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Full Length Research Paper

New protocol for ex vivo lung perfusion prior to transplantation

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Normothermic ex vivo lung perfusion (EVLP) is a technique where the retrieved donor lung can be perfused in an ex vivo circuit, providing an opportunity to reassess its function before transplantation. The hyper-oncotic reconditioning fluid helps to mobilize and remove excess interstitial and alveolar fluid. In addition, recruitment of atelectatic areas has an important role. The value of applying EVLP is to widen the range of donation by allowing lungs of brain death victims, which suffers the associated neurogenic edema in addition to possible traumatic contusions and or infections, and the lungs of circulatory death donors, to be reconditioned and reassessed before final decision of acceptance or rejection. EVLP is already applied within different institutions worldwide, using various application protocols. EVLP is an important technology in the practice of lung transplantation. This paper represents a newly suggested EVLP protocol ‘’Shehata Protocol’’ with theoretical proof of its superiority over one of the most successful and applicable protocols nowadays, Toronto Protocol.

Keywords: Ex vivo lung perfusion, lung conditioning, lung preservation, lung repair, lung transplantation and primary graft dysfunction.

INTRODUCTION

The majority of donor lungs could be potentially injured and cannot be considered suitable for transplantation (Richard et al., 2009). Usually the organ donors are victims of brain death. Brain death results in neurogenic lung edema. A common cause of brain death is accident of multiple trauma, which could be associated with lung injuries (chemical / physical) and or infection. Cardio-vascular causes of death would also be associated with lung complications and edema. All these factors would result in lung grafts not meeting the acceptance criteria for donation, though they would be otherwise accepted. Ex Vivo Lung Perfusion (EVLP) keeps the lungs metabolically active prior to transplantation (Richard et al., 2009), which may allow pulmonary cells and tissues to retain the regenerative processes. During this period assessment and improvement of injured donor lungs could be tried (Dirk et al., 2014). The improvement could be achieved through several mechanisms:

- Dehydration of lung tissue by the high oncotic pressure in the perfusate.
- Removal of harmful and toxic waste products (blood clots, neutrophils, inflammatory cytokines) with filters and membranes in the circuit.
- Recruitment of atelectatic areas for better ventilation/ perfusion matching.
- Application of delivered therapy.

Therefore, many teams worldwide have considered application of EVLP according to Toronto protocol in the clinical settings of lung transplantation (Dirk et al., 2014; Edouard et al., 2014; Massimo et al., 2014). However, all transplantation teams, including Toronto team, are still facing two major problems; graft failure and rejection, and

development of bronchiolitis obliterans.

Aim of work

To introduce a new EVLP protocol, which aims at decreasing K^+ efflux and oxidative stress, and accordingly, decreasing the cytokines production within the graft. This would be expected to result in superior results over the most widely applicable protocol, Toronto protocol, through the decreased incidence of rejection and or bronchiolitis obliterans.

Selection of donors

The lung grafts from ideal or standard donors, which would undergo traditional transplantation as well as grafts from marginal (high risk) donors could be subjected to EVLP for reassessment before transplantation. Criteria for donor classification are presented in figure 1. Other high risk factors would be poor lung compliance during donor operation, history of multiple blood transfusion, and or history of aspiration.

Selection of recipients

Recipients for single or double lung transplantation and re-transplantation could be considered eligible for EVLP.

Protocol of EVLP

1. The donor lungs would be retrieved in the donor hospital, flushed with cold (4 C^0) Steen solution supplemented with 7.8 mg recombinant human gelsolin and anti-oxidants*, and transported to the recipient hospital under cold static preservation (4 C^0) using Steen solution™.
2. The graft selected for EVLP would be placed in the circuit.
3. The circuit would be primed with 2 liters of Steen solution supplemented with 500 mg methyl Prednisolone, 500 mg imipenem/ cilastatin, 3000 IU heparin, RBCs (hematocrite 20%), iron chelators (e.g. 2,2`- dipyridyl)*, and anti- oxidants*. (*using commercially available, medically recommended doses)
4. After one hour, 500 ml of the circulating perfusate would be removed and replaced with fresh 500 ml. Afterwards, 250 ml would be replaced every hour.
5. Once the lungs would be transferred to the circuit chamber, the pulmonary artery and left atrium would be connected to specifically designed cannulas, which are connected to the circuit and anterograde flow would be started at 150 ml/min with the perfusate at room temperature.
6. The temperature of the perfusate would be gradually increased to 37 C^0.
7. When 32 C^0 would be reached, ventilation would be started and the perfusate flow rate would be gradually increased to reach the target flow (40% of cardiac output throughout the maintenance, to be gradually increased to 100% during the last 2 hours of EVLP) (Table 1).
8. The gas flow used to deoxygenate and provide carbon dioxide to the inflow perfusate via a gas exchange membrane would be then initiated at 1 L/min.
9. The mean pulmonary artery pressures would be maintained between 8 and 15 mm Hg.
10. A positive left atrium pressure would be maintained between 3 and 5 mm Hg, through the pressure adjustor around which the left atrium is reconstructed (see Appendix I).
11. A protective mode of mechanical ventilation would be applied using a tidal volume of 7 ml/kg (based on donor ideal body weight) at 7 breaths/min (increased to 12 during last 2 hours of EVLP), positive end-expiratory pressure of 5 cmH_2O, and FiO_2 of 21%.
12. The lungs would be recruited with inspiratory holds to a peak airway pressure of 20 cmH_2O every hour.
13. At 1 hour and 3 hours, administration of 0.5 mg plasmin (dissolved in 0.5 ml Steen solution), and application of surfactant (surfactant protein B and C containing surfactant diluted with saline at 37 C° to a final concentration of 16 mg/ml. A volume of 2.5 ml/ kg surfactant would be used for lavage ’40 mg/kg’. Diluted surfactant would be applied into the lung via flexible bronchoscope).
14. At 3 hours, perfusate would be supplemented with 7.8 mg recombinant human gelsolin.
15. For the evaluation of lung functions, FiO_2 would be increased to 100%, tidal volumes would be increased to 10 ml/kg, and respiratory rate would be increased to 10 breaths/min for 5 minutes.
16. The pH, pCO_2, electrolytes, and glucose would be maintained at physiological levels in the perfusate.
17. At the end of EVLP, the lung block would be cooled down in the circuit to 10 C^0 in a 10 minute period.
18. Thereafter, perfusion and ventilation would be stopped (FiO_2 would be increased to 50% for sake of lung storage), and the trachea would be clamped to maintain the lungs in an inflated state.
19. The lungs would be then statically preserved at 4 C^0 in supplemented Steen solution until transplantation.
20. Infusion of 7.8 mg gelsolin immediately and 8 hours after transplantation might be considered.

Lung functions would be evaluated every hour through:

- PaO_2/FiO_2 in pulmonary vein effluent (mm Hg)
- Pulmonary artery pressure (mm Hg)
- Lung dynamic compliance (ml/cmH_2O)
- Peak airway pressures (cmH_2O).

And at 1 hour and 3 hours through:

- Plain ex vivo lung x-rays.
- Flexible bronchoscopy.

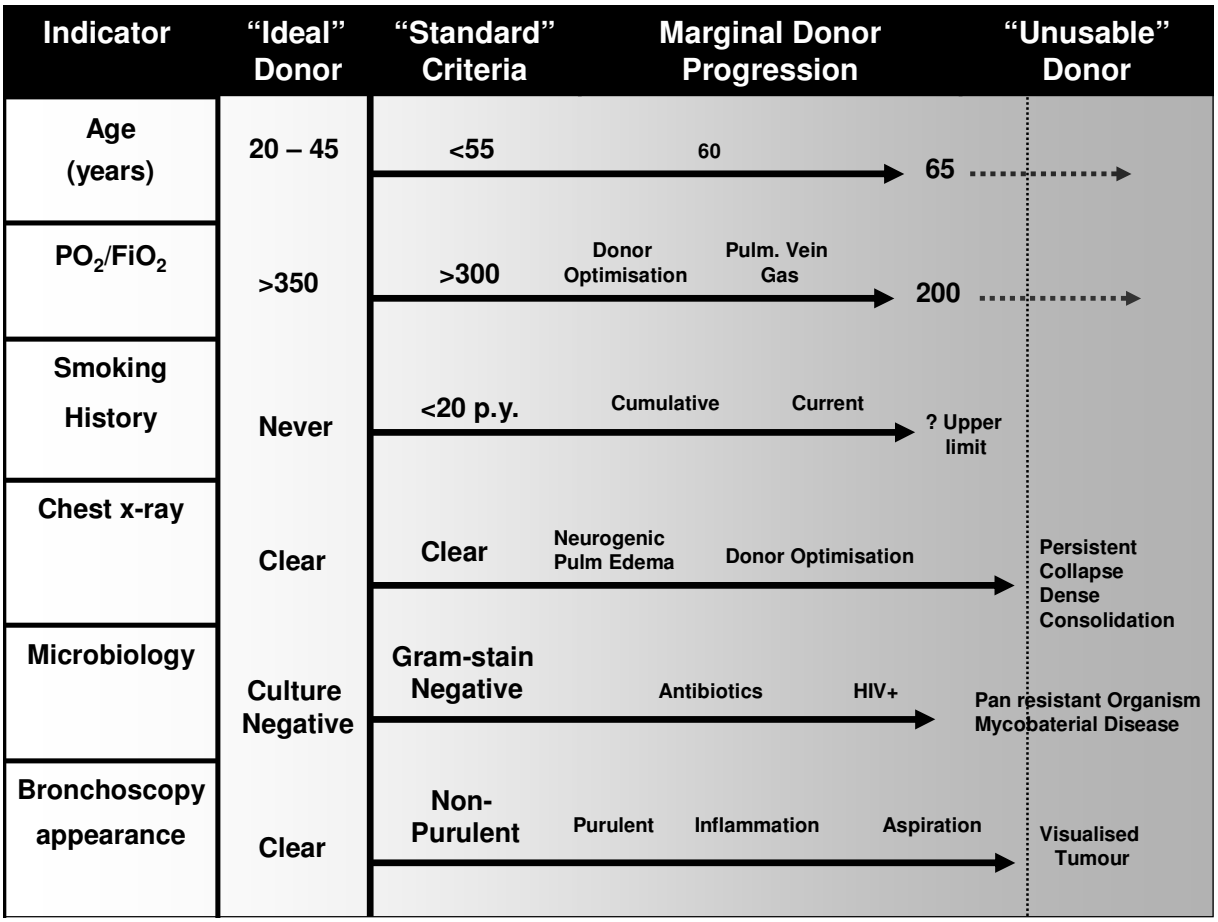

Figure 1. Criteria of graft classifications
Botha Phil, Fisher Andrew J, Dark John H (2006). Marginal Lung Donors: A Diminishing Margin of Safety? Transplantation Journal. 82(10): 1273-1279. Copyright: © 2006 Lippincott Williams and Wilkins, Inc.

Table 1. Target parameters of EVLP Toronto protocol

Parameter	Shehata Protocol
Perfusion	
Target Flow	40% gradually increased to 100% cardiac output
Pulmonary artery pressure	Flow dictated
Left Atrium	Closed
Perfusate	Supplemented Steen solution™
Ventilation	
Start temperature (C^0)	32
Tidal volume	7 ml/ kg body weight
Respiratory rate (bpm)	7 increased to 12 during last 2 hours of EVLP
PEEP	5 cm H_2O
FiO_2 %	21

The initial total duration of EVLP would be 4 hours, unless the graft required longer reconditioning due to unsatisfactory parameters.

An additional new modification in this protocol would be the restitution of bronchial vessels during the transplantation operation and the inclusion of bronchial vessels in the ex vivo perfusion. In other words, stumps of bronchial vessels would be maintained during excision of the graft from the donor, these stumps would be connected to special cannulas, and the necessary

Table 2. Toronto protocol versus Shehata protocol

Toronto	Shehata
1. Acellular perfusate	Cellular (RBCs)
2. 40% cardiac output target flow	100% cardiac output target flow
3. Supplementation with Prednisolone + Heparin + Antibiotics	Supplementation with Prednisolone + Heparin + Antibiotics
	+ Surfactant instillation
	+ Plasmin infusion
	+ Gelsolin infusion
4. Cold static preservation (with Perfedax), followed by EVLP (with Steen solution™), followed by cold static preservation (with Perfedax)	Steen solution™ supplemented with iron chelators, K^+ channel agonists and anti- oxidants would be used for cold static preservation and EVLP

modifications would be applied to the EVLP system to allow perfusion through bronchial vessels using the same perfusate, with a target flow of 3- 5% cardiac output. (See Appendix I)

The K^+ level of the preservation and perfusion Steen solution would be always maintained at the physiological level. Supplementation with drugs that increase K^+ channels activity would be considered during both cold preservation and perfusion.

Cytokines filter will be included within the EVLP circuit, in addition to leukocytes filter, to insure filtration of cytokines and leukocytes. (See Appendix I)

RESULTS

The primary effectiveness endpoint

A composite of patient and graft survival at day 30 and absence of primary graft dysfunction grade 3 (PGD3) within the first 72 hours post-transplantation as defined by the International Society for Heart and Lung Transplantation (ISHLT)

Secondary effectiveness endpoint

Incidence of PGD 2-3 at 72 hours post-transplantation and patient and graft survival at day 30 post-transplantation.

Items to be considered are:

- Need of extracorporeal life support
- Period of mechanical ventilation
- Length of ICU and hospital stay
- Incidence of pulmonary complications
- 30 days mortality
- One year survival

DISCUSSION

The introduction of EVLP into the practice of lung transplantation by many teams worldwide allowed the successful recruitment of circulatory death donor grafts and the high risk grafts which were otherwise declined (Cypel et al., 2012). The progress achieved by Toronto team in particular showed breakthrough of the ability of maintaining viable and well functioning grafts up to 12 hours at 37 C^0. In two published studies of Toronto team (2010 and 2012), the results between EVLP and standard lung transplantation groups were comparable with no significant differences. However, there was an observable improvement in the application of EVLP in the more recent study. This was expressed in the percent of incidence of primary graft dysfunction (PGD) at 72 hours of transplantation. In 2010, the incidence of PGD was 15% compared to 2% in 2012. Such improvement resulted from the improving protocol supplementation with medications, diagnostic measures and or gene therapy (Cypel et al., 2012; Marcelo et al., 2011; Yeung et al., 2012).

The main differences between the newly suggested protocol and Toronto protocol involves four points which are summarized in Table 2.

According to Prof. Keshavjee, the chief of Toronto team for EVLP and lung transplantation, the studies comparing acellular and cellular perfusates did not show significant differences in the results. In addition, they think that cells would be destroyed in the circuit which might result in iron overload. However, the inclusion of RBCs in the perfusate may provide better oncotic and oxygenation assessment characteristics to the perfusate. The analyses of blood gases during EVLP with an acellular perfusate could be unreliable and could camouflage oxygenation deficits during assessment, especially when ventilation–perfusion mismatch is present. A

previous study showed that the shunting effect would be obvious when erythrocytes are included into the perfusate, and would be vanished again when the perfusate is replaced with fresh acellular Steen solution™ (Yeung et al., 2012). In addition, the point of cell destruction within the circuit has no clear evidence and if happened, inclusion of iron chelators within the perfusate would be protective (Pizanis et al., 2011). Other EVLP protocols, Lund and OCS protocols still use cellular perfusates (Richard et al., 2009; Dirk et al., 2014).

In Toronto protocol, the target flow is 40% of cardiac output because they think it would be better to maintain, but not to stress the graft ex vivo. However, the proposed protocol aims at testing the graft with the physiological flow expected to be met after transplantation. While other protocols are using 100% cardiac output throughout EVLP (Richard et al., 2009; Dirk et al., 2014), the proposed protocol aims at maintaining the graft with 40% target flow and increasing it to 100% during the last two hours of EVLP. This would also abolish the effect of the sudden increase in mechanical stress from 40% ex vivo to 100% after transplantation.

Investigating the extracellular matrix of lung biopsies taken from recipients of lung transplantation showed significant increase in versican, a major extracellular glycosaminoglycan, at 6 and 12 months, compared to healthy individuals (Annika et al., 2011). Furthermore, the versican level was found to be significantly higher in the recipients who developed bronchiolitis obliterans compared to recipients who did not develop that complication (Annika et al., 2011)

Versican decreases the elastic properties of the tissue and binds toll- like receptor 2 triggering fibroblast activation and secretion of pro-inflammatory cytokines (Kim et al., 2009). In myocardial infarction, versican was found also to increase 6 hours after coronary artery ligation and reach its peak within 2 days, however, the source of the initial increase was surprisingly the invading monocytes (Toeda et al., 2005).

To initiate immune rejection and inflammatory cell invasion, chemotactants and inflammatory cytokines play an essential role. The static cold preservation of the graft would result in inhibition of Na^+ / K^+ ATPase leading to increased K^+ efflux. Both K^+ efflux and oxidative stress (with the associated impairment of ATP-sensitive K^+ channels activity) were found to stimulate and activate cellular macromolecules called inflammasomes (Kathy and Martha, 2014; Kawaguchi et al., 2011). Upon activation, inflammasomes activate caspase 1, which in turn proteolytically activates pro-IL1-β (which was previously shown to induce IL6) and IL18, starting a cascade of immune reaction and immune cells infiltration (Kawaguchi et al., 2011).

While Perfadex solution contains 6 mmol K^+/L, the used Steen solution™ would contain also physiological K^+ level. However, the inclusion of antioxidants and the relief of oxidative stress might help to minimize the associated inhibition of K^+ channels activity. Also, the supplementation with K^+ channels agonists would be considered because the activation of K_{ATP} channels was found to antagonize the opening of mitochondrial permeability transition pore (which is a non-specific channel of the inner mitochondrial membrane that opens during the first few minutes of post-ischemic reperfusion and is a critical determinant of both apoptotic and necrotic cell death in the setting of ischemia-reperfusion injury), thereby preventing uncoupling of the mitochondria. In addition, K^+ channels enhanced activity would decrease the production of ROS induced through cellular membrane depolarization (Shampa et al., 2014). In addition, the presence of dextran and albumin in Steen solution would provide a better environment for cold preservation.

Although previous studies recommended the utilization of the low K^+ preservation solutions over the high K^+ ones (Rosemary et al., 2003), the reasons for that recommendation might be secured in the new protocol. High K^+ content was claimed to result in depolarization of pulmonary endothelial and smooth muscle cells, leading to increased production of oxygen species (Rosemary et al., 2003). The inclusion of anti-oxidants and K^+ channels agonists in the proposed protocol might antagonize such risks. This goes with the results of a previous study conducted by Pizanis and his colleagues, which recommended the utilization of N-custdiol dextran solution over Perfadex because it showed better results, though N-custdiol dextran solution contains more K^+ (10 mmol/ L) than Perfedax solution (Pizanis et al., 2012).

Gelsolin is an actin binding protein, which present intracellular and plays a Ca^{2+} dependent regulatory role of cell migration (through that it helps wound healing) (Patricia et al., 2004). Gelsolin present also in a secretory plasma form, which drops significantly as a result of burn injury, inflammatory injury and brain death injury (Patricia et al., 2004). It was reported that drop of plasma gelsolin results in pulmonary micro-vascular dysfunction, where the circulating actin filaments could wound the micro-vascular wall, followed by recruitment of platelets and release of inflammatory mediators. The infusion of recombinant human gelsolin was found to protect against those events (Patricia et al., 2004). In addition, plasmin administration was proved to dissolve thrombi in the graft, allowing better reconditioning of the lungs (Hideki et al., 2013).

While ischemic- reperfusion injury was found to be associated with diminished surfactant production, surfactant instillation was found to improve graft reconditioning outcome, especially after acid aspiration (Ilhan et al., 2013).

Although it would be logic to start EVLP immediately as rapidly as possible after donor's death, so that, the ischemic injury would be minimized as described by Hannover team and OCS™ protocol (Gregor et al., 2012; Anders et al., 2014), sometimes this could not be

applicable especially if international graft transportation would be considered. In addition, a previous study compared the results of immediate and delayed (preceded with cold static preservation) EVLP, and recommended the application of delayed EVLP because its results were superior to that of immediate EVLP (Mulloy et al., 2012). However, the presented protocol considers both immediate and delayed EVLP for further comparative studies to find out the technique with superior results.

Inclusion of antibiotics in the perfusate could be modified from case to case, or be fixed for a combination of broad spectrum drugs that cover all known organisms, with the possibility of addition of anti- tubercles and fungicidal drugs to protect against overwhelming infections in immunocompromised patient after transplantation.

Under physiological conditions, the systemic bronchial arteries receive 3 to 5% of cardiac output, which is low compared with pulmonary artery blood flow. However, this relatively little arterial flow has a significant importance to the vitality of the airways, the fluid balance of pulmonary tissue, and the metabolic functions of the lungs (Kentaro et al., 2014). In addition, bronchial artery re-vascularization was found to protect pulmonary endothelium and type II pneumocytes in the early phase after lung transplantation (Kai et al., 2002). Accordingly, the inclusion of bronchial vessels in lung transplantation as well as EVLP would be of value.

With the application of all these measures included within this newly suggested ''Shehata'' EVLP protocol, better reconditioning of lung grafts would be expected, and accordingly, better prognosis for lung transplantation. Clinical studies (on animal models, then on patients) as well as molecular studies should be conducted for further experimental comparison between the results of ''Shehata'' protocol and the other EVLP protocols. (See Appendix II)

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Appendix I

A modified model for ex vivo lung perfusion device. Global Advanced Research Journal of Medicine and Medical Science Vol. 3(10) pp. 342-346, October 2014

Appendix II

A modified model for ex vivo lung perfusion device. Global Advanced Research Journal of Medicine and Medical Science Vol. 3(10) pp. 342-346, October 2014

Note