Ex Vivo Lung Perfusion

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Abstract

Purpose of the Review Lung transplantation is a lifesaving therapy for patients suffering from end-stage lung diseases. The number of patients waiting for lung transplantation greatly exceeds the number of donors available. Currently, only 20% of lungs donors are used for transplantation. Ex vivo lung perfusion (EVLP) has been developed as a tool to assess and also potentially repair lungs before transplantation. This article will review the rationale for EVLP and the different EVLP methods including the Toronto EVLP method, describe technical details of EVLP, report on the clinical results of EVLP, and describe the use of EVLP as a platform to deliver different therapies.

Recent Findings EVLP has been demonstrated to be a safe method of assessing high-risk donor lungs. The long-term survival and graft function of patients that received high-risk donor lungs assessed and treated with EVLP have been shown to be comparable to those receiving conventional donor lungs. Preclinical studies demonstrate that EVLP can serve as a platform for the delivery of specifically targeted therapies to repair injured lungs.

Summary EVLP has demonstrated to be a promising tool for the assessment and recovery of injured donor lungs using pharmacologic agents as well as gene and cellular therapies.

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Introduction

Lung transplantation (LTx) is a lifesaving therapy and is currently the only curative treatment for many patients suffering from end-stage lung diseases. Despite the increasing number of transplants being performed each year, the number of patients waiting for a LTx greatly exceeds the number of donors available. Currently, only 15–20% of lungs from multiorgan donors are considered usable for transplantation [1]; the rest are generally considered unsuitable due to lung injury that occurs with brain death and ICU-related complications (i.e., ventilator-acquired pneumonia, neurogenic and hydrostatic pulmonary edema, or barotrauma).

Although significant improvements in lung preservation, surgical technique, immunosuppression, and post-transplantation management have led to improved outcomes, the median survival after lung transplantation is only 6 years. Primary graft dysfunction (PGD) with an incidence of 11–25% is the most serious early complication after lung transplantation. It is associated with a 20–30% mortality rate in the first month after lung transplant and also associated with inferior long-term survival [1, 2]. In an attempt to avoid PGD, transplant teams are usually conservative when selecting donors. Mortality can be as high as 30% in patients awaiting transplantation [3, 4].

Normothermic ex vivo lung perfusion (EVLP) has emerged as a novel strategy to not only allow for more accurate lung assessment but to also potentially treat and repair injured donor lungs. Its application in high-risk donor lungs has been successful and has resulted in expansion of the donor pool [5••].

Rationale for EVLP

The current standard clinical practice of organ preservation is cold static preservation (CSP). During donor lung retrieval, a cold pulmonary flush using low-potassium dextran preservation solution is performed with topical cooling and lung ventilation [6, 7]. Lungs are then stored and transported at 4 °C in a static inflated state. Most of the detrimental effects of ischemia/reperfusion are driven by chemical reactions, and reducing the organ temperature also reduces the enzymatic activity that drives these processes. Hypothermia has proven to reduce cellular oxygen and nutrient requirements and cellular metabolism, slowing down cell death processes and maintaining cell viability. However, hypothermia also reduces reparative organ functions, which in effect prevents recovery of the lungs from injury.

In contrast, normothermic EVLP has proven to enable the preservation of metabolic function and lung homeostasis [8]. EVLP provides an excellent platform not only for lung preservation but also for more detailed assessment and treatment of donor lungs [9]. The idea of organ perfusion is not new. Alexis Carrel and Charles Lindberg performed experiments in normothermic organ perfusion 80 years ago [10]. Steen readdressed the idea of the ex vivo lung perfusion mostly to perform a short functional evaluation of the lung in donors after cardiac death (DCD). He achieved his goal of successfully performing short-term perfusion using a buffered extracellular solution with a colloid osmotic pressure (Steen solution) combined with blood as the lung perfusate [11–13]. Steen's EVLP preservation technique however could not provide a preservation period longer than 120 min due to increased pulmonary vascular resistance and airway pressures which led to circuit-induced lung injury. The Toronto group introduced the concept of extended EVLP (>12 h) by modifying the EVLP technique using an optimal lung protective strategy now known as the Toronto EVLP method. Appling this strategy, EVLP-treated donor lungs can remain in a functional physiological state for extended periods of up to 12 h [8]. The main achievement of the Toronto EVLP technique is the maintenance of donor lungs in a state where they are able to function in an optimal environment: using protective flow parameters to prevent mechanical stress, protective ventilation to avoid circuit-induced lung injury, and protective composition of the perfusate to achieve cell homeostasis. This extended EVLP technique provides a platform to not only assess but also for treatment delivery in the normothermic state [14].

EVLP Protocols

All strategies for EVLP are not the same. It is important to note this in comparing results and in clinical application of the act of ex vivo lung perfusion. There are three different protocols that have recently been used and examined in clinical trials. They include the Toronto EVLP technique, the Lund (Vivoline, Lund, Sweden) technique, and the Organ Care System technique (OCSTM, Transmedics, Andover, MA, USA). These protocols share certain characteristics yet each has unique features that characterize it. They vary in the equipment, the ventilation, perfusion settings, and the perfusate composition (see Table 1).

The Toronto and Lund techniques use perfusate based on an extracellular solution with human albumin added (Steen Solution™; XVIVO Perfusion, Denver CO, USA). This solution maintains a colloid osmotic pressure; it also has dextran 40 to protect the microcirculatory endothelium and inhibit coagulation and platelet adhesion [15, 16]. The difference between them is that Lund also has red blood cells with a hematocrit of 14%. The perfusate in the OCSTM protocol is based on low-potassium dextran 40-based solutions without additional human albumin: OCSTM Solution (Transmedics) or Perfadex (XVIVO Perfusion, Denver, CO); they also add red blood cells with a hematocrit of 15–25%. Whether red cells should be added or not continues to be controversial.

Another difference between the three protocols is the target flow during EVLP. This is set at 100% of cardiac output in the Lund protocol, 40% in the Toronto Protocol and a fixed level of 2–2.5 L/min in the OCSTM protocol.

Lund and OCSTM protocols leave the left atrium open for drainage of pulmonary effluent into a drainage pan. The Toronto Protocol utilizes a closed atrial cuff technique with a specially designed cannula to allow the maintenance of a positive LA pressure between 3 and 5 mmHg. This is done to avoid the development of vascular stress injury. The prevention of hydrostatic pulmonary edema is further facilitated by maintaining the PAP below 12 mmHg at targeted flow all the time [17–19]. The foundations of the Toronto Technique for clinical EVLP are summarized in Table 2.

EVLP Technique

Cannulation

After the donor flush preservation, the lungs are stored in the inflated state and transported to the transplant center at 4 °C where the dissection on the back table starts. The left atrial (LA) cuff is trimmed and attached to a specially designed cone-shaped cannula with a 4-0 polypropylene running suture. If the pulmonary artery (PA) is long enough, a simple PA cannula is inserted into the PA making sure it is placed before the bifurcation and secured with simple silk ties. If the main PA happens to be cut short, a specifically designed cone-shaped cuffed PA cannula is attached to the PA with a 5-0 polypropylene running suture. The trachea is clamped above the carina, the tracheal stapler line is opened, and a regular

Table 1 EVLP protocols

	Lund	Toronto	OCS
Perfusate	Extracellular solution albumin + dextran 40 (Steen™) + RBC hct 14%	Extracellular solution albumin + dextran 40 (Steen™)	Low-potassium dextran 40 (OCS™) solution + RBC hct 15–25%
Flow			
Type	Continuous	Continuous	Pulsatile
Initiation	100 mL/min	10% of target	200 mL/min
Target	100% cardiac output	40% cardiac output	2–2.5 L/min
Left atrium	Open	Closed	Open
PAP	≤20 mmHg	≤15 mmHg	≤20 mmHg
Ventilator			
Start temp	32 °C	32 °C	34 °C
RR	20/min	7/min	10/min
TV	5–7 mL/kg donor BW	7 mL/kg donor BW	6 mL/kg donor BW
PEEP	5 cmH$_2$O	5 cmH$_2$O	5–7 cmH$_2$O
FiO$_2$	50%	21%	21%
EVLP time	2 h	4–6 h	Transport time

RBC red blood cells, *PAP* pulmonary artery pressure, *RR* respiratory rate, *TV* tidal volume, *PEEP* positive end expiratory pressure, *FiO$_2$* inspired fraction of oxygen, *BW* body weight

endotracheal tube is inserted and secured with two heavy sutures. The cannulas are attached to the LA and PA with the pressure tubing facing the trachea to facilitate the subsequent identification of a twisted cannula when the lung is placed into the circuit. The endotracheal tube is clamped and the tracheal clamp is then released. After the cannulation, a second retrograde flush is performed using 1 L of low-potassium dextran solution. This ensures that there is no leakage at the cannula anastomoses and also backwashes out any small clots or emboli. The lungs are then transferred to the EVLP circuit. If one of the lungs is too damaged, they can be divided, making sure to have adequate arterial and tracheal/bronchus lengths and atrial cuffs for both sides and one lung can be perfused alone. It is also possible to perfuse each lung in a separate circuit.

Essentially, the EVLP circuit is composed of a circuit with a centrifugal pump, a leukocyte filter, a hollow-fiber oxygenator, a heat exchanger, and a reservoir. It is primed with 2.0 L of Steen solution (XVIVO, Vitrolife), 500 mg methylprednisolone (Solumedrol; Sandoz Canada, Boucherville, Canada), 3000 IU of unfractionated heparin (Organon, Canada), and

Table 2 Toronto EVLP principles

1 Acellular perfusate solution with albumin and dextran
2 Gradual rewarming to achieve normothermia
3 Gradual increase on flow until 40% of donor predicted cardiac output
4 Protective lung ventilation parameters
5 Closed atrium

an antibiotic (500 mg imipenem/cilastatin (Primaxin) 500 mg, Merck, Whitehouse Station, NJ).

Initiation and Maintenance Phase

The lungs are placed into the chamber. The PA and LA pressure cannulas are connected to the pressure monitor and de-aired. The sterile portion of the tubing is opened and cut to an appropriate length between two clamps.

The outflow clamp is then removed. Following the Toronto Technique, the target perfusion flow consists of 40% of the donor predicted cardiac output. The procedure initiates with lungs on room temperature and perfusion with 10% of the calculated target flow. After 10 min, the flow is raised to 20% of predicted and the temperature is set to 30 °C. The flow is increased to 30, 50, 80, and 100% of target each 10 min. The temperature is set to 37 °C. At 20 min, the clamp is removed from the trachea and ventilation (7 mL/kg, PEEP 5 cm H2O, and 7 of respiratory rate/min) is initiated when the temperature reaches 32 °C, the gas mixture (86% N$_2$, 8% CO$_2$, and 6% O$_2$) is turned on at a sweep of 1 L/min. The post-membrane pCO$_2$ target is between 35 and 40 mmHg and is achieved by titrating the sweep gas. The left atrial pressure is maintained between 3 and 5 mmHg by adjusting the level of the reservoir. Once the lungs are normothermic, ventilation and target flow is achieved, recruitment maneuvers are performed up to a peak airway pressure of 25 cm H$_2$O until atelectatic areas are recruited. The recruitment maneuvers should be performed by increasing the tidal volume by 100cm^3 each breath and holding during inspiration for 10–15 s.

After the first hour, 500 mL of Steen solution is exchanged from the circuit followed by a 250 mL exchange hourly.

Evaluation on EVLP

The assessments are performed every hour. For this purpose, ventilation parameters are set to 10 mL/kg tidal volume, 10 breaths per minute, and FiO_2 1.0 for 5 min. Post-membrane and post-lung perfusate gases are analyzed to calculate the delta pO_2. Additional physiologic parameters—static compliance, dynamic, compliance, PA pressure, LA pressure, peak airway pressure, and plateau pressure—are recorded. The ventilation parameters are changed back to the maintenance parameters after the hourly evaluation. A lung x-ray is performed routinely at 1 h of EVLP and then every 2 h. Pulmonary recruitment maneuvers are performed every 30 min after each assessment by increasing the tidal volume by 100 cm^3 with subsequent inspiratory hold maneuvers up to 25 cm H_2O (and a maximum of 15 cm^3/kg) for 10 s.

The Toronto's clinical protocol generally entails EVLP for 4 to 6 h. The decision to transplant or not is not made prior to 3 h of EVLP. At this point, the functional gas exchange data taken from three assessments and two lung x-rays are evaluated for the decision paying specific attention to trends in the important physiologic parameters. Criteria for lung acceptance for transplantation after EVLP are shown in Table 3. We have shown that EVLP should proceed for at least a few hours to assess the trend of physiologic parameters (compliance, gas exchange, airway pressures). The deterioration of those parameters suggests the development of edema as an early lung injury marker. The drop in compliance usually precedes a drop in perfusate pO_2 [20].

Termination of EVLP

After 3–6 h of EVLP when the decision has been made to terminate perfusion, the lungs are cooled to 15 °C and ventilated with a FiO_2 of 0.5. Once 15 °C is reached both cannulae (inflow and outflow) are clamped and cut. The endotracheal tube is also clamped and the lungs should be kept inflated. The trachea can also be stapled off. An anterograde flush with 1 L of low-potassium dextran solution is then performed to preserve the lung for the second cold static phase for transport to the donor OR and during implantation. The vascular cannulae are removed and the trachea is stapled just below the endotracheal tube. The lungs are then placed in cold Perfadex solution inside an ice cooler and transported to the recipient OR.

Clinical Experience of Lung Transplantation after EVLP

Clinical Experience Using the LUND Protocol

The first use of EVLP was to assess lungs from DCD donors in an attempt to expand the donor pool. Steen et al. in 2001 published the first case report of a successful clinical EVLP used to assess lungs from a Maastricht category II DCD donor for a 1 h perfusion. After this, Steen et al. reported in 2007 their EVLP experience in assessing and reconditioning six clinical double lung transplantations using initially rejected lungs from brain-dead donors. In all these patients, the postoperative course was reported to be uncomplicated with an observation period of 3 to 6 months. The lungs were reconditioned during 1 to 2 h of ex vivo perfusion after which the lungs were kept in a topical ECMO system at 8 °C until transplantation the next day. The time from harvesting until the start of reperfusion of the transplanted second lung was approximately 20 h [21].

Henriksen et al. published the first Danish experience with ex vivo lung perfusion of donor lungs before transplantation using the Lund Protocol [22]. Since the first EVLP in Denmark in 2012 until the study was published (2014), seven previously rejected donor lungs were transplanted after showing improvement at the end of EVLP. Their results suggest that lungs treated with EVLP have similar outcomes after transplantation as lungs transplanted in the conventional way [22].

Table 3 Toronto EVLP acceptance and exclusion criteria

Acceptance criteria	Exclusion criteria
PLAO$_2$/FiO$_2$ ratio ≥ 400 mmHg	PLAO$_2$/FiO$_2$ ratio < 400 mmHg
Stable or decreasing pulmonary artery pressure	>15% deterioration on pulmonary artery pressure
Stable or decreasing airway pressures	>15% deterioration on airway pressure
Stable or improving pulmonary compliance	>15% deterioration on pulmonary compliance

Table modified from [1, 2]

1. Van Raemdonck D, Neyrinck A, Cypel M, Keshavjee S. Ex-vivo lung perfusion. Transpl Int. 2015;28(6):643–56

2. Machuca TN, Cypel M. Ex vivo lung perfusion. J Thorac Dis. 2014;6(8):1054–62

EVLP ex vivo lung perfusion, *PLAO$_2$* partial pressure of oxygen measured in effluent from left atrium, *FiO$_2$*, inspired fraction of oxygen

In 2014, Wallinder et al. reported the initial experience from the University of Gothenburg group. They compared the results between 11 initially rejected donor lungs that were transplanted after EVLP (eight double lung transplants and three single lung transplants) with conventional lung transplant recipients. EVLP recipients using their technique had longer time on mechanical ventilation and longer intensive care stay but no differences were found in the hospital stay. Two patients in the EVLP group and six in the control group had primary graft dysfunction post-operatively. All recipients of EVLP lungs were discharged alive from the hospital compared with three patients in the control group, which died before discharge [23].

The DEVELOP-UK Trial from Newcastle was a multicenter trial involving five centers in the UK. This utilized the Vivoline device (Lund, Sweden) and a modified Lund perfusion protocol with Steen solution, blood perfusion, and an open atrium. Unfortunately, the results from this trial were negative with a number of patients experiencing serious adverse events (ECMO or death) and the trial was prematurely closed. This was reported at the International Society for Heart and Lung Transplantation meeting in 2016 (reference Andrew Fisher abstract at the last ISHLT). But the full results are not in publication yet.

Clinical Experience Using the Toronto Protocol

The Toronto Lung Transplant Program with more than 280 clinical post-EVLP transplants has the largest experience worldwide. Cypel et al. published the first clinical trial in 2011. High-risk donor lungs were placed in the EVLP circuit and perfused for 4–6 h following the previously described Toronto normothermic ex vivo protocol. The trial was able to demonstrate that the recipients of EVLP-treated lungs had similar results (primary graft dysfunction and 30-day mortality) as recipients of conventional donor lungs [5••, 24].

Toronto also published a study comparing outcomes between recipients that were transplanted with DCD lungs without EVLP to DCD with EVLP recipients [25]. They were able to show that patients who received lungs from DCD donors after being treated with EVLP had a significantly shorter hospital length of stay and a trend toward shorter length on mechanical ventilation requirements. Early survival curves were comparable among groups, but interestingly, the 3-year survival rate was higher in the DCD with the EVLP group than the DCD-no EVLP group [25].

Tikkanen et al. published last year a study addressing the functional outcomes and quality of life after normothermic ex vivo lung perfusion lung transplantation of the first 63 patients receiving lungs assessed using EVLP and compared them to 340 conventional donor lung recipients transplanted during the same period of time [26••]. The 5-year survival rate in the EVLP group was 71 vs. 57% in the control group

($p > 0.05$). In addition to survival, the CLAD rate, the lung function, and the quality of life outcomes of recipients of EVLP donor lungs were comparable with those recipients who were transplanted using conventional donor lungs [26••].

In 2012, the Vienna group published their results of a prospective study of lung transplantation using only initially unacceptable donor lungs, which were improved by EVLP for 2–4 h using the Toronto Technique. They compare the results of the lungs transplanted after EVLP assessment from March 2010 to June 2011 [9] with recipients transplanted during the same period of time with conventional preservation lungs (119). They found no significant differences between the two groups in terms of PGD at 24 h, duration on mechanical ventilation, ICU length of stay, hospital stay, and 30-day and 280-day survival rates [27].

In 2014, Boffini et al. from Torino, Italy, published the results of a study comparing the incidence of PGD immediately after lung transplant and after 72 h in patients receiving standard lungs ($n = 28$) with patients receiving grafts after EVLP with acellular perfusion according to the Toronto Technique ($n = 8$) [28]. No differences in the incidence of PGD were found between the two groups [28].

The French clinical EVLP experience was published by Sage et al. from Paris-Foch Hospital in 2014 [29]. Thirty-two lungs determined to be unsuitable for transplantation and rejected by the 11 French lung transplant teams were put on an EVLP circuit following the Toronto EVLP protocol. After EVLP, 31 pairs of lungs with acceptable function were transplanted. During the same period, 81 double lung transplants were used as controls. The authors did not find any differences between the two groups in regard to the incidence of primary graft dysfunction 72 h after lung transplant, median time to extubation, intensive care unit and hospital lengths of stay, 30-day mortality, and 1-year survival rates [29].

In 2012, Zych et al. from the Harefield group in the UK published a retrospective study evaluating their experience of 13 consecutive EVLP runs between January 2009 and December 2010 [30]. Lungs rejected for routine transplantation were placed into the EVLP circuit and reperfused using acellular Steen solution as per the Toronto Technique. Six pairs of lungs were accepted for transplantation showing a much lower utilization rate (46%) compared with the one published by Toronto (87%) [30]. The six transplanted patients showed similar early, 3-month, and 6-month survival rates and ICU and hospital length of stay to their contemporary transplant population [30].

Clinical Experience Using the OCS Protocol

In 2012, the Hanover and Madrid Groups together published the first combined experience using the OCS [31]. Between February 18 and July 1, 2011, 12 patients were transplanted at the two centers. Lungs were flushed with low-potassium

dextran solution, explanted, and immediately connected to the Organ Care System (OCS) (Transmedics, Andover, MA, USA), perfused with Steen's solution supplemented with two donor blood-derived red cell concentrates. All lungs met the ISHLT standard donor lung acceptability criteria prior to placing them on the OCS system. The authors concluded that standard donor lungs can be safely preserved with the OCS, and all lungs resulted in successful transplantation. A clinical trial randomizing standard criteria lungs to OCS vs. cold static preservation is underway, but final results have not been published yet.

Treatment during EVLP

Only 20% of the potential donor lungs are being used for transplantation. Lungs are injured by a number of very different mechanisms including brain death, contusion, aspiration, ventilator induced lung injury, infection, edema, and atelectasis. Ex vivo perfusion has shown to be an excellent platform to repair most of these issues.

The EVLP for lung treatment will likely require longer perfusion times. While 3–4 h may be enough time to assess a lung and clear lung edema, a perfusion with intent for more advanced repairs will conceivably require stable perfusion for a longer period of time [32]. A number of experimental studies have been recently published examining the use of EVLP as a treatment tool to repair damaged lungs.

Pulmonary Edema

Pulmonary edema in donor lungs is one of the most common reasons to reject an organ. The lung appears to be one of the most vulnerable organs to the hemodynamic, and histopathological changes that take place during the development of brain death. The ICU fluid management can also lead to overload that leads to pulmonary edema. EVLP has shown to stimulate alveolar fluid clearance in the organ donor.

Beta2-agonist therapy (terbutaline) during perfusion has also been used to increase edema fluid clearance in rejected donor lungs with acute lung injury and pulmonary edema [33].

Another study has been published showing that β2-adrenoreceptor agonist inhalation (procaterol) during EVLP attenuates lung injury in an animal DCD model [34]. Inhaled procaterol significantly elevated lung tissue cAMP levels and CFTR gene expression, decreasing in the wet to dry lung weight ratio and improving lung function during 4 h of EVLP.

Infection

Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in the ICU. VAP affects up to 28% of all intubated patients in the ICU [35–37]. Between 46 and 89% of the donor lungs have positive bronchoalveolar lavage (BAL) bacterial cultures according to published data [38, 39•]. Several studies have addressed the issue regarding whether the transmission of donor bronchial microorganisms may result in recipient pneumonia and poor post-transplant outcomes [40, 41].

EVLP is an ideal platform to treat infected lungs by giving them high doses of antibiotics in the perfusate without concern of systemic side effects that are normally seen when such drugs are delivered to the whole patient.

Andreasson et al. published a study from 18 human donors considered unusable for transplantation that underwent EVLP with a perfusate containing high-dose, broad-spectrum antibiotics. Six lungs were ultimately transplanted, all of whom survived to hospital discharge. They showed that EVLP with high-dose, anti-microbial agents in the perfusate is associated with an effective reduction in the microbial burden of the human donor lung [42].

Nakajima et al. recently published another study with 15 human donor lungs that were declared unsuitable for transplantation because of clinical suspicion of infection. The lungs were randomized into two groups, control ($n = 7$) or treatment with high doses of antibiotics: meropenem 2 g, vancomycin 15 mg/kg, and azithromycin 500 mg in five cases, and meropenem 2 g, vancomycin 15 mg/kg, and ciprofloxacin 400 mg were used in three cases. They confirmed that broad-spectrum antibiotics in the perfusate reduces the pathogen burden and endotoxin levels as well as diminishes the inflammatory injury [39•].

Gastric Aspiration

Evidence of aspiration is a major concern when assessing a potential donor and remains a major reason for declining donor lungs for transplantation. Aspiration of gastric contents is seen very frequently in not only in DCD donors after withdrawn of life support but also in donors with neurological damage. Several pulmonary syndromes may occur after aspiration, depending on the amount the type of and type of aspirated material and the frequency of aspiration [43]. Aspiration pneumonitis, caused by the inhalation of gastric content, can lead to surfactant dysfunction [43].

Inci et al. from Zurich in 2008 studied a pig model using gastric juice infused through a flexible bronchoscope to investigate ways to repair caustic lung injury during EVLP [44].

In 2011, Meers et al. from Lueven, Belgium, developed a pig model to assess and treat aspiration in an EVLP setting. Lung injury was induced with 5-mL/kg administration of a betaine HCl/pepsin mixture via a flexible bronchoscope. After injury, animals were randomly assigned to three study groups ($n = 6$ each)—saline lavage during EVLP (control), surfactant lavage EVLP (SL-EVLP), and surfactant lavage

before harvest (SL-Pre)—and a normal group (*n* = 4), with no lung injury. They showed that EVLP surfactant lavage improves of the graft function of lungs injured by gastric acid aspiration [44].

In 2014, Khalife-Hocquemiller and the group from Marie Lannelongue in Paris investigated the effects of (EVLP) reconditioning with surfactant administration on lung injury secondary to gastric aspiration [45]. Pigs were randomized into the following groups: gastric acid-induced injury +4 h EVLP, gastric-induced injury + surfactant lavage immediately before EVLP, and saline infusion alone or saline infusion + by EVLP. Pigs that received surfactant before EVLP improved the functional parameters (PaO2, pulmonary vascular resistance, and apoptotic cell percentage) and partially improved the histologic severity score [45].

Nakajima et al. in 2015 showed in a gastric aspiration lung injury pig experiment that lung lavage followed by trans-bronchial administration of exogenous surfactant during EVLP improves the post-transplant lung function. After lung injury was induced with gastric juice (pH = 3.0), pigs were ventilated for 6 h, donor lungs were retrieved. Following 10 h of cold ischemic time, lungs were randomized into four groups (*n* = 5 each group): (1) no treatment (control), 2) lung lavage (LL), (3) surfactant administration (SF), and (4) surfactant administration following lung lavage (SL). Lungs underwent EVLP for 6 h. Following a 2-h second cold ischemic time (post-EVLP), the left lung was transplanted and reperfused for 4 h in all the groups. Surfactant administration alone significantly improved lung function during EVLP, but it was not enough to reduce inflammatory activity. Lung lavage followed by trans-bronchial administration of exogenous surfactant during EVLP provided superior post-transplant function [46].

Pulmonary Embolism (PE)

Pulmonary embolism and pulmonary infarction are a frequent finding in donor lungs [47]. Organ donors are at high risk for venous thromboembolism. The finding of pulmonary embolism has been associated with primary graft failure and poorer outcomes in recipients after lung transplantation [48].

A few cases have been reported where some treatment has been implemented in lungs with PE after organ donor surgery, including pulmonary embolectomy and thrombolytic therapy [49–51]. We published the first case report of therapeutic ex vivo thrombolysis followed by successful clinical transplantation. The donor had clinical history, hemodynamic data, and macroscopic findings that were compatible with extensive acute PE. Thrombolysis was performed during EVLP with alteplase and once recovery was confirmed the lungs were successfully transplanted [52].

After that first published case, there have been two other clinical cases reporting the use of fibrinolytic agents including alteplase or urokinase added into the perfusate during EVLP for donor PE, followed by successful lung transplantation without any major complications related to the fibrinolysis [53, 54].

Gene Therapy

In the early post-operative lung transplant period (first 72 h), primary graft dysfunction (PGD) represents an important risk factor for morbidity and mortality [1]. Severe PGD can affect up to 25% of all lung transplant [4]. The specific pathophysiologic mechanisms resulting in PGD remains unknown, but evidence suggests that injury mediated by endogenous inflammatory mediators during ischemia/reperfusion period may play an important role.

Clinical and experimental studies have shown that lung reperfusion induces a rapid release of cytokines. During cold ischemic time and after reperfusion pro- and anti-inflammatory cytokines such as TNF-α, IFN-γ, IL-8, IL-10, IL-12, and IL-18 are present and can be measured in lung tissue [55].

One potential therapeutic approach to be applied to injured donor lungs in an attempt to reduce the inflammatory injury is to use interleukin-10 (IL-10) gene therapy. IL-10 is predominantly produced by lymphocytes and monocytes, and it plays important roles in immune-regulation. It mainly exerts its anti-inflammatory effect by inactivating neutrophils and macrophages consequent downregulation of pro-inflammatory cytokine secretion [56]. Interleukin-10 gene has been proven to ameliorate lung dysfunction by decreasing cell death after transplantation [57, 58]. Adenoviruses have been used as vectors, because their ability to accomplish the gene transfection in non-replicating cells. The gene transfer using adenovirus occurs without direct integration into the host chromosomes (epichromosomal).

In preclinical studies, EVLP has proven to be the ideal platform for gene therapy delivery to the lung. In a study published in 2009 from Cypel et al., ten human brain death lung donors clinically rejected for transplantation were perfused for 12 h in an EVLP following the Toronto Protocol. They showed that lungs treated with intrabronchial delivered AdhIL-10 had significant improvement in lung function over controls, the inflammatory cytokine gene expression associated with brain death was changed to anti-inflammatory profile, and the alveolar-blood barrier integrity was recovered [9].

Yeung et al. in 2012 compared ex vivo with in vivo intratracheal delivery of adenoviral vector encoding IL-10 or green fluorescent protein to pig lung donors. Lungs were perfused in EVLP for 12 h. The lung function remained stable in the IL-10 group and decreased in the controls or lungs that received green fluorescent protein [59•].

Mesenchymal Stem Cell (MSC)-Based Therapy

Mesenchymal stem cells are multipotent stromal cells that can differentiate into a variety of cell types including osteoblasts, chondrocytes, myocytes, fibroblast, and adipocytes.

The International Society of Cellular Therapy in 2006 reported three minimal criteria to define human MSCs: (1) Under standard culture conditions, MSC must be adherent to plastic. (2) MSC must express CD105, CD73, and CD90 as cell surface markers and should not express CD45, CD34, CD14 or CD11b, CD79alpha or CD19, and HLA-DR surface molecules. (3) MSC must be able to differentiate to osteoblasts, chondroblasts, and adipocytes in vitro [60]. MSCs modulate the immune system after migrating to the sites of inflammation where they produce an immunomodulatory and anti-inflammatory effect through cell-cell interactions between MSCs and lymphocytes or by the production of soluble factors.

One of the most important effects for MSCs is the inhibition of T cell proliferation and cytokines secretion. They also regulate the immune system through modulation of dendritic cells, B and T cells, neutrophils, and prostaglandin (PGE_2). MSCs secrete cytoprotective growth factors (keratinocyte growth factor, vascular endothelial growth factor, and hepatocyte growth factor), and angiopoietin-1 (endothelial barrier protective effect).

Several models have been used to study MSC in lung diseases showing less inflammation in the lungs, less apoptosis, and reduced lung injury [61–64]. Treatment of the lung by MSCs can be achieved by systemic (intravenous) or local (intratracheal) delivery.

The University of California San Fransisco group has published some studies of MSC treatment using rejected human lungs in an ex vivo perfusion system [61, 65] (intrabronchial, intravenous, and adding MSC into the perfusate); they have shown reduction in the edema by maintenance of alveolar epithelial barrier integrity and by restoring alveolar fluid clearance. They also showed inflammation suppression and epithelial-specific growth factor production [65–69].

We have recently published work describing the optimal route and dose of MSCs to be delivered to the lung on EVLP using a swine model [70]. We found that intravascular delivery of MSCs was most effective and demonstrated a better outcome compared to airway delivery. We also found increased endothelial growth factor levels in lung tissue and a decrease in IL-8 levels (measured in EVLP perfusate) in MSC-treated lungs [70].

Conclusion

Normothermic ex vivo lung perfusion has been applied as a novel and very innovative strategy to assess high-risk donor lungs before transplantation increasing the donor pool. Recipients of EVLP-treated lungs have shown to have at least similar results as recipients of transplanted with regular donor lungs.

More importantly, ex vivo lung perfusion represents an ideal platform to treat and repair lungs with different injuries. Specifically targeted therapies can be administered directly to the lung intrabronchially or into the perfusate avoiding any systemic side effects. Thus EVLP will allow us to actively recondition and treat donor lungs, which hopefully will translate in to increased donor lung utilization with improved post-transplant outcomes.

References

Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance

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