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Ex vivo lung evaluation and reconditioning

Avaliação e recondicionamento pulmonar ex vivo

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Abstract

Objective: **Only about 15% of the potential candidates for lung donation are considered suitable for transplantation. A new method for ex vivo lung perfusion (EVLP) has been developed and can be used for evaluation and reconditioning of "marginal" and unacceptable lungs. This is a report of functional evaluation experience with ex vivo perfusion of twelve donor lungs deemed unacceptable in São Paulo, Brazil.**

Methods: **After harvesting, the lungs are perfused ex vivo with Steen Solution, an extra-cellular solution with high colloid osmotic pressure. A membrane oxygenator connected to the circuit receives gas from a mixture of nitrogen and carbon dioxide and maintains a normal mixed venous blood gas level in the perfusate. The lungs are gradually rewarmed, reperfused and ventilated. They are evaluated through analyses of oxygenation capacity, pulmonary vascular resistance (PVR), lung compliance (LC).**

Results: **The arterial oxygen pressure (with inspired oxygen fractions of 100%) increased from a mean of 193.3 mmHg in the organ donor at the referring hospital to a mean of 495.3 mmHg during the ex vivo evaluation. After 1 hour of EVLP, mean PVR was 737.3 dynes/sec/cm^5, and mean LC was 42.2 ml/cmH_2O.**

Conclusions: **The *ex vivo* evaluation model can improve oxygenation capacity of "marginal" lungs rejected for transplantation. It has a great potential to increase lung donor availability and, possibly, to reduce the waiting time on the list.**

Descriptors: **Lung transplantation. Organ preservation. Lung injury.**

Resumo

Objetivo: **Apenas 15% dos pulmões doados são aproveitados para transplante. Um novo método de Perfusão Pulmonar *Ex Vivo* (PPEV) foi desenvolvido e pode ser usado para avaliação e recondicionamento de pulmões "marginais" e rejeitados para o transplante. Esse trabalho relata nossa experiência com a avaliação funcional da PPEV.**

Métodos: **Foram estudados pulmões de 12 doadores considerados inapropriados para transplante pulmonar. Após a captação, os pulmões são perfundidos *ex vivo* com Steen Solution, uma solução de composição eletrolítica extracelular com alta pressão coloidosmótica. Um oxigenador de membrana ligado ao circuito recebe uma mistura gasosa (nitrogênio e dióxido de carbono) e**

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This study was carried out at the Heart Institute (InCor) of the University of São Paulo Faculty of Medicine Clinics Hospital, São Paulo, SP, Brazil.

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"desoxigena" o perfusato, mantendo uma concentração de gases semelhante a do sangue venoso. Os pulmões são gradualmente aquecidos, perfundidos e ventilados. A avaliação dos órgãos é feita por gasometrias e medidas como a resistência vascular pulmonar (RVP) e complacência pulmonar (CP).

Resultados: **A PaO_2 (FiO_2 100%) passou de um valor médio de 193,3 mmHg no doador para 495,3 mmHg durante a PPEV. Após uma hora de PPEV, a RVP média era de 737,3 dinas/seg/cm^5 e a CP era de 42,2 ml/cmH_2O.**

Conclusões: **O modelo de avaliação pulmonar *ex vivo* pode melhorar a capacidade de oxigenação de pulmões "marginais" inicialmente rejeitados para transplante. Isso denota um grande potencial do método para aumentar a disponibilidade de pulmões para transplante e, possivelmente, reduzir o tempo de espera nas filas.**

Descritores: **Transplante de pulmão. Preservação de órgãos. Lesão pulmonar.**

INTRODUCTION

Since the first lung transplant has been performed in humans in the 60s, there has been a significant improvement in surgical and anesthetic techniques, as well as the development of new methods of lung preservation and immunosuppressant drugs. Such changes have made transplantation an effective therapy for many well-established end-stage lung diseases. Unfortunately, the increased number of transplants did not keep pace with the great increase in the number of patients included in the waiting lists. This imbalance caused mainly by lack of donors, resulted in increased mortality in the waiting list [1].

Currently, only 15 to 20% of lungs available for transplantation (brain-dead donors) are actually used [2]. National data show an even worse situation. In São Paulo State, only 4% of the lungs available for transplantation (brain-dead donors) were used in 2006. In the same period, the utilization rate of both liver and kidney was higher than 90%, according to the Secretary of Health of the State of São Paulo [3]. The most frequent reasons for refusal were Poor Gas analysis results (30.1%) and the presence of infection (23.7%). This is due to increased susceptibility of the lungs to the deleterious effects of brain death (endocrine insufficiency, hemodynamic instability, hypothermia, inflammatory response) and to complications of ICU stay (prolonged mechanical ventilation, nosocomial pneumonia, barotrauma, excessive crystalloid infusions).

In recent years, several strategies have been proposed aiming at to increase the number of lung transplants, including living donor's transplants, use of non-heart beating donors and "marginal" donors. These strategies, however, were limited to a small number of patients due to technical and ethical limitations. They did not have the expected impact on the number of transplants [4].

The ex vivo lung perfusion (EVLP) is a new method that allows the careful inspection of the lungs removed, besides hemodynamic and ventilator measures, gas exchange and evaluation. This technique was first used in humans, when Steen et al. [4], in Sweden, transplanted a lung from a donor with a non-beating heart in 2000. The EVLP then began to be used for reconditioning of lungs rejected for transplantation. These researchers published in the early 2009s [5] a study regarding the first six lung transplants initially rejected (the mean PaO2 mmHg donors = 158.25) and reconditioned through EVLP (mean PaO2 after EVLP = 515.25 mmHg).

The present study demonstrates a survey with the functional assessment of EVPL. It aims to assess the EVLP application in human lungs rejected for transplantation and its capability to restore them, i.e., to improve gas exchange.

METHODS

In the present research, lungs from brain-dead donors reported by the Transplant Center of the State of São Paulo from April to July 2009 were used. The research included those patients who were in admitted within the Organ Procurement Organizations (OPO) area hospitals, University of São Paulo Faculty of Medicine Clinics Hospital, and the Santa Casa de Misericórdia (State of São Paulo Holy House of Mercy). They were rejected for lung transplantation teams based on selection criteria recommended by the International Society of Heart and Lung Transplantation (ISHLT). All the patients whose next of kin, relatives, or their legal representatives refused to participate in the research were excluded. The Local Institutional Ethics Committee approved all the protocol, including the written informed consent.

The organ harvesting is performed based on the daily basis technique followed by the pulmonary transplant team, shortly described next.

The donor is placed in the supine position with a pad under the shoulder blades. A median sternotomy is performed making an incision through the midline of the sternum to gain access to the pericardium and mediastinal pleurae. At this stage, if pleuropulmonary adherences are present, they are cut away. The superior and inferior vena cavae are dissected and repaired. The aortopulmonary window is dissected, releasing it from the posterior plane

between the aorta and pulmonary artery, to allow the placement of a hemostatic forceps seizing the aorta. A purse-string suture is sewn in the pulmonary trunk using a 4-0 polypropylene thread and the perfusion cannula is inserted. After performing the therapeutic administration of heparin on the donor it is useful to give alprostadil (prostaglandin E1) (250 to 300 u/Kg; 0.5 mg) is administered in the trunk of pulmonary artery. The SVC is ligated and the IVC is sectioned superior to the diaphragm. After clamping the ascending aorta, the pulmonary perfusion starts administrating Perfadex, 50 to 60 ml/kg, (Vitrolife, Gothenburg, Sweden) at 4°C. The left auricle should be opened in order to drain the solution from the lungs. As an alternative to the section of the left auricle, the left atrium may be opened along with the pulmonary veins; however, without endangering them. Cold saline solution should be injected into the pleural cavities for cooling the organs, while the entire donor's blood is aspirated.

Throughout the process of perfusion, the ventilation is maintained to enable perfect distribution of the preservation solution and to prevent atelectasis. After the infusions are completed, the cannulas are removed and the organ harvesting starts. On each side, the pulmonary veins should be attached through an atrial cuff, with the presence of the subtle muscle layer of the left atrium. The SVC is cut just below the ligature, releasing it from the right pulmonary artery. The aorta is sectioned near the site of clamping. The pulmonary trunk is sectioned prior to its bifurcation, and the heart is removed permanently. The release of the lungs through the pulmonary ligament is initiated. Gently, all the anterior mediastinal tissue of the esophagus is released up to two or three rings above the Carina. The en bloc resection of the thoracic structures, including the entire pericardium and adjacent structures in order to avoid injury to the trachea, artery, and pulmonary veins is performed. As soon as the lungs were released and the distal trachea dissected, this is clamped with the lungs inflated, sectioned at this level, and the bloc then removed from the operative field. The lungs are immersed in cold saline solution and transported on ice.

EVLP was started after 10 hours of cold ischemia. The perfusion system consists of several tubes, venous reservoir, membrane oxygenator, and all fitted to pediatric profile (Figure 1). The system is composed of a heat exchanger and a centrifugal pump (Braile Biomedica, Sao Jose do Rio Preto, Brazil). During our initial experiment, two technical modifications were important for the improvement of the protocol: the use of a pediatric cardiopulmonary bypass system which allowed a considerable reduction in the volume of perfusate, and the use of a centrifugal pump for more precise control of the pulmonary artery flow [6].

The lung block is placed into a transparent plastic container (Vitrolife, Gothenburg, Sweden). An orotracheal tube is inserted and fixed into the trachea. A perfusion cannula (Vitrolife, Gothenburg, Sweden) is inserted into the pulmonary trunk (Figure 2). This cannula has a built-in tube for coupling into a pressure transducer, permitting continuous monitoring of pulmonary artery pressure (PAP). The return of the solution through the pulmonary veins is performed directly to a hard shell reservoir and drained into the venous reservoir by gravity. The system is filled with 1500 ml of STEEN Solution (Vitrolife, Gothenburg, Sweden). This is a solution of extracellular electrolyte composition, which contains human serum albumin and dextran. It has been our option not to use blood into the circulation, similar to what the Toronto group does. The circulating red blood cells undergo mechanical damage during the perfusion, which may aggravate the ischemia-reperfusion injury.

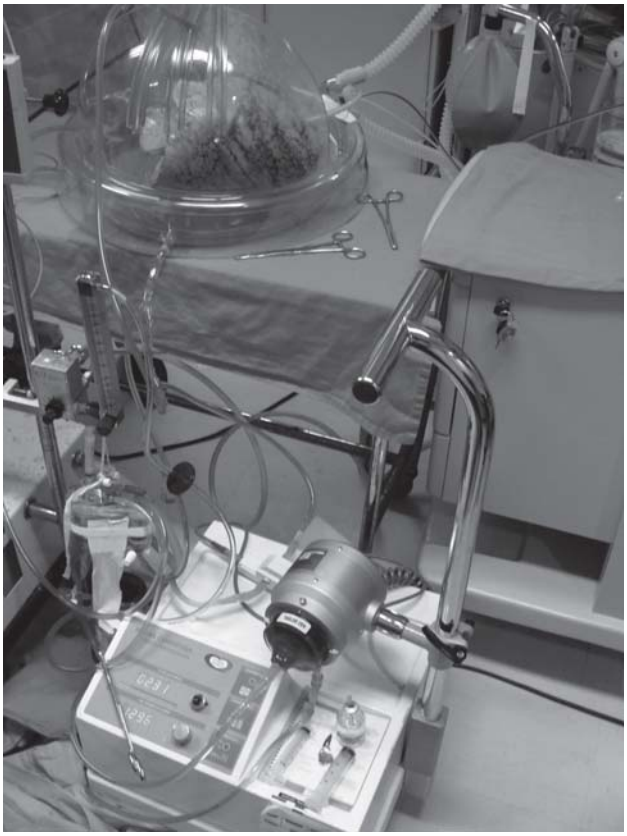

Fig. 1 – Complete circuit with pulmonar hard shell reservoir, centrifugal pump, reservoir and membrane oxygenator filled with a perfusion solution (STEEN Solution)

As a perfusion flow, it was used 40% of the estimate cardiac output (CO) (CO is 3 times the body surface area). EVLP is initiated with the solution at 20ºC and 10% of the estimated initial flow. The temperature was gradually raised to 37°C in the first 30 minutes. The flow also increased

slowly, reaching 30% of the estimated flow at 30 minutes, and reaching the maximum flow around 60 minutes. If the PAP reached 20 mmHg before the estimated maximum flow, this is maintained at a lower value. It is vital that the PAP is maintained below 20 mmHg to minimize edema. When the temperature reaches 32ºC (usually 20 minutes after EVLP is started), the ventilation is initiated. At this time, a flow of gas (7% CO2 + 93% N2) is administered to the system through the membrane oxygenator to "deoxygenate" the perfusate. Even if the perfusate enters the pulmonary artery, it will have a gas composition similar to the venous blood. Recruitment maneuvers up to a maximum pressure of 25 cm H2O can be performed to repair any atelectasis.

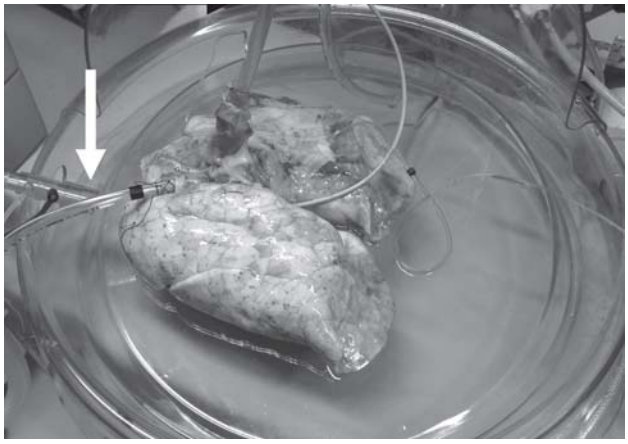

Fig. 2 – Details of the lungs in the transparent plastic container during EVLP: tracheal tube for ventilation (white arrow) and arterial cannula (black arrow) for infusion and monitoring of pulmonary artery pressure

The ventilator is kept up at the following settings: tidal volume = 6-8 mL/kg, frequency = 7/min, FiO2 = 100% and PEEP = 5 cm H2O. This protective ventilation strategy is to avoid any additional trauma. After 60 minutes of EVLP, the perfusate leaving the pulmonary veins is collected for blood gas analysis. The following parameters are analyzed: PaO2, PaCO2, pulmonary vascular resistance (PVR) and pulmonary compliance (PC). The PVR was calculated by the PAP minus left atrial pressure (always zero once there was no atrium and the drainage of the pulmonary veins was free) multiplied by 80 and divided by the perfusion flow. The PC was calculated from tidal volume divided by the difference between airway pressure ($P_{plateau}$) and PEEP.

Data analysis was performed using the statistical software SPSS (SPSS, version 17.0). Continuous variables are expressed as mean (± standard error of the mean). The comparison between the measurements before and after EVLP was assessed using a paired Student's *t*-test. We considered $P < 0.05$ as statistically significant.

RESULTS

The study population included 12 lung donors (six men and six women) with a mean age of 50.4 ± 6.9 yrs. The main cause of death was hemorrhagic stroke (CVA) (seven cases). Most donors (10 cases) were rejected for lung transplantation due to poor blood gas analysis results, i.e., PaO2 < 300 mmHg with FiO2 = 100% and PEEP = 5 cm H2O. The average time for patients requiring intubation and mechanical ventilation was 6.1 ± 1.2 days. The characteristics of the donor are described in Table 1.

Table 1. Characteristics of the donors.

Donor	Gender	Age (years)	Cause of Death	Reason of Refusal	Time (days) on mechanical ventilation
1	F	70	HS	Poor Gas analysis results	6
2	M	48	HS	Pneumonia	3
3	F	60	HS	Poor Gas analysis results	8
4	F	22	Anoxic Encephalopathy	Donor-recipient incompatibility	6
5	F	41	SH	Poor Gas analysis results	1
6	M	74	HS	Poor Gas analysis results	12
7	M	26	CCT	Poor Gas analysis results	7
8	F	61	HS	Poor Gas analysis results	4
9	M	52	HS	Poor Gas analysis results	11
10	M	68	HS	Poor Gas analysis results	10
11	M	20	CCT	Poor Gas analysis results	1
12	F	63	SH	Poor Gas analysis results	4

HS: hemorrhagic stroke; SH: subarachnoid hemorrhage; CCT: Craniocerebral trauma traumatismo cranioencefálico

Mean PaO2 (FiO2 = 100%) measured from the donor shortly before the procurement was 193.3 mmHg (± 42.16). After 60 minutes of EVLP, the mean PaO2 (FiO2 = 100%) was 495.3 mmHg (± 13.27). The difference between the two samples (PaO2 *in situ* vs PaO2 ex vivo) was significant (p <0.001) (Figure 3). The mean partial pressure of O2 in the perfusate that entered the pulmonary artery (venous) during EVLP was 83.3 mmHg (± 12.66), demonstrating that the gas mixture applied to the system really "deoxygenated" the perfusate. Table 2 shows the individual values of PaO2.

The mean pulmonary vascular resistance during EVLP was 737.3 (± 130) dynes/sec/cm^5 and the mean pulmonary compliance was 42.2 (± 7.42) ml/cm H2O.

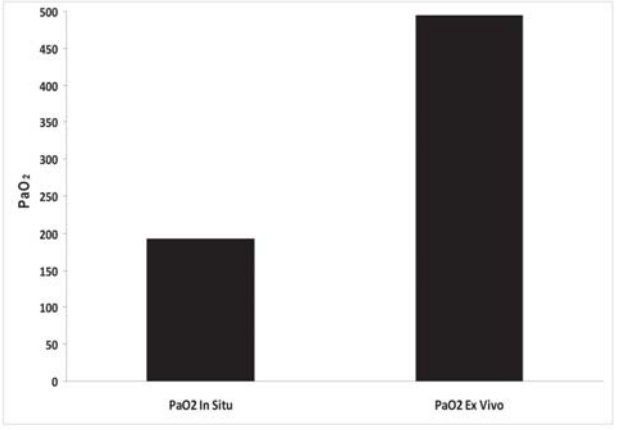

Fig. 3 – Mean PaO_2 before procurement and after EVPL

Table 2. PaO_2 measured *in situ* in donor immediately before and after organ harvesting and EVLP, respectively.

Donor	PaO_2 (mmHg) *in situ* FiO_2 100%	PaO_2 (mmHg) *ex vivo* FiO_2 100%
1	92.0	457.0
2	365.6	463.0
3	179.9	515.0
4	350.0	489.0
5	100.0	458.0
6	286.0	548.0
7	216.8	507.0
8	58.0	547.0
9	174.0	416.0
10	254.4	529.0
11	174.3	477.0
12	69.0	538.0
Média ± EPM	193.3 ± 42.16	495.3 ± 13.27

PaO_2: arterial oxygen partial pressure; FiO_2: inspired oxygen fraction; SEM: standard error of the mean

DISCUSSION

The low rate of utilization of lungs donated for transplantation is responsible for the increased mortality on the organ waiting lists. Only 15% of the lungs donated are removed and transplanted worldwide. Several strategies, such as the use of "marginal" donors were proposed to try increasing the number of transplants, but none was actually effective.

The ex vivo lung perfusion appears as a method capable of making a more accurate assessment of these organs, including hemodynamic and ventilatory measures. It can also retrieve them, making it possible its use in patients in the waiting list. This is particularly useful in the case of lungs rejected due to a poor blood gas analysis (PaO2 < 300 mmHg), a reflection of inadequate gas exchange.

In our study, 10 organ donors were rejected because a poor gas analysis. Two had satisfactory blood gas analysis. One was rejected due to pneumonia (purulent secretion at bronchoscopy), and one for lack of a compatible recipient. After EVLP, there has been a significant improvement in the oxygen index (PaO2/FiO2 ratio) in all cases. The ten cases rejected due to poor blood gas analysis started to have a PaO2 above 400 mmHg, which would allow its use for transplantation (in the absence of other contraindications). The explanation for success of the method seems to lie in the fact that the perfusion is done at normothermia (hypothermic perfusion increases PVR), in a slow and gradual way (PAP is always kept below 20 mmHg), which prevents the formation of edema. Besides, the high colloidal osmotic pressure [oncotic pressure] of the perfusion solution (STEEN Solution) allows the mobilization and removal of alveolar and interstitial fluid, thus reducing pulmonary edema. The EVLP also enables inspection and palpation of the entire lung, and by recruitment maneuvers, one can undo persistent atelectasis. Without interference from the rigid structures of the chest and diaphragm, the total volume supplied by the ventilation is distributed directly to the lungs, improving the ventilation/perfusion.

In 2006, Wierup et al. [7] published a study with a similar methodology. From the six donors studied, three reached a PaO2 > 400 mmHg after EVLP. Two had a PaO2 between 300 and 400 mmHg, and one remained with PaO2 < 300 mm Hg. In this study, the mean PVR during EVLP was 400 dynes/sec/cm5. Our cases had a higher mean PVR (737 dynes/sec/cm5). This can be explained by the longer cold ischemia time in our sample (10 hours vs 7 hours in the study of Wierup et al. [7]), and by a larger selection of donors. Our study included patients with pneumonia and prolonged intubation (mean time on mechanical ventilation of 6 days vs 2 days in the study of Wierup et al. [7]).

Ingemansson et al. [5], in 2009, reported six cases of lung transplantation, whose organs were initially rejected

and reconditioned through EVLP. Survival at 90 days was 100%. One patient died of sepsis 95 day after the operation and another one due to rejection nine months later. Four patients are alive and well without signs of bronchiolitis obliterans 24 months after transplantation [5]. In Toronto, Cypel et al. [8] have developed a strategy of ventilation and pulmonary perfusion ex vivo that allowed the maintenance of EVLP at normothermia for 12 hours without formation of edema with a PVR, airway pressure, and oxygen capacity stable throughout the 12-hour period. This new methodology allows not only the evaluation and preservation of an ex vivo lung, but also the application of new molecular or drug therapies, in an attempt to recover the lungs donated and rejected for transplantation. Possible applications include the use of perfusates with high osmotic pressure and beta-adrenergic receptors to accelerate the removal of edema, in addition to perfusion with high doses of antibiotics to treat pneumonia and fibrinolytic agents to remove thrombus in the pulmonary circulation.

The EVLP has a great potential for reconditioning lungs donated and rejected for transplantation. This method can increase the number of lungs for transplantation accepted by retrieving the cases rejected due to unsatisfactory PaO2. Further studies are needed to determine possible histological changes in organs undergoing EVLP.

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