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Review

THE PRESENT AND FUTURE OF *EX-VIVO* LUNG PERFUSION

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Summary

A current limiting factor within the field of lung transplantation is the availability of viable donor lungs to meet the increasing recipient demand. *Ex-vivo* lung perfusion (EVLP) is a novel technology which involves the active ventilation of lungs in a physiologic-like environment outside of the body. During EVLP, high risk donor lungs can be assessed, treated and repaired prior to transplantation. Indeed, many transplant centers have now reported comparable outcomes of using these extended criteria lungs to conventional donors through the use of EVLP. Pre-clinical studies and case reports have demonstrated EVLP as an ideal intervention for targeted therapeutics to address donor-related complications such as infection, pulmonary emboli, and aspiration-injury. In addition, increasing evidence has been put forth demonstrating that EVLP usage can extend the total length of pulmonary preservation, which may serve a variety of clinical purposes.

Key words: *ex-vivo* lung perfusion, lung transplantation, organ reconditioning, preservation, therapeutics

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BACKGROUND

Since the advent of lung transplantation, the gold standard procedure for pulmonary graft preservation has been hypothermic static storage at 4C. This technique involves flushing the lungs with a cold low-potassium dextran solution, mildly inflating the lungs, and subsequent storage on ice. While on ice, cellular processes within the organ are slowed down and the metabolic requirements of the lung is significantly reduced. The major limitation of cold static storage is that there are limited opportunities to assess and recondition the graft at such low temperature ranges. Therefore, there is a strong rationale for maintenance of the lungs under normothermic and more physiological conditions.

The concept of normothermic *ex-vivo* organ perfusion has been described as early as 1935. Using cats and rabbits as research models, *ex-vivo* preservation of thyroid glands for one week was achieved using a glass-chambered perfusion pump [1]. This study served as evidence that *ex-vivo* maintenance of organs may be possible. In 1987, the usage of normothermic *ex-vivo* lung perfusion (EVLP) was proposed for extended pulmonary preservation during distant procurements. This concept was soon abandoned due to the development of circuit-induced lung injuries after prolonged perfusion periods [2]. In 2001, Steen and his colleagues proposed to perform EVLP for lung evaluation purposes in a DCD donor. Lung assessment was successfully

performed within the *ex-vivo* platform in which the recipient displayed exceptional post-transplant outcomes [3]. In 2008, the Toronto team published a modified protective approach to EVLP which enabled prolonged maintenance of lungs during extended perfusion periods [4]. In 2011, the same group performed a prospective non-randomized clinical trial demonstrating the safety and feasibility of EVLP to assess and recondition of high risk donor lungs using their approach [5]. This was proceeded by a rapid growth of literature involving EVLP within both clinical and experimental settings. Indeed, usage of EVLP is increasingly becoming a part of clinical practices within North America, Europe, and Australia [6-15].

THE *EX-VIVO* LUNG PERFUSION SYSTEM

Currently, there are 4 commercialized devices which are available to perform clinical EVLP. These include: the Organ Care System™ Lung (OCS); the XPS™ (XVIVO Perfusion AB); the Lung Assist® (Organ Assist); and the Vivoline® LS1. Each system differs in regards to their individual components, design and clinical usage. The OCS™ Lung is the only system which is used for transportation of the organ, while the primary usage of the other devices are for in house organ assessment and potential treatment.

Although these systems may differ in individual components, the basic components of a standard EVLP system include: a solution to perfuse the lungs, a ventilator, a heater to control temperature, a pump to control flow, a reservoir to control circuit volume, a leukocyte filter to deplete immune cells and a gas-exchange membrane. The ventilator parameters, type of pump, pressures and flows may differ based on the technical protocol being used.

EVIDENCE-BASED

Ex-vivo lung perfusion for organ reconditioning

One of the greatest advantages of EVLP is the opportunity to assess, treat and repair the graft prior to transplant. This can be quite significant considering that the percentage of lungs used from multi-organ donors is between 15-20%, inferring that up to 85% of lungs are rejected in some regions for transplantation [16]. During EVLP, graft function can be monitored through trends in ventilator parameters, analysis of the perfusate, and qualitative inspection-as. Through analyses of these parameters, trends regarding organ function can be monitored. Many reports have described the ability of EVLP to recondition the donor graft prior to transplantation. Reconditioning of the lung may occur through removal of airway secretions, lung recruitment, resolution of pulmonary edema, the washout of immunological components and the activation of innate repair mechanisms within the lung. Herein, we describe key studies which have described the concept of *ex-vivo* lung reconditioning of injured donor lungs within a clinical setting.

In 2007, Steen et al. described the first human transplantation of a nonacceptable donor lung after reconditioning from EVLP. The donor lung P/F ratio was just 96.4 mmHg, even after ventilator treatment and attempts to clean the airways. The left lung was reconditioned using EVLP, and the lungs were subjected to transplantation in a high-risk recipient [17]. The patient showed good recovery, and after 3 months, a computed tomographic thoracic scan and transbronchial biopsies showed a normal left lung [17].

In the 2011 study published by the Toronto group, 23 lungs considered to be high risk for transplantation were subjected to 4 hours of EVLP within a prospective non-randomized trial [5]. High-risk donor lungs were defined by specific criteria, including pulmonary edema, a P/F ratio of less than 300 mm Hg or other concerns [5]. 20 of these lungs proceeded to transplant, in which no differences in PGD after 72 hours was found in comparison to contemporary donors, and no differences were seen in 30-day mortality, bronchial complications, duration of mechanical ventilation, and length of stay in the intensive care unit and hospital [5].

In 2012, the Vienna group published a prospective study which showed the utilization of a similar classification of high-risk donors within their center. Of 13 lungs which met the inclusion criteria of the study, 9 lungs were successfully reconditioned and were deemed acceptable for transplantation. These lungs demonstrated a median donor PaO_2 of 216 mmHg which improved to a median PaO_2 of 466 mmHg at the final assessment of EVLP [6]. Of the patients who received these lungs, none developed grade 2 or 3 PGD, and patients showed no 30-day mortality [6].

Later that year, the Harefield group published a retrospective review of 13 consecutive EVLP over a span of a little under two years. Ultimately, 6 lungs were deemed suitable for transplantation. 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 [13].

Two years later, Wallinder et al. published the EVLP experience from the Gothenburg group. In this case-control study, 11 donor lungs were proceeded to EVLP due to either inferior P/F ratio, bilateral infiltrate on chest X-ray or ongoing extra corporeal membrane oxygenation [18]. Donor grafts showed improved oxygenation with a median P/F ratio of 209 mmHg in the donor and to 447 mmHg at the end of EVLP [18]. The authors found a longer median time

to extubation and median intensive care unit stay to be significantly higher in recipients receiving lungs from the EVLP groups, *versus* contemporary controls [18]. Despite this finding, no difference was seen in length of hospital stay, and all recipients of EVLP lungs were discharged alive [18].

In 2014, Sage et al. published the French EVLP experience. During EVLP, 31 out of 32 lungs initially rejected for transplant recovered from a median P/F ratio of 274 mmHg to 511 [8]. There were no significant differences in PGD incidence after 72-hours, median extubation time, intensive care unit and hospital lengths of stay, 30-day mortality, and 1-year survival rates amongst reconditioned lungs transplanted in comparison to lungs transplanted from standard criteria donors [8].

In the same year, Henriksen et al. shared the first Danish experience using EVLP. In the study period, seven of 33 Danish lung transplantations were made possible due to EVLP. All lungs showed an improved median P/F ratio of 173.2 mmHg within the donor to 441 mmHg at the end of EVLP. No patients died to EVLP-related causes by the end of the registration period [14].

A lung transplant group from Italy evaluated the impact of reconditioning of lungs through EVLP on post-transplant outcomes within their center. Within the study, the authors were able to recover 8/11 lungs which would have otherwise been declined by using EVLP [12]. For those lungs transplanted, the incidence of PGD3 at 72 hours was 0% in comparison to 25% for those lungs from standard criteria lungs (n = 28) which were transplanted during the study period [12]. Similar findings were observed in a recent international non-inferiority, randomised, controlled, open-label phase 3 trial. Within the trial, normothermic machine preservation of lungs using the OCS system was compared to conventional cold storage techniques (n = 151 OCS and n = 169 control). Incidence of PGD3 within 72 h was reported in 25 (17·7%) of 141 patients in the OCS group (95% CI 11·8 to 25·1) and 49 (29·7%) of 165 patients in the control group (p = 0·015).

There are upcoming single center and multi-center trials which are continuing to evaluate the reconditioning potential of EVLP within their respective transplant practices. The generation of such data will complement the existing literature and allow for a deeper understanding of the generalizability of EVLP in different countries.

Ex-vivo lung perfusion for organ preservation

Of recent, one of the futuristic indications of EVLP have been described to be for the purposes of optimizing pulmonary preservation lengths [19]. Using the current gold standard practice of cold ischemic preservation, average preservation times are limited to approximately 6-8 hours. Going beyond the current preservation window may have several implications- some of which include the overcoming of geographical hurdles within organ donation, better donor-recipient matching, and optimizing transplant logistics. Within the setting of lung preservation, EVLP may have important implications. During prolonged cold ischemia, disruption of important cellular processes may occur which can ultimately lead to an accumulation of cell death and ultimately inadequate function of the graft [20]. A large animal pre-clinical study published in 2015 demonstrated that the intervention of EVLP during cold ischemic preservation could effectively allow for an extension of total preservation time, which otherwise would not be possible using standard methods [21].

In 2014, the Leuven group published a successful case report of a combined liver-lung transplantation. In anticipation of a longer lung preservation time due to the liver transplantation, the authors subjected the donor lungs to preservation on the EVLP platform. The total normothermic perfusion times was approximately 11 hours in which the lungs were subsequently transplanted. The total *ex-vivo* preservation time of the lungs were 13 hours and 32 minutes first lung and 16 hours for the second [22]. The patient was extubated 7 days after the surgery, and no rejection was seen ten months later [22].

Within a large cohort (n = 906), the Toronto group published a retrospective analysis comparing outcomes of transplant recipients whom received lungs which were preserved for less than 12 hours *versus* those which were preserved for more than 12 hours [23]. Preservation times greater than 12 hours was primarily achieved through the usage of EVLP for the assessment of extended criteria donor grafts. Interestingly, results of this analysis showed no differences in hospital stay, intensive-unit length of stay, primary graft dysfunction grade, and overall survival amongst the two groups [23].

Given this information, the thoughtful use of EVLP in the setting of lung preservation can be a feasible option to successfully extend total preservation periods. This idea is even more attractive considering that a prospective randomized trial and another multicenter international study have both shown that EVLP can be used for standard criteria donor lungs without compromising lung quality [7,24]. Prospective trials evaluating the feasibility and efficacy of this approach are still required.

Ex-vivo lung perfusion for targeted therapeutics

One of the major advantages of EVLP is that every individual lung can be diagnosed and assessed while being actively ventilated and perfused. Donor lungs present themselves with a variety of complications which can may include the presence of emboli, concerns of aspiration-injury, and infection. Once these concerns are identified, they can be addressed by using intervening therapeutics during EVLP. Currently, the data generated using targeted therapeutics has mostly been pre-clinical. Data from clinical trials evaluating the feasibility and efficacy of these approaches are still required.

Immunomodulation

Previous studies have shown that the onset ischemia-reperfusion injury is correlated with a rapid release of endogenous inflammatory mediators [25,26]. Therefore, promoting an anti-inflammatory environment prior to transplantation may have protectives effectives in regards to lung injury. In 2009, Cypel et al. investigated the functional repair of human donor lungs using Interleukin-10 (IL-10) gene therapy [27]. IL-10 is an anti-inflammatory cytokine that has been shown to reduce the release of pro-inflammatory cytokines [28]. In this study, the authors delivered adenoviral vector encoding human IL-10 bronchoscopically within the EVLP platform. Throughout the time course of perfusion, treated lungs showed significant improvements in pulmonary vascular resistance, alveoli oxygenation capabilities, and a favorable switch in cytokine expression [27].

Another example of an immunomodulatory intervention trialed on the platform is Mesenchymal Stem Cell (MSC)-based therapy. MSCs are well known to play an immunomodulatory role by migrating to sites of inflammations, in which they participate in cell-to-cell interactions or secrete soluble factors [29]. By injecting these cells into the EVLP perfusate, lower levels of circulating pro-inflammatory Interleukin-8 (IL-8) were found within the EVLP circuit [30].

Aspiration injury

Inci et al. put forth an aspiration-injury model which they instilled a mixture of betaine-HCl/pepsin into the airways [31]. Surfactant lavage within these injured lungs during EVLP resulted in a higher P/F ratio, and lower pulmonary vascular resistance in comparison to controls [31]. Likewise, Meers et al. described a model of aspiration-induced injury by instilling gastric juice into the airways, rather than an acidic substitute mixture [32]. Using a similar gastric-juice induced lung injury model, it was later shown that exogenous surfactant administered immediately before EVLP was able to improve PaO_2, lower pulmonary vascular resistance, and decrease the amount of apoptotic cell death [33]. Within another similar model, the Toronto group trialed a strategy of performing a saline lavage immediately followed by the administration of exogenous surfactant for the treatment of an aspiration-induced [34]. The lungs were treated during EVLP, and were subsequently transplanted into a recipient animal [34]. Results of the study showed reduction of circulating inflammatory cytokines post-transplantation and superior lung function in comparison to controls, represented by a greater P/F ratio [34].

Infection

In 2013, Lee et al. investigated the use of MSCs to recover endotoxin-induced acute lung injury within donor human lungs rejected for transplantation. The use of clinical-grade human MSCs restored alveolar fluid clearance to a normal level, decreased inflammation, and were associated with increased bacterial killing and reduced bacteremia This was achieved in part through increased alveolar macrophage phagocytosis and secretion of antimicrobial factors [35].

Another study examined the effect of perfusing lungs declined for transplantation with a perfusate containing high-dose, empirical, broad-spectrum anti-microbial agents. Out of the 18 lungs, 13 of them had positive bacteria cultures (72%), in which the bacterial load significantly decreased during EVLP [36]. Ultimately, they deemed six lungs suitable for transplantation, in which all patients survived hospital discharge [36].

In 2016, a study was published randomizing donor human lungs rejected for transplantation due to the clinical concern of infection, in which they received 12 hours of conventional perfusion, or perfusion supplemented with high-dose antibiotics. Perfusate endotoxin levels at 12 hours were significantly lower in the antibiotic group compared with the control group [37]. In addition, the treatment group showed significant improvements in pulmonary oxygenation and compliance and reduced pulmonary vascular resistance during EVLP [37].

In a recent publication, Zinne et al. put forth a *Pseudomonas aeruginosa* pneumonia pig model [38]. The authors investigated whether two hours of EVLP using circulating colistin followed by autotransplantation could be advantageous in comparison to conventional methods of daily intravenous colistin administration and controls. In the control and conventional treatment groups, the mortality rate related to infection after five days was 66.7% [38]. However, in the EVLP group, there was only one infection-related mortality and one procedure-related mortality, resulting in an overall mortality rate of 33.3% [38]. In addition, the authors found that lungs treated with EVLP displayed less clinical symptoms of infection [38].

Pulmonary edema

Studies have shown that the rate of alveolar epithelial ion and fluid transport can be upregulated through the use of β_2-adrenergic agonists [39,40]. Franck et al. showed that by putting human lungs rejected for transplantation on the EVLP circuit, there was a natural alveolar fluid clearance and reduction in pulmonary edema [41]. In addition, by adding a β_2-selective adrenergic agonist (terbutaline) to the EVLP perfusate, rates increased significantly by more than twofold [41].

Along a similar idea, another group tested whether a β-adrenergic receptor agonist (salbutamol) which is known to upregulate fluid transport in the lung would be effective in reducing pulmonary edema [42]. Their primary indicator of edema formation was lung glucose consumption during EVLP, which they validated within a previous

study [43]. In the study, the authors randomized donor pig lungs to salbutamol infusion during EVLP, or a placebo. The results showed that glucose concentration in the perfusate was affected by salbutamol, and salbutamol infusion was associated with lower pulmonary pressures and better lung mechanics [42].

Pulmonary emboli

The first case report treating pulmonary emboli (PE) during EVLP involved the administration of alteplase to the EVLP perfusate [44]. Improvements in pulmonary artery pressure and pulmonary vascular resistance during EVLP indicated successful thrombolysis, and the lungs proceeded to clinical transplantation [44]. These results were reconfirmed in 2015, in which another case report was published showing alteplase as a successful adjunct for thrombolysis during EVLP, leading to subsequent clinical LTx [45]. Urokinase, a known plasminogen activator, has also shown positive results in the treatment of pulmonary emboli [46].

Virus inactivation

In a paper published in 2019, Galasso et al. examined whether targeted approaches on the EVLP platform could be used to inactivate and eliminate hepatitis C virus from NAT+ human donor lungs. In this paired study, the authors compared performing a complete circuit exchange, irradiating the perfusate with ultraviolet C light (UVC), and photodynamic therapy (PDT) to internal controls [47]. Exchange of the circuit and PDT resulted in a significant decrease in perfusate and tissue viral load examined by qPCR. Despite UVC showing no significant effects based on qPCR counts, further in vitro studies demonstrated that the virus was no longer virus after UVC irradiation [47]. Another recent study demonstrated the use of a fusion-toxin protein to eliminate cells infected with CMV in donor lungs [48].

EVLP ALLOWING DAYS LONG LUNG PRESERVATION

Very recently Ali and colleagues explored the concept of multi-day lung preservation by pairing 10°C lung preservation with short cycles (4 h) of EVLP. For the first time, they demonstrated successful 3-days of lung preservation with exceptional immediate post-transplant graft function. Furthermore, they identified the importance of EVLP to re-vitalize metabolites lost during the cold storage period and reduce mitochondrial injury. Extension of preservation periods to days will allow for improved transplant logistics, the opportunity to explore organ banking concepts, allow for better immunological matching amongst donor and recipients and the opportunity to perform time-dependent therapeutics. Further metabolic rehabilitation through enhancement of this protocol may allow for stronger lung recovery during these periods which could revolutionize the landscape of current lung transplantation practices [49].

CONCLUDING STATEMENTS

EVLP is part of the present and future of lung transplantation. It will not only impact donor organ availability, but will also provide the means for organ modification leading to improved short and long term outcomes in transplantation.

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Conflict of interest statement

MC and SK are co-founders and shareholders in Traferox Technologies Inc. They are also consultants for Lung Bioengineering.

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Author contributions

AA, SK, and MC wrote and approved this manuscript.

Ethical consideration

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