figure 1, *A*) and that of PH group was in the right lateral decubitus position (Figure 1, *E*). A left-sided thoracotomy was performed at the fourth intercostal space.

Hilum treatment. The recipient's left lung was pulled out of the thorax with a clamp to expose the AH or PH. Vessel isolation was achieved by gentle and blunt dissection using the forceps tips. Since the PA was relatively robust and resistant to stretching,[7,8] the isolation could be achieved with ease. By contrast, the isolation of PV was relatively tricky. It was performed with gentle spreading movements cautiously, starting from the part adjacent to the left atrium, and leaving an open space of one third of the longitudinal connection between PV and Br (Figure 1, *B* and *F*). The connective tissue and adhering fat around the vessels were removed to avoid cutting a false lumen before donor introduction. After dissecting the recipient's hilar structures, a one-throw slipknot of 9-0 silk was placed at the base of the PA and PV (in that order) to occlude the blood flow to the left lung[5] and the Br was unclamped[7] in AH group (Figure 1, *C*). In PH group, a microvessel clamp was placed on the left pulmonary vessels and Br horizontally adjacent to the heart (Figure 1, *G*).

Anastomosis and reperfusion. An incision was then made in each of these structures in preparation for the cuff insertion. The excess fluid was removed from the Br by a cotton swab before insertion. The donor lung was implanted into the respective recipient structures through the previously made incisions. Anastomosis was performed in the following order: PA, Br, PV (AH group) or PV, Br, PA (PH group). The cuffs were secured with 9-0 sutures.

The transplanted lung was reinflated and reperfused by releasing the microvascular clamp or slip knots (Figure 1, *D* and *H*). After positioning the transplanted lung back into the recipient thorax, the native lung was excised, the thoracic wall was closed by 2 layers, and the orotracheal tube was removed after spontaneous respiration had resumed. Food and water were allowed ad libitum postoperatively. More surgical details are available in Videos 1 and 2.

Evaluation of Techniques

Operation time. Donor operation time was basically the same in both groups. The time of the recipient procedure, including recipient operation time (the interval from recipient skin incision until the thoracic cavity closing), back table time (the interval from beginning the preparation of the hilar structures until the completion of cuff placement), and warm ischemia time (the interval from the removal of the graft from LPD storage until perfusion restoration), was compared between the 2 groups.[7]

Thoracic radiographic examination. Radiographs of the chest were taken at 24 hours posttransplantation to document changes in the thoracic cavity.

Lung functions. At the time of sacrifice, the animals were anesthetized, intubated, and mechanically ventilated on inspired oxygen fraction (F_{IO_2}) of 1.0. Pulmonary compliance was calculated by the PowerLab

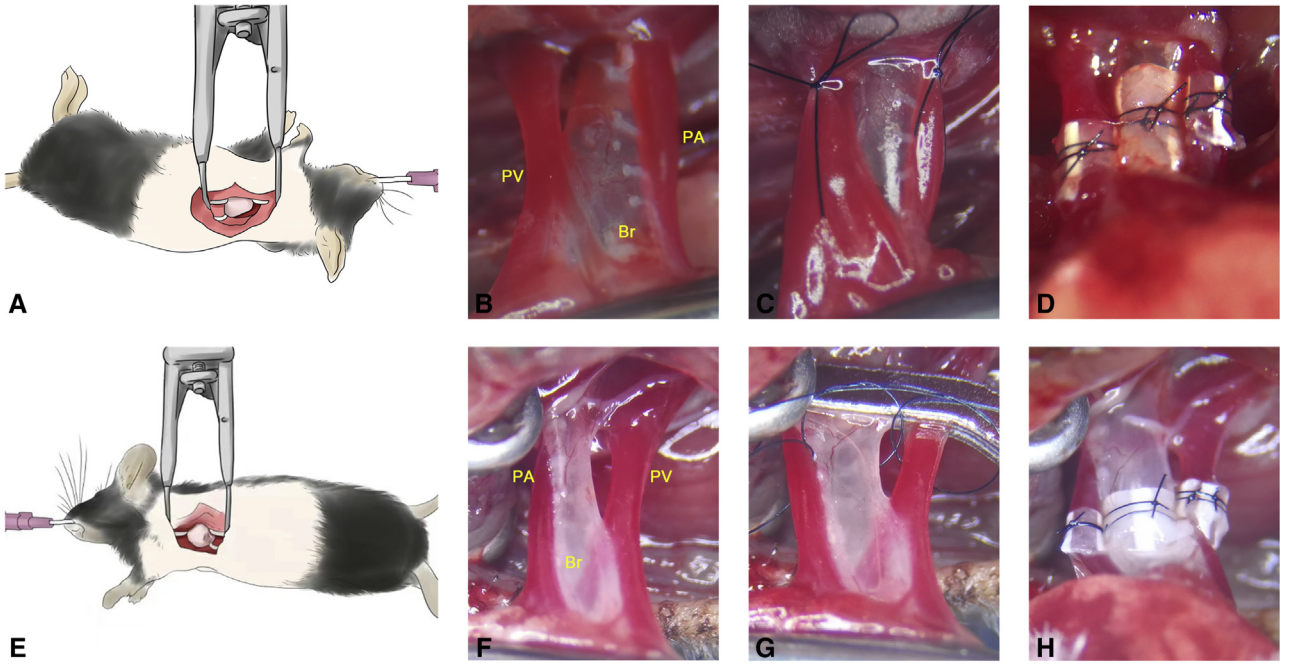


FIGURE 1. Schematic illustrations of the operative position, dissection, occlusion, and anastomosis of the recipient's hilum in the AH group (A-D) and PH group (E-H). *PV*, Pulmonary vein; *PA*, pulmonary artery; *Br*, bronchus.

data acquisition system (8/35; AD Instruments). The right pulmonary hilum was occluded for 3 minutes and subsequently no less than 150 μL of blood was aspirated directly from the ascending aorta for blood gas analysis (iSTAT Portable Clinical Analyzer, Abbott).

Hematoxylin and eosin (H&E) staining. The left lungs were harvested after being inflated and flushed with LPD, then postfixed in formaldehyde, sectioned, and stained with H&E.

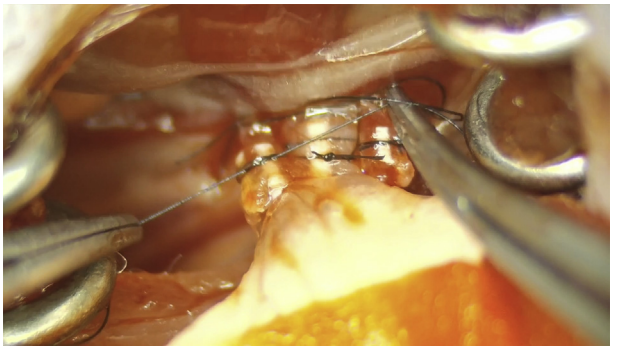
Statistics

Statistical analysis was performed by the Student *t* test and Fisher exact test based on the properties of the data using SPSS, version 22.0 (IBM Corp).

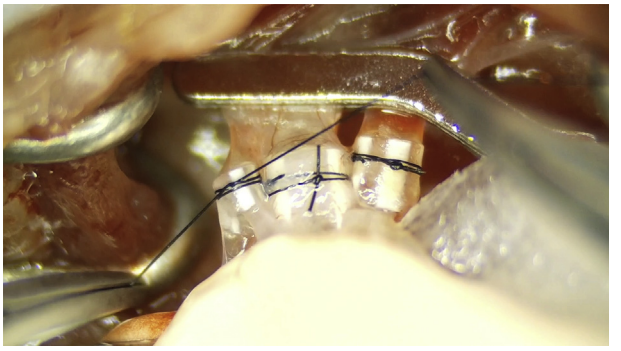
RESULTS

A total of 20 LTxs were performed in AH group, with a success rate of 85% (17/20). One case failed intraoperatively due to PV rupture during implantation and 2 due to pulmonary venous thrombosis (Figure 2, *A*) and atelectasis (Figure 2, *B*) respectively, at 24 hours after LTx. In PH group, 28 LTxs were performed with a success rate of 89% (25/28). Two cases failed due to PV rupture and one due to PA torsion intraoperatively (Figure 2, *C*). No complications were found postoperatively in PH group.

The perioperative indicators are shown in Table 1. The recipient operation time and back table time in AH group were longer than those in PH group ($P < .05$), but the warm ischemia time did not differ significantly between the 2 groups ($P = .258$). At 24 hours after surgery, chest radiography showed that grafts in both groups were well



VIDEO 1. Anterior hilum anastomosis in mouse lung transplantation model. Video available at: https://www.jtcvs.org/article/S2666-2507(22)00254-1/fulltext.



VIDEO 2. Posterior hilum anastomosis in mouse lung transplantation model. Video available at: https://www.jtcvs.org/article/S2666-2507(22)00254-1/fulltext.

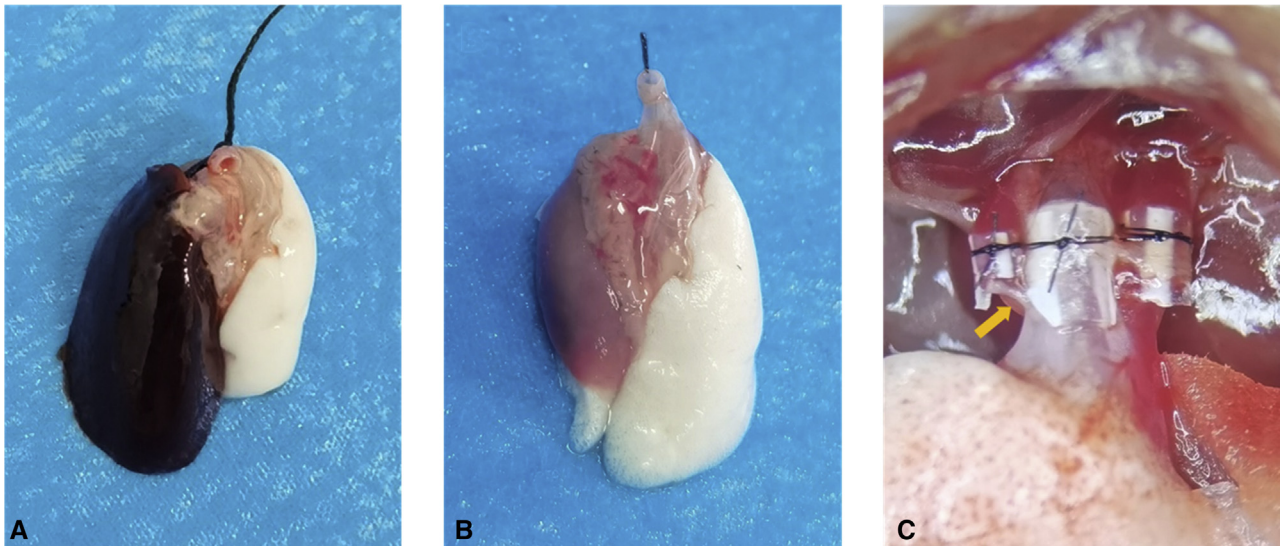


FIGURE 2. Representative pictures of failed cases. A, pulmonary venous thrombosis at 24 hours after LTx; B, atelectasis at 24 hours after LTx; C, PA torsion after reperfusion (*yellow arrow*: site of torsional PA).

inflated (Figure 3, *A* and *B*). The 2 groups had no difference in arterial oxygen tension/F_{IO_2} (362.5 ± 65.1 vs 392.2 ± 86.4 mm Hg, $P = .52$) and pulmonary compliance (28.8 ± 1.7 vs 29.3 ± 1.9 μL/cm H_2O, $P = .67$) (Figure 3, *E* and *F*). After removing the graft lungs, the grafts showed minor lung injury and perfect perfusion (Figure 3, *C* and *D*). Histologically, syngeneic grafts in both groups presented normal appearance with slightly increased alveolar septum thickness and essentially no evidence of cellular infiltration (Figure 3, *G* and *H*).

DISCUSSION

Since the first transplantation in animals in the early 1940s,[11] experimental models have enormously contributed to the development of surgical procedures and the prevention of associated complications, such as rejection or ischemia–reperfusion injury. The mouse model is progressively prevailing because it has a wide range of strains, which enables the study of genetic factors that may affect transplantation.[3] By using mouse models of orthotopic lung transplantation, the researchers can obtain samples (like arterial blood gases, bronchioalveolar lavages, and lung parenchymal tissue) to investigate the responses in human lung transplant recipients.[6] However, orthotopic pulmonary transplantation in mice using cuff techniques is rather formidable because superb microsurgical skills are required due to the diminutive situs and the extremely fragile tissue. Globally, only a few centers can carry out this technique routinely (Table 2).[4,7,10,12,13] For novices, acquiring microsurgical skills needs an extended training period, usually spanning several months.[6,9] Hence, selecting the appropriate surgical techniques could shorten the learning time and reduce the waste of laboratory animals. To the best of our knowledge, there has been no literature comparing anterior and posterior hilar anastomosis in mouse LTx to date.

In the present study, no significant difference was observed in the recipients' postoperative performance (thoracic radiographs, macroscopic appearance, arterial oxygen tension/F_{IO_2}, pulmonary compliance, H&E staining) between AH group and PH group. However, the recipient operation time and back table time in AH group were longer than those in PH group, and the warm ischemia time did not differ significantly, meaning that the time discrepancies were predominantly caused by the management of hilar structures before anastomosis.

TABLE 1. Comparison of perioperative indicators between the 2 groups

Variables	AH group	PH group	*P* value
Success rate (%)	85% (17/20)	89% (25/28)	.683
Operation time, min, mean ± SD			
Recipient operation time	52.8 ± 5.0	47.3 ± 5.7	.003
Back table time	27.8 ± 3.9	25.3 ± 2.8	.034
Warm ischemia time	13.1 ± 2.1	12.2 ± 2.6	.258

AH, Anterior hilum; *PH*, posterior hilum; *SD*, standard deviation.

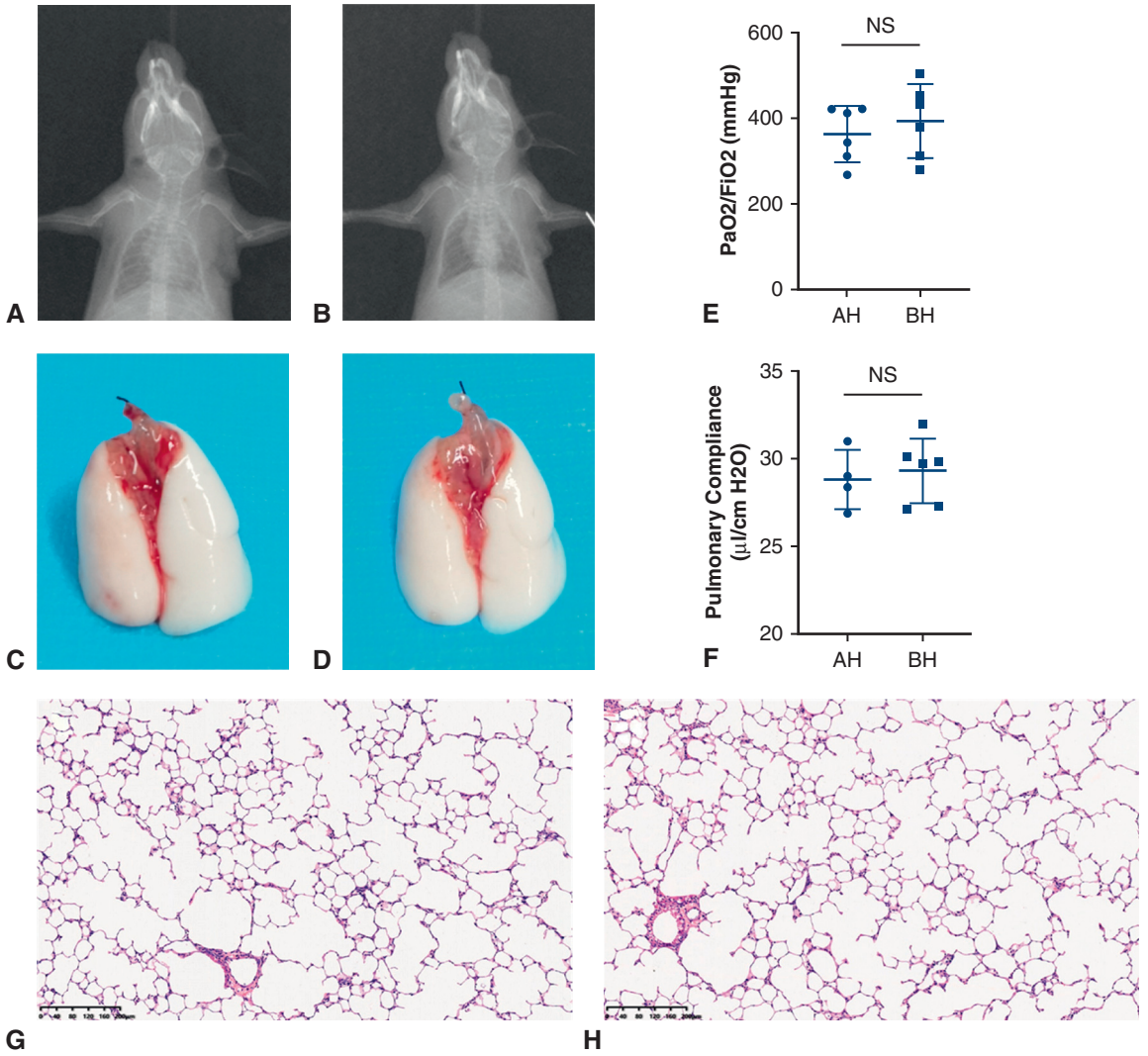


FIGURE 3. Characteristics of recipients in AH and PH groups after 24 hours of reperfusion. A and B, The chest radiographs illustrate well-inflated grafts. C and D, The gross appearance of graft lungs presents no or minor lung injury. No difference in P_{AO_2}/F_{IO_2} (E) and pulmonary compliance (F) are shown between the 2 groups (n = 4-6/group). *NS*, No significant difference ($P > .05$). G and H, Histologic observations of graft lungs present normal appearance. *AH*, Anterior hilum; *PH*, posterior hilum; P_{AO_2}/F_{IO_2}, Arterial oxygen tension/inspired oxygen fraction.

In the PH group, we used a microvascular clamp to occlude the hilar horizontally adjacent to the heart (Figure 1, *G*), which was easy to operate, and no atelectasis occurred after the surgery. Importantly, the structures were flattened by the clamp, making the incision clearer and thus facilitating the implantation. The mouse physiological structure shows that the AH is shorter than the PH, so occluding the central hilar structures with a microvessel clamp horizontally in AH group is not realistic. Generally, the PV and PA were occluded with microvessel clamps vertically or slip knots in AH group. These 2 occlusion methods have their own advantages and disadvantages.[8] The advantages of using clamps lie in its easy and quick application and flexibility of the occluding position. However, the width of the clamp may sacrifice a part of the vessel length, thus making anastomosis more difficult. Slip knots can maintain the length of the structure, but may increase the cumbersomeness and thus prolong the operation time. Moreover, slip knot suture is often entangled with cuff-fixed suture. Jungraithmayr and colleagues[7] suggested that the Br should be unclamped during the operation to avoid injuring the fragile bronchial tissue. However, this move may add to the operation difficulty. The cuff may be pushed out by the airflow of the ventilator during Br insertion in AH anastomosis. Pausing the ventilator, completing cuff placement and securing the silk within 10 seconds could improve insertion stability. However, prolonged breathing suspension could cause

TABLE 2. Comparison of mouse lung transplantation protocols

Technique	Institution	Occlusion of hilum	Survival
Vascular and bronchial cuffs			
Anastomosis in anterior hilum	Washington University[4-6]	Br: clamped with a microvascular clamp vertically PA and PV: occluded with a slip knot	90.8% (109/120)[4]
	University Hospital Zurich[7-9]	Br: remained unclamped PA and PV: clamped with a microvessel clamp vertically	87.5% (21/24)[7]
	Huazhong University of Science and Technology[12]	Br: remained unclamped PA and PV: clamped with a microvessel clamp vertically	96.7% (29/30)
Anastomosis in posterior hilum	Indiana University[10]	Br and PA and PV: clamped with a microvessel clamp horizontally adjacent to the heart	96% (96/100)[10]
Suture	University of Pennsylvania[13]	–	–

Br, Bronchus; *PA*, pulmonary artery; *PV*, pulmonary vein.

death from hypoxia. In addition, since PV is above Br (Figure 1, *C*), the airway fluctuation immediately after Br anastomosis may impact PV inserting, possibly resulting in PV tear.

There are several potential benefits for PH anastomosis. For one thing, when the recipient's left lung was pulled out of the thorax in PH group, the heart was positioned below the hilum (Figure 1, *F*), and thus the beating of the

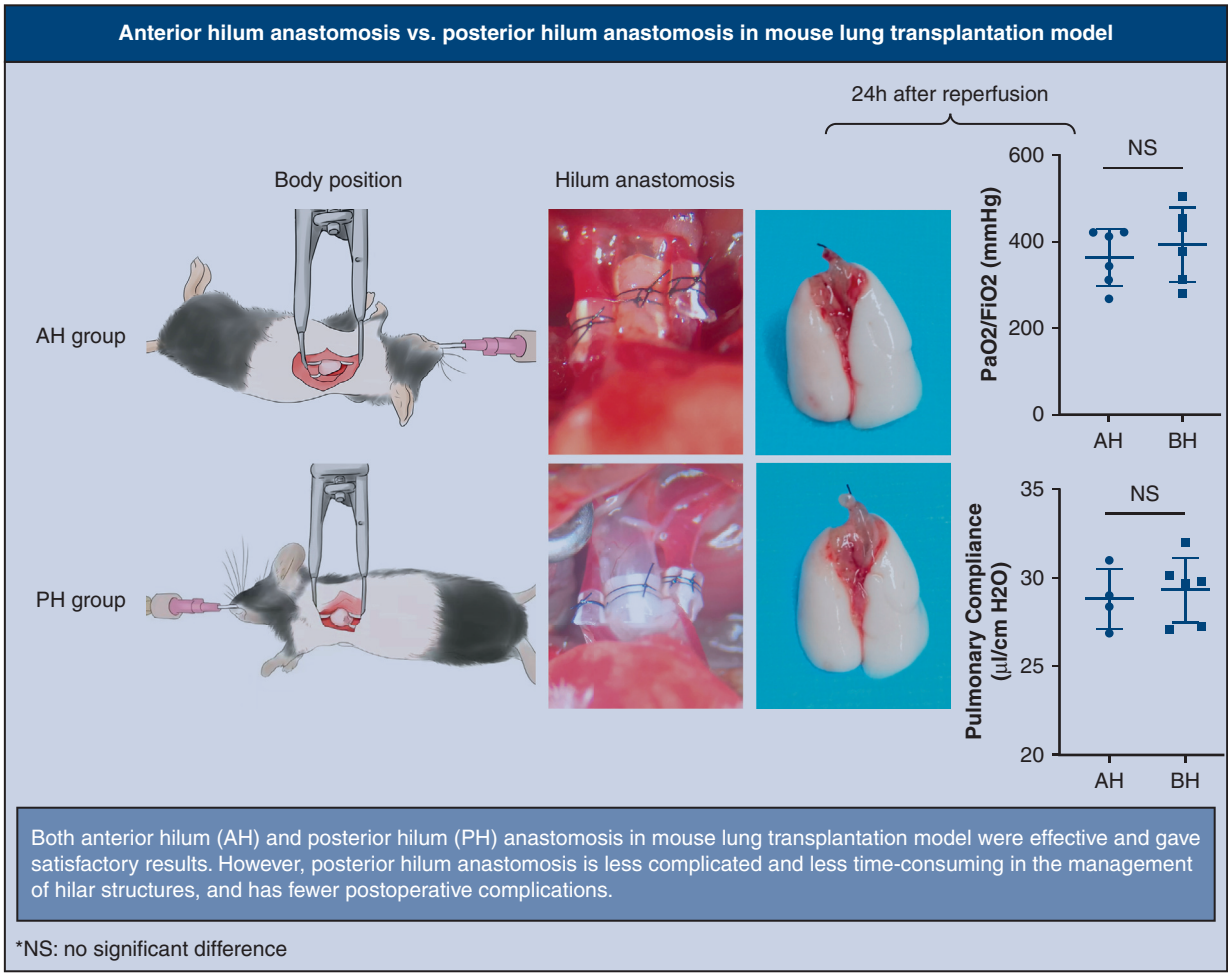


FIGURE 4. Both AH and PH anastomosis in mouse lung transplantation model were effective and gave satisfactory results. However, posterior hilum anastomosis is less complicated and less time-consuming in the management of hilar structures and has fewer postoperative complications. *AH*, Anterior hilum; *PH*, posterior hilum; P_{AO_2}/F_{IO_2}, arterial oxygen tension/inspired oxygen fraction.

heart could hardly interfere with the operation. For another, the mice were in the prone position (lying on their stomach) when in daily activities and the cuff tails were above the hilum in PH group, which had little effect on vessel compression. This may partly explain the absence of complications (such as thrombosis and atelectasis) in PH group.

There are some limitations in this study. First, all the mouse LTxs were performed by a single surgeon skillful at microsurgical techniques, so the discussion about the difficulty of the surgery is relatively subjective. Second, since all samples collected after LTx in this study were used for another experiment of ischemia-reperfusion injury (an early postoperative pathological process), the evaluation time of grafts was 24 hours after surgery. Therefore, the long-term function of the grafts was not assessed. Third, the success rates of mouse LTxs in this study are lower when compared to rates reported in other literature, which might be attributed to the small sample size and the alternation of anastomosis approach.

CONCLUSIONS

This study has determined that both anterior and posterior hilar anastomoses in mouse LTx are effective and can produce satisfactory results, and there is no significant difference between the 2 methods for surgeons skilled in microsurgical techniques (Figure 4). Compared with anterior hilar anastomosis, PH anastomosis using a microvascular clamp is less complicated and less time-consuming in the management of hilar structures, and has fewer postoperative complications.

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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