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ORIGINAL PRE-CLINICAL SCIENCE

In vivo lung perfusion for prompt recovery from primary graft dysfunction after lung transplantation

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KEYWORDS:
lung transplantation;
in vivo lung perfusion;
ischemia-reperfusion injury;
primary graft dysfunction;
organ recovery

BACKGROUND: No proven treatment after the development of primary graft dysfunction (PGD) is currently available. Here, we established a novel strategy of in vivo lung perfusion (IVLP) for the treatment of PGD. IVLP involves the application of an in vivo isolated perfusion circuit to an implanted lung. This study aimed to explore the effectiveness of IVLP vs conventional post-lung transplant (LTx) extracorporeal membrane oxygenation (ECMO) treatment using an experimental swine LTx PGD model.

METHODS: After 1.5-hour warm ischemia of the donor lungs, a left LTx was performed. Following the confirmation of PGD development, pigs were divided into 3 groups (n = 5 each): control (no intervention), ECMO, and IVLP. After 2 hours of treatment, a 4-hour functional assessment was conducted, and samples were obtained.

RESULTS: Significantly better oxygenation was achieved in the IVLP group ($p \leq 0.001$). Recovery was confirmed immediately and maintained during the following 4-hour observation. The IVLP group also demonstrated better lung compliance than the control group ($p = 0.045$). A histologic evaluation showed that the lung injury score and terminal deoxynucleotidyl transferase dUTP nick end labeling assay showed significantly fewer injuries and a better result in the wet-to-dry weight ratio in the IVLP group.

CONCLUSIONS: A 2-hour IVLP is technically feasible and allows for prompt recovery from PGD after LTx. The posttransplant short-duration IVLP strategy can complement or overcome the limitations of the current practice for donor assessment and PGD management.

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Background

Successful recovery of the donor lungs is the primary and most crucial step in posttransplant survival for transplant candidates. Considerable efforts have been made over the last decade with evolving technologies for pretransplant evaluation and reconditioning of donor lungs.[1-4] However, the offered lungs are not fully utilized; thus, the benefits of transplantation are still limited by the number of suitable available organs. According to the latest US statistics provided by the Organ Procurement Transplant Network/Scientific Registry of Transplant Recipients, the actual utilization rate of recovered but not transplanted lungs remains > 5% and has not improved.[5] Accordingly, the effective use of extended-criteria donor lungs remains an ongoing challenge.

Deceased lung donors frequently have clinical factors related to the risk of primary graft dysfunction (PGD), a major morbidity leading to critical complications after lung transplantation (LTx).[6] There is no proven strategy for the treatment of PGD. Extracorporeal membrane oxygenation (ECMO) is used in clinical situation for supporting patients through PGD, but this population still has significantly lower long-term survival than patients without severe PGD.[7] A variety of approaches to preventing PGD have been investigated,[7] but most are still not used clinically. Of these, ex vivo lung perfusion (EVLP) is one of the few technologies that has been successfully applied in clinical practice.[8-10] The concept of pretransplant intervention in EVLP is entirely reasonable in terms of patient safety and clinician judgment. However, the role of EVLP is currently limited to pretransplant evaluation and preparation for reversible edematous pathologies of donor lungs. In addition, controversy persists regarding cost issues and appropriate indication criteria for EVLP application.[11,12] Overall, the management of PGD remains a clinical challenge.[7]

We hypothesized that a quick recovery from PGD can be achieved through posttransplant in vivo lung perfusion (IVLP), in which an isolated circuit for the implanted lung perfused with Steen solution (XVIVO AB) is installed after the confirmation of PGD development. The therapeutic IVLP circuit should have beneficial effects in diluting cytokines and mitigating alveolar edema as observed in EVLP. The IVLP technique has been experimentally developed for the treatment of acute respiratory distress syndrome and localized intensive chemotherapy for metastatic lung malignancies.[13-15] We believe that this technique could also be applied to lung transplant recipients and may enable a prompt recovery from PGD. This study aimed to investigate whether post-LTx IVLP could contribute to recovery from PGD using an experimental swine LTx model.

Material and methods

Study design

Fifteen pairs of Landrace male pigs were used as donors and recipients. All donors were euthanized, and the heart-lung block was procured after a 1.5-hour hands-off time to produce warm ischemic damage. Subsequently, a single left LTx was performed. Following the confirmation of PGD development, we randomly divided the objective recipients into 3 groups and performed 2-hour different interventions (n = 5 each): no treatment (control group), veno-arterial ECMO (ECMO group), and IVLP (IVLP group). After the 2-hour treatment, graft function was observed for the following 4 hours (Figure 1). All animals received humane care in compliance with the Principles of Laboratory Animal Care (formulated by the National Society for Medical Research) and the Guide for the Care and Use of Laboratory Animals (prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health [NIH publication no. 86-23 revised in 1996]). The study protocol was approved by the Animal Care and Use Committee of Okayama University (protocol #OKU-2022319).

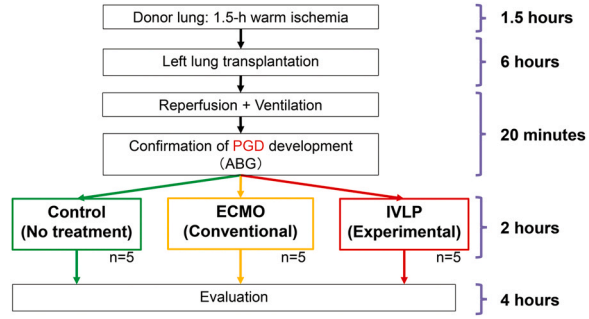

Figure 1 The grafted lung was injured by 1.5-hour ischemia. After left lung reperfusion, we divided the recipients into 3 different arms (control, extracorporeal membrane oxygenation, in vivo lung perfusion). It took approximately 30 minutes from reperfusion to the start of treatment. After the 2-hour intervention, we moved to the 4-hour evaluation phase. ABG, arterial blood gas analysis; ECMO, extracorporeal membrane oxygenation; IVLP, in vivo lung perfusion; PGD, primary graft dysfunction.

Animal preparation and anesthesia

All donors and recipients were premedicated with intramuscular ketamine and atropine sulfate. The airway was secured via tracheostomy. They were then anesthetized with 100% oxygen, sevoflurane, and rocuronium. A volume-controlled ventilator was used with a tidal volume of 12 ml/kg, respiratory rate of 15/min, and positive end-expiratory pressure of 5 cm H_2O. A femoral artery line was inserted to measure arterial blood pressure (ABP) and perform an arterial blood gas (ABG) analysis. A Swan-Ganz catheter was placed in the main pulmonary artery (PA) via the internal jugular vein to measure pulmonary artery pressure (PAP) and cardiac output (CO). Systemic cardiopulmonary parameters were obtained over time.

Donor preparation

The median sternotomy was performed for each donor. Each was euthanized through electric cardioversion, followed by systemic heparinization. After a 1.5-hour warm ischemic phase with the lungs semi-inflated, the heart-lung block was procured in the standard manner. The lungs were flushed with 1,000 ml of low-potassium dextran glucose solution chilled at 4°C (EP-TU; Nipro, Osaka, Japan), and the heart-lung block was stored in a refrigerator at 4°C until implantation.

Recipient operation and evaluation of posttransplant graft function

After being prepared in the same manner as the donor, each recipient underwent single left LTx through left semi-clamshell thoracotomy using standard techniques. PGD development was confirmed 20 minutes after lung reperfusion. No systemic administration of steroid was conducted. Subsequently, we divided the animals into 3 groups as mentioned above. After the preparation procedure for ECMO or IVLP was performed, the treatment was started. It took about 30 minutes from the graft reperfusion to implement ECMO or IVLP. Following the experimental intervention, the ECMO or IVLP circuit was removed, and the data collection continued for 4 hours. ABG, peak inspiratory pressure, ABP, and PAP were recorded every 30 minutes. At each measurement, the right PA was intermittently clamped to obtain accurate left graft function data. Dynamic airway compliance was measured using double-lung ventilation. To mitigate the impact of the healthy right lung on the evaluation of the left lung, we utilized the concept of delta airway compliance. This involved calculating the difference between the baseline data obtained immediately after reperfusion and the subsequent measurements. Dopamine or sodium bicarbonate was used to maintain the ABP or pH, if necessary. At the end of the experiment, tissue samples were collected, and the animals were euthanized.

ECMO group

In the ECMO group, a purse string suture was placed at the ascending aorta and right atrium. After the intravenous administration of heparin, a 16 Fr arterial cannula and 28 Fr venous cannula were placed in the ascending aorta and right atrium, respectively. The circuit was established using a membrane oxygenator and centrifugal pump. ECMO was initiated with a target mean PAP of 15 to 25 mm Hg, systemic artery pressure of 65 mm Hg, and a flow of half the CO, using a flow setting that had been clinically well-verified in a previous report on ECMO for preventing PGD.[16] Ventilation was continued in the initial setting (a tidal volume of 12 ml/kg, respiratory rate of 15/min, and positive end-expiratory pressure of 5 cm H_2O with 100% oxygen). After completion of the treatment, the ECMO flow was slowly reduced and stopped with the maintenance of hemodynamic stability, and the circuit was removed.

IVLP group

A purse string suture was placed at the pulmonary trunk and left atrium (LA), and intravenous heparin was administered. A left PA cannulation was performed before pulmonary vein (PV) cannulation. As for the PA, a 14 Fr inflow cannula was inserted at the trunk, and the tip was placed into the left main PA, where a tourniquet was applied over the cannula to block native blood inflow from the heart. Regarding the PV, the single cannulation method was applied because we employed pigs with a body weight of 30 kg, which have smaller hilar structures compared to animals used in previous IVLP studies. An 18 Fr 2-stage drainage cannula was inserted at the LA appendage, and the tip was placed into the inferior PV. The tourniquet was applied at the PV orifice of the LA, which was on the central side of the PV anastomosis line. This procedure allowed for the drainage of both the upper and lower PV outflow with a single cannula and prevented the PV outflow from returning to the systemic circulation. The IVLP configuration consisted of a de-oxygenator, leukocyte filter, reservoir, centrifugal pump, and primed perfusate of 500 ml of Steen solution supplemented with cefazolin, methylprednisolone, and heparin at 37.0°C (Figure 2). The inflow was gradually increased to a target inflow pressure of 25 mm Hg. The pressure monitoring sensor of outflow was attached to the connector positioned in the middle of the drainage lines of the circuit, which was located approximately 6 to 7 cm higher than the heart. Then the outflow pressure acquired by the sensor was targeted to be between −5 and 0 mm Hg to maintain the estimated actual LA pressure within the range of 0 and +5 mm Hg. Ventilation was continued in the initial setting (tidal volume of 12 ml/kg, respiratory rate of 15/min, and positive end-expiratory pressure of 5 cm H_2O with 100% oxygen).

The perfusate exchanged after 1 hour of IVLP treatment. The inflow and outflow pressures, flow rate, reservoir volume, ABG of the IVLP circuit inflow, outflow, and systemic circulation were monitored every 30 minutes. The oxygenation capacity of the grafted lung was calculated as the difference between the outflow and inflow partial pressure of oxygen per fraction of inspired oxygen (PaO_2/FiO_2). After the IVLP treatment, the left lung was reperfused via the systemic circulation. The first 100 ml of pulmonary drainage through the LA cannula was discarded as an intrapulmonary residual perfusate before the circuit was removed.

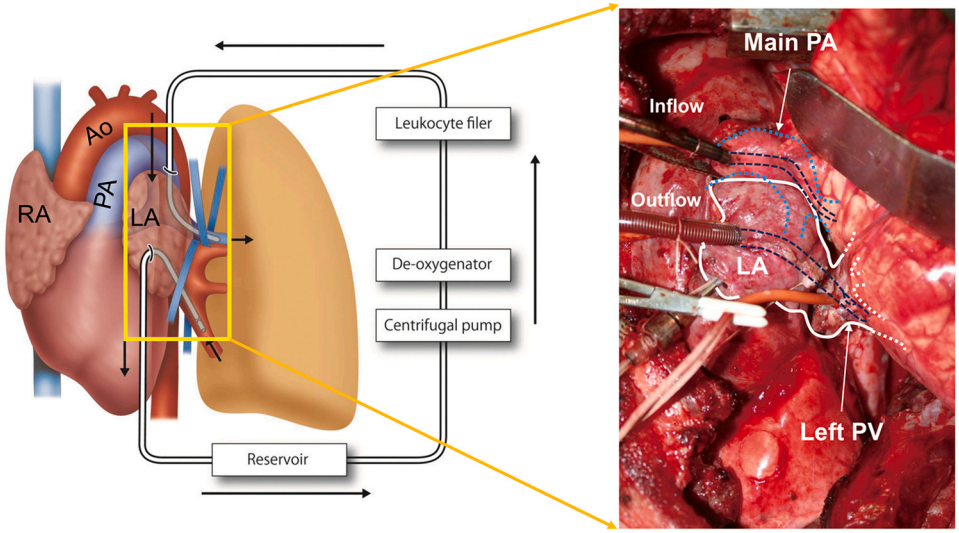

Figure 2 Configuration of In vivo lung perfusion circuit. Ao, aorta; LA, left atrium; PA, pulmonary artery; PV, pulmonary vein; RA, right

Histologic evaluation

Lung tissue samples were collected from the basal and front edges of the left lower lobe tips at the end of the evaluation. The samples were fixed in 10% buffered formalin and embedded in paraffin. The sectioned samples were stained with hematoxylin and eosin for histologic examination. Two blinded pathologists assessed the slides for the presence of lung injury in a randomized manner. Each slide was scored based on the histopathological lung injury score. We then added the two-slide scores of each case as the total scores. The lung injury score included interstitial edema, intra-alveolar edema, hemorrhage, cell infiltration, and hyaline membrane formation. The severity of these findings was graded from 0 (absent) to 4 (severe).[17]

Wet-to-dry weight ratio

The wet-to-dry weight (W/D) ratio of the grafted tissue was analyzed to evaluate pulmonary edema. After lung tissue samples were obtained, the remaining graft tissue was weighed and placed in an oven at 80°C for 10 days.

Analysis of cell death in lung tissue

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) was performed to assess cell death within lung tissue collected from the left lower lobe tips as described above using a commercially available kit (DeadEnd Fluorometric TUNEL System; Promega, Madison, WI). We evaluated 5 randomly selected views per tissue slide and calculated the positive cells/total cells for each. Cell counts were performed using ImageJ 1.53k (National Institutes of Health, Bethesda, MD).

Statistics

The differences in baseline parameters among the 3 groups were assessed using the Kruskal-Wallis rank sum test (Table 1). The differences in the treatment effect on the post-LTx graft physiological function among the groups were analyzed through repeated measures analysis of variance. The analysis of variance tested the overall treatment effect, averaged across all measurement timings. Statistical distinctions between the 2 groups in lung injury score, W/D ratio, and TUNEL assay were determined employing the Mann-Whitney U test. The data are presented as mean ± standard deviation. No adjustments were made for multiple group comparisons. A significance level of $p < 0.05$ was considered statistically significant. All statistical analyses were conducted using EZR version 1.37, developed at Saitama Medical Center, Jichi Medical University, Saitama, Japan. EZR serves as a user-friendly graphical interface for R, version 3.4.1, developed by The R Foundation for Statistical Computing, Vienna, Austria.

Results

Baseline data

There were no significant differences in the baseline conditions among the 3 groups. The oxygenation performance of the graft lung was equally impaired in all 3 groups before the intervention (Table 1). In the ECMO group, the median flow rate was 2.0 liter/min (50.0% CO), and the median PAP (mean) was 16 mm Hg (range 15-23 mm Hg). In the IVLP group, the median perfusion flow was 160 ml/min (5.8% CO), and the median inflow pressure was 23 mm Hg (range 17-25 mm Hg). The mean PAP during treatment in the ECMO group could be managed at around 20 mm Hg during treatment. This indicates that the reperfusion pressure to the transplanted lungs was controlled at an equivalent or lower level than the IVLP group.

Evaluation of graft function

The changes in graft physiological function after LTx are shown in Figure 3. The IVLP group demonstrated significantly greater improvement in the PaO_2/FiO_2 ratio (a) than the control ($p < 0.001$) and ECMO ($p = 0.001$) groups. The recovery of gas exchange in the IVLP group was

Table 1 Baseline Parameters

Parameters	Control	ECMO	IVLP	*p* value
Donor				
Body weight (kg)	29.3 ± 3.6	25.5 ± 1.9	27.2 ± 2.0	0.21
PaO_2/FiO_2	478.2 ± 45.7	501.7 ± 51.5	474.7 ± 37.1	0.69
CO (liter/min)	3.8 ± 1.2	3.2 ± 0.3	3.4 ± 0.5	0.73
Total ischemic time (min)	329.6 ± 46.8	370.6 ± 19.0	382.2 ± 39.3	0.26
Cold ischemic time (min)	164.2 ± 30.9	197.8 ± 34.8	207.6 ± 21.9	0.16
Recipient				
Body weight (kg)	29.7 ± 4.2	29.8 ± 2.8	27.9 ± 3.3	0.62
PaO_2/FiO_2	361.8 ± 106.5	500.4 ± 41.1	474.3 ± 84.2	0.17
CO (liter/min)	4.3 ± 1.0	4.6 ± 0.9	4.0 ± 0.7	0.72
Graft function just after reperfusion				
PaO_2/FiO_2 (with contralateral PA clamped)	90.0 ± 20.1	96.5 ± 37.7	98.5 ± 33.9	0.93
Dynamic airway compliance (ml/mm Hg)	21.7 ± 2.1	20.9 ± 2.6	18.5 ± 1.9	0.13

CO, cardiac output; ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; IVLP, in vivo lung perfusion; PaO_2, partial pressure of oxygen.

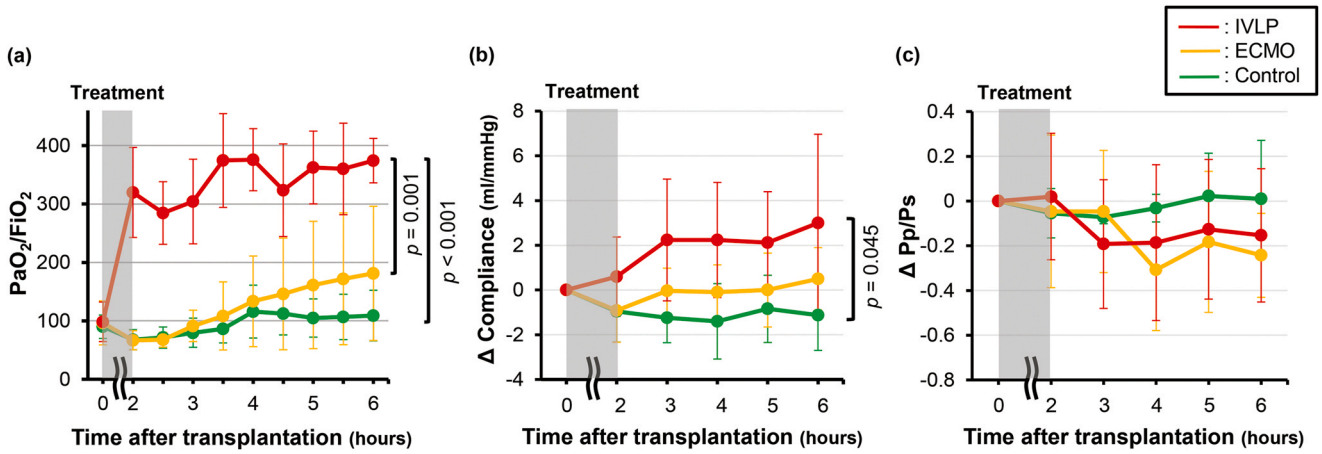

Figure 3 (a) In vivo lung perfusion group showing rapid improvement and reaching almost fully recovered in partial pressure of oxygen per fraction inspired oxygen. (b) Total lung compliance also improved after in vivo lung perfusion. (c) Extracorporeal membrane oxygenation and in vivo lung perfusion groups showed a tendency toward an improvement in pressure/systemic arterial pressure. ECMO, extracorporeal membrane oxygenation; FiO_2, fraction of inspired oxygen; IVLP, in vivo lung perfusion; PaO_2, partial pressure of oxygen; Δ Pp/Ps, Δ pulmonary artery pressure/systemic arterial pressure.

confirmed immediately after the treatment and was maintained during the following 4-hour observation. Delta airway compliance and Δ pulmonary artery pressure/systemic arterial pressure (Pp/Ps) were calculated as the difference compared to that at the time of just after reperfusion. Dynamic airway compliance also improved after IVLP compared to the control group ($p = 0.045$) (b). There was no significant difference in ΔPp/Ps among the 3 groups, although the ECMO and IVLP groups showed an improving tendency (c).

Evaluation of graft function during IVLP treatment

Figure 4a shows $\Delta PaO_2/FiO_2$ during IVLP treatment. Graft oxygenation ability constantly improved during treatment. Concerning dynamic lung compliance (Figure 4b), delta compliance also improved rapidly in the first 30 minutes and persisted until the end of treatment. Delta pulmonary vascular resistance peaked during the first hour and then decreased in the latter half of treatment (Figure 4c). Delta compliance and delta pulmonary vascular resistance were calculated as differences compared to the start of IVLP treatment. The pretreatment gross findings of edema and atelectasis in the lower lobe were promptly relieved and almost disappeared at the end of IVLP treatment (Figure 4d), which was not observed in the control (Figure 4f) and ECMO (Figure 4e) groups.

Histologic findings and lung injury score

Figure 5a shows representative histologic findings of the graft tissue stained with hematoxylin and eosin. Alveolar edema and cell infiltration were less severe in the IVLP group than in the other groups. Figure 5b-f compares the lung injury scores. Intra-alveolar edema, hemorrhage, and cell infiltration were significantly less severe in the IVLP. The hyaline membrane formation score was 0 for all samples. Overall, the IVLP group and ECMO group showed a lower total lung injury score than the control group ($p = 0.012$, 0.043).

W/D ratio

Figure 5g shows the W/D ratio. Although the difference did not reach statistical significance ($p = 0.064$), the IVLP group demonstrated a comparatively lower value than the other 2 groups.

TUNEL assay

Figure 6a shows representative images of the TUNEL staining results. The number of TUNEL-positive cells/total cells in the IVLP group was significantly lower than that in the control ($p = 0.008$) and ECMO ($p = 0.008$; Figure 6b).

Discussion

Solid management to avoid high-grade PGD is key to successful organ utilization and posttransplant outcomes.[18,19] Procedures for donor lung management, evaluation, optimization, organ procurement, and preservation are well standardized.[1] For posttransplant intervention, however, no proven treatment for the development of PGD is available.[7] PGD is typically managed through protective mechanical ventilation[20] and fluid management.[21] Additionally, the utilization of extracorporeal cardiopulmonary support[22] is necessary in certain instances to ensure adequate oxygenation until autonomic recovery of graft function is attained. In this study, we explored the potential of IVLP as a novel posttransplant treatment strategy for PGD triggered by severe graft ischemia-reperfusion (IR) injury. The experimental IVLP group demonstrated a significantly quick recovery of lung function not seen in the ECMO and control groups. Thus, this study shows that a brief treatment with IVLP

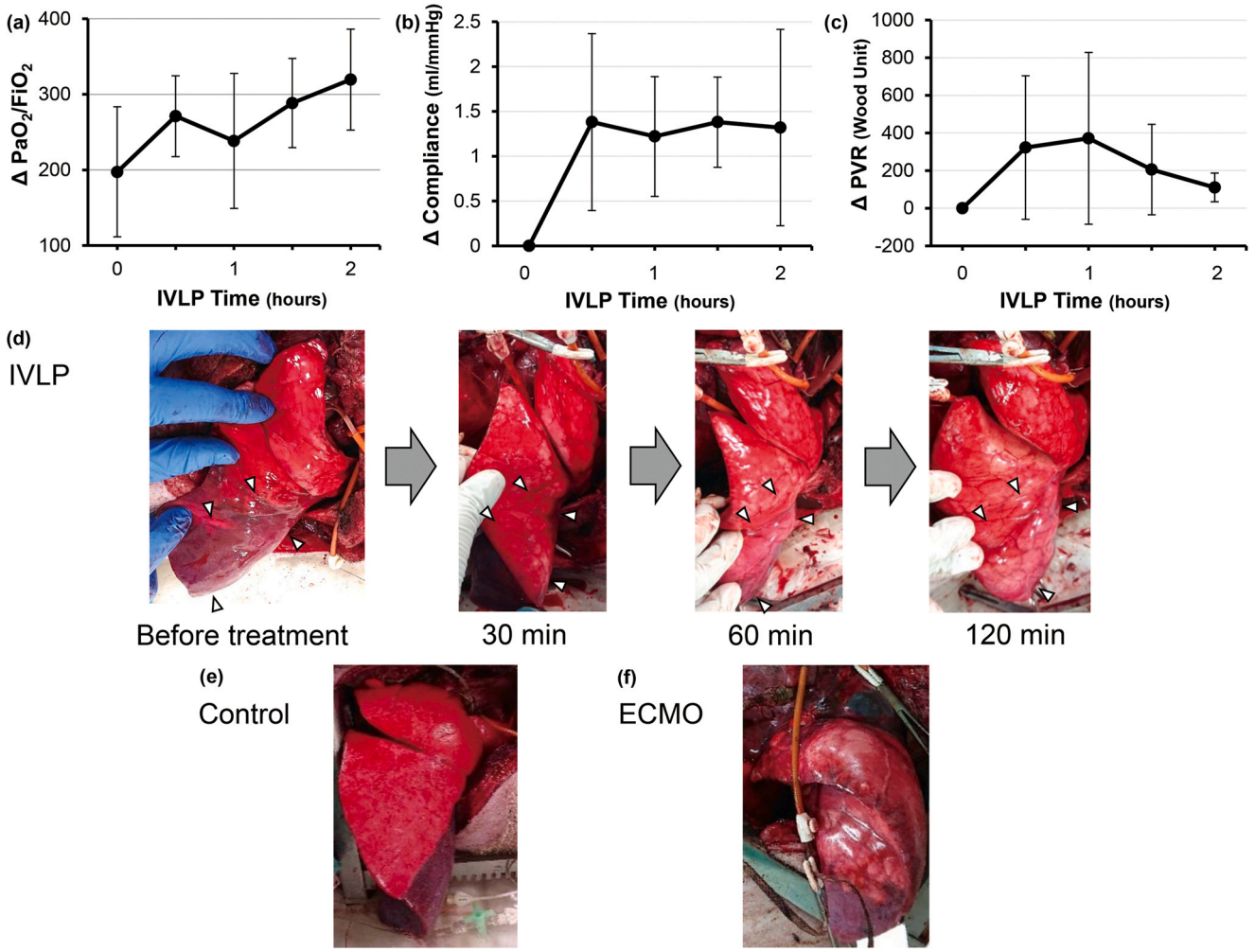

Figure 4 (a) During in vivo lung perfusion treatment, Δ partial pressure of oxygen per fraction inspired oxygen improved rapidly in the first 30 minutes and continued until the end. (b) Δ compliance improved in the first 30 minutes and remained better until the end. (c) Δ pulmonary vascular resistance was increased in the first hour and decreased in the second hour. (d) The severely edematous appearance and atelectasis, especially in the lower lobe (arrowhead), at the start of circulation was rapidly improved by in vivo lung perfusion treatment. (e, f) The gross findings of control, and ECMO group after 4-hour evaluation.

is technically feasible and contributes to a prompt improvement of lung function in the post-LTx PGD setting.

PGD is multifactorial and ultimately attributed to IR-induced lung injury.[23] Acute inflammation provoked by lung reperfusion and the production of multiple cytokines are essential pathologies of IR injuries.[24] Inflammation results in subsequent alveolar epithelial and vascular cell injuries,[25,26] interstitial and intra-alveolar edema, hypoxia, and cell death. Considering the treatment time provided by the standard EVLP protocol,[8] the 2-hour IVLP intervention was relatively short. However, the study results indicate that postoperative IVLP could alleviate PGD progression despite its brief duration. We believe there are several reasons regarding the effectiveness and advantages of short-duration IVLP shown in this study. The first point is the prompt initiation of therapeutic intervention during the surgery immediately after confirming the onset of PGD. We consider that initiating IVLP early after the development of PGD is crucial in stopping the progression of sequential IR pathologies. This approach helps prevent irreversible cell death in the lung tissue and ultimately promotes a faster recovery from PGD. Another potential procedural benefit of IVLP is its process with no second graft ischemia time, which was reportedly regarded as a potential risk factor in the EVLP setting.[27] The IVLP circuit can be installed with the graft circulating and ventilated in vivo. In addition, final reperfusion can be performed shortly after completion of the therapeutic intervention by simply releasing the vascular snares. Therefore, the influence of extra ischemia is negligible in this situation. A further important aspect for IVLP is the implementation of low-flow pulmonary artery perfusion with strict control of inflow pressure. To achieve sufficient therapeutic effectiveness within a short duration, we established a treatment protocol that emphasizes protective perfusion with low flow and includes the addition of similar additives used in common EVLP protocol to the closed-circuit fluid. We consider that the advantages mentioned above could be relevant to the reasons why IVLP resulted in dramatic therapeutic effects even within a short duration. Achieving a positive effect with short-term treatment is crucial to minimize the potential side effects associated with it.

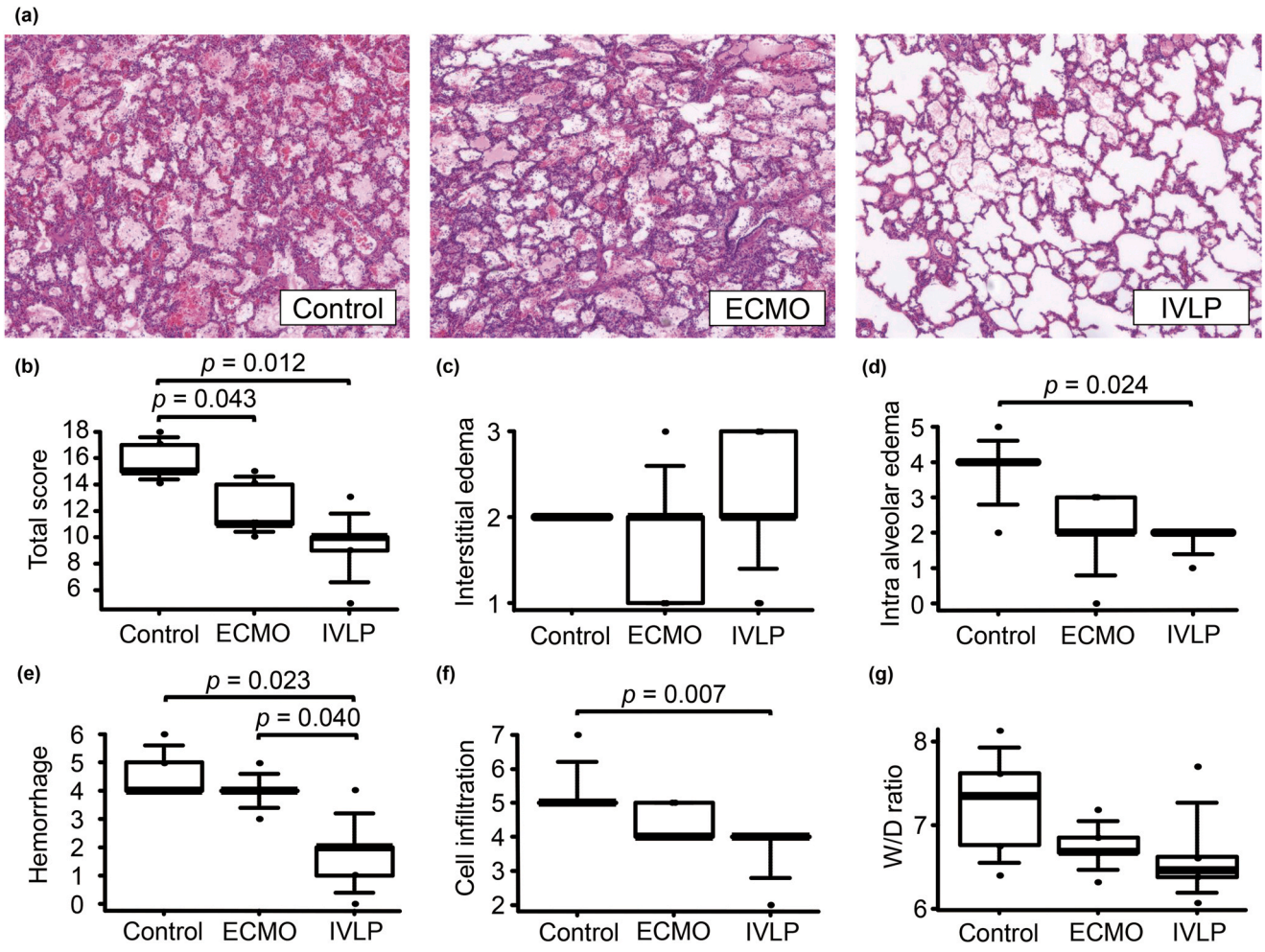

Figure 5 (a) Representative histologic findings in the graft tissue stained with hematoxylin and eosin (objective lens, ×10). (b-f) Comparison of lung injury scores among the 3 groups. (g) Comparison of the wet-to-dry weight (W/D) ratio. The in vivo lung perfusion group tended to have lower values than the other groups. ECMO, extracorporeal membrane oxygenation; IVLP, in vivo lung perfusion.

It is recognized that avoiding an increase in return pressure to the transplanted lung is important for the prevention and management of PGD. Clinical efforts are being made to minimize PGD by controlling the perfusion rate to the transplanted lung using posttransplant ECMO. In this study, we established the ECMO group as the standard arm using a flow setting that had been clinically well-verified in a previous report on ECMO for preventing PGD.[16] The ECMO protocol adopted in this study reasonably simulates the commonly applied condition, with a median flow rate of 2.0 liter/min or 40% to 50.0% of CO. In this setting, the mean PAP during treatment in the ECMO group could be managed at around 20 mm Hg, which means the reperfusion pressure to the transplanted lungs was controlled to an equivalent or lower level than the IVLP group. Consequently, in the design of this study, the perfusion flow to the lungs differs between the ECMO group and the IVLP group, but the pulmonary inflow pressures are set to be approximately equal, aiming for a comparable setup in terms of providing lung-protective reperfusion. Accordingly, the results indicate that IVLP therapy yields therapeutic benefits in the management of PGD that exceed the effects achieved solely by reducing lung perfusion rates and pressure.

In terms of the technical aspects of IVLP, in the current research model, only a limited perfusion flow was achieved during the treatment. One of the potential reasons for this could be attributed to the drainage system used in IVLP. In IVLP, the PV drainage had to be conducted through small-bore venous cannulas due to anatomic constraints. There is a clear distinction in the method and device used for LA drainage between the IVLP and EVLP systems or physiological pulmonary venous circulation in the ECMO system. Furthermore, the research settings for inducing lung injury in this study differed from those in other studies. In our current study setting, severe lung edema and persistent atelectasis developed after the experimental warm ischemic intervention and could significantly affect pulmonary vascular resistance. These factors limited the amount of IVLP flow that could be achieved. To mitigate congestion, we prioritized the control of pulmonary artery inflow pressure over increasing the flow rate. We believe that the low perfusion flow utilized in IVLP may have ultimately contributed to its positive effects in the current study. When applying it to individuals with larger body sizes, achieving a more stable outflow drainage can be simply facilitated by increasing the cannula diameter and employing dual venous drainage. However, the technical trials of achieving further

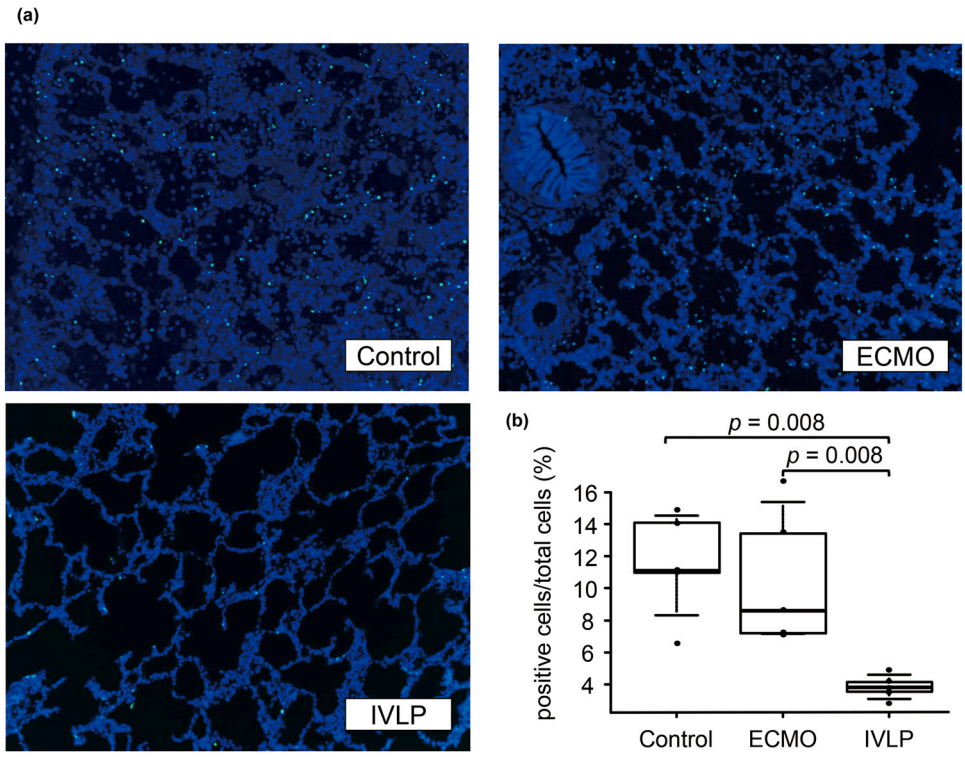

Figure 6 (a) Representative pictures of Terminal deoxynucleotidyl transferase dUTP nick end labeling staining (objective lens, ×10). (b) Comparison of positive cell/total cell ratio among the 3 groups. ECMO, extracorporeal membrane oxygenation; IVLP, in vivo lung perfusion.

secure LA drainage may be future challenges of the IVLP methodology to increase the perfusion flow and improve overall effectiveness.

EVLP techniques and protocols have been extensively studied and implemented in clinical practice, leading to improved rates of donor lung recovery and prevention of PGD after LTx. However, PGD can occur not only due to factors related to the donor lung but also due to surgical factors and recipient-related factors. Therefore, even if a lung is evaluated and deemed transplantable through EVLP before transplantation, the occurrence of PGD after transplantation can be influenced by the surgical procedure and the recipient's condition. In addition, the decision to accept a donor lung can vary among transplantation centers and cases, and the criteria for determining the need for EVLP in each donor case lack sufficient validation. Some arguments suggest that certain lungs meeting the current EVLP indication criteria could be safely transplanted without employing EVLP.[11,12] This situation complicates the decision of whether to indicate EVLP in actual LTx cases. IVLP, as a therapeutic concept, holds potential benefits in that it allows for timely intervention in response to the development of clinically problematic PGD after LTx. Considering those perspectives, IVLP is believed to be an important therapeutic strategy that can complement the role of EVLP.

We consider that posttransplant IVLP is a clinically applicable strategy, but there remain several challenges related to the study limitations. Although left single LTx and unilateral IVLP treatment were performed under contralateral single-lung ventilation in this study, the technical feasibility of right-sided or bilateral IVLP remains unclear. In addition, the results of lung compliance in this study should be carefully interpreted, as they were obtained under bilateral lung ventilation conditions. Another potential argument would be that the majority of clinical LTx candidates are unable to tolerate unilateral ventilation. Thus, cardiopulmonary support such as ECMO is necessary during IVLP treatment in most cases. We consider that it is highly unlikely that the brief ECMO during the 2-hour IVLP would significantly impact graft and patient conditions. From a technical standpoint, IVLP can be practically implemented by introducing concomitant ECMO through femoral cannulation. However, there might be a certain extent of the potential negative effect of the concomitant use of ECMO during IVLP that was not examined in this study. Another potential concern might be compromised bronchial circulation due to low-flow graft perfusion during IVLP. We specifically confirmed the impact of IVLP in a short duration setting, which is unlikely to significantly affect bronchial healing. However, if applied in a clinical setting, it is something that should be noted with caution. Furthermore, the in vivo use of Steen solution is currently not approved. In this study, we isolated the therapeutic circuit from the systemic circulation during IVLP. In addition, the first 100 ml of pulmonary drainage was discarded as intrapulmonary residual perfusate at the end of the IVLP. However, the acceptability of this technique should be evaluated based on results from clinical trials of IVLP currently being conducted for therapeutic purposes in metastatic lung tumors (NCT02811523 and NCT05611034). The above issues should be addressed when the IVLP strategy is applied in a clinical setting.

In conclusion, IVLP resulted in a drastic improvement in transplanted lung function with severe IR injuries. The procedure can be applied immediately after reperfusion intraoperatively, and 2-hour therapeutic circulation is technically feasible and effective. The posttransplant IVLP strategy can gain a novel practical role differentiated from EVLP in the treatment of PGD, thereby increasing the organ use and decreasing posttransplant early mortality rates.

Author contributions

K. Matsubara and K. Miyoshi were responsible for study conception and design. K. Matsubara, K. Miyoshi, S.K., Y.K., D.S., Y.T., T.S., H.Y., and T.K. collected the data. K. Matsubara, K. Miyoshi, T.O., and A.M. analyzed the data. S. Tanaka, M.O., S.S., and S. Toyooka interpreted data. K. Matsubara and K. Miyoshi wrote the article. All co-authors approved the paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.healun.2023.10.011.

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