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Ex vivo lung perfusion in lung transplantation

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Abstract

Introduction. *The number of lung transplants performed worldwide is not enough because of a shortage of suitable (ideal) donors, missed chances to use lungs from donors who died of cardiac arrest, the lack of resources to perform this technically complex operation in poor, developing countries and due to a number of other reasons.) The world literature sources contain information about an increase in the number of lung transplantations by using organs from non-ideal (suboptimal and marginal) donors. This became possible thanks to the technology of* ex vivo *normothermic perfusion of donor lungs.*

Aim. *To demonstrate the possibilities in the assessment, therapy and restoration of the function of non-ideal (suboptimal and marginal) donor lungs by using the technique of* ex vivo *lung perfusion.*

Material and methods. *We reviewed scientific articles published in the period from 2003 to 2023 in the PubMed and Google Scholar databases for the key query "ex vivo lung perfusion".*

Conclusion. *The ex vivo lung perfusion technique is a promising and effective procedure for lung evaluation, recondition and regeneration for transplantation. A rapid development of technologies for this treatment modality makes it possible to increase the number of lungs suitable for transplantation, reduce the number of post-transplant complications and mortality rates on the waiting list, and improve the outcomes of lung transplantations.*

Keywords: lung transplantation, non-ideal donor, ex vivo lung perfusion, primary graft dysfunction, lung graft

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ARDS, acute respiratory distress syndrome
BAL, bronchoalveolar lavage
cAMP, cyclic adenosine monophosphate
CFTR, cystic fibrosis transmembrane conductance regulator
CO, cardiac output
CSP, cold static preservation
ECMO, extracorporeal membrane oxygenation
EVLP, ex vivo lung perfusion - extracorporeal normothermic perfusion of the lungs
FBS, fibrobronchoscopy
FiO_2, fraction of inspired oxygen (in the inhaled gas mixture)
HBD, heart-beating donor
Hct, hematocrit
hMSC, human mesenchymal stem cells
LA, left atrium
LLL, left lower lobe

MAPC, multipotent adult progenitor cell
MAP-kinase, mitogen-activated protein kinase
MLV, mechanical lung ventilation
NF-kB, nuclear factor kappa-light-chain-enhancer of activated B cells
NHBD, non-heart-beating donor
OCS, organ care system
PEEP, positive end-expiratory pressure
RBCS, red blood cell suspension
RLL, right lower lobe

Introduction

Unfavorable environmental conditions, the spread of smoking, infections, and the inaccessibility of timely medical care in some regions are the causes for the constant increase in chronic diseases of the respiratory system [1]. In far from all cases, modern medicine can provide effective methods of treatment or prevention of incurable lung diseases in the terminal stage. Despite the development of new drug and non-drug therapies, lung transplantation for such patients is the only possible treatment option [2] that can prolong life and improve its quality. At the same time, limited donor resources lead to an insufficient number of such operations [3], and therefore survival during the waiting period is no more than 50% within 2 years if the operation was not performed during this period [4].

An increase in the number of lung transplants is possible through the use of organs from non-ideal – suboptimal – donors [5-7]. This, in turn, increases the risks of postoperative complications and deaths [7, 8]. One of the possible ways to reduce risks when using organs from suboptimal donors is normothermic ex vivo lung perfusion (EVLP) [9].

This review presents data on the technique of normothermic donor lung perfusion ex vivo to improve the quantity and quality of lung transplants from non-ideal donors. This is mainly possible by assessing the functional qualities of the donor organ [10, 11].

The objective was to acquaint the reader with new opportunities in the assessment, conditioning and functional regeneration of non-ideal (suboptimal) donor lungs through the use of ex vivo lung perfusion techniques.

Method of normothermic ex vivo lung perfusion

Many transplant teams began using EVLP in clinical practice by employing a proprietary perfusion system assembled from individual components. The technique was based on two main processes: ventilation and perfusion, so the main components were a mechanical lung ventilation (MLV) device and the elements of an extracorporeal support device.

The basic components of the system are a ventilator, a modified endotracheal tube, a lung organ chamber, a reservoir containing a perfusion solution, a perfusion (centrifugal) pump, a heater, an extracorporeal membrane oxygenation (ECMO) device with a membrane oxygenator, lines, oxygen and carbon dioxide saturation control sensors, perfusate pressure sensors with the system, a gas mixture N_2 86%; CO_2 8%; O_2 6%) for deoxygenation, a leukocyte filter, and cannulas for connecting the EVLP lines to the pulmonary artery and the open left atrium [12].

The main one of the most important consumable components of the system is the perfusion solution. Most often, various authors mention in their studies the Steen's solution (Steen^{TM}), which is used in most perfusion protocols. It has optimal osmolarity, high dextran content, and essential antioxidant properties, which protects the endothelium of pulmonary vessels from macrophages and activation of leukocytes [13, 14].

Regardless of the perfusion protocol, the solution goes through a certain cycle. First, it is removed from the lungs, enriched with oxygen,

then from the left atrium it enters the reservoir when the left atrium is open or into the cannula when it is closed. Then, through the lines by means of a centrifugal pump, it enters a membrane oxygenator, which, by deoxygenating the gas mixture, performs the function of body tissues. In a membrane oxygenator, the solution is deoxygenated and enriched with carbon dioxide, after which it is returned to the ventilated lungs through a cannula inserted into the pulmonary trunk. During the perfusion process, the heat exchanger gradually heats the perfusate up to 37°C from the very beginning of the procedure, then a constant temperature of the circulating solution and organ is maintained [15] (Fig. 1). Thus, unlike classical cold storage (4° C), which is still the gold standard for graft preservation, the ex vivo allows the lungs to function and maintains them at physiological temperature, this reduces ischemia and extends the period of lung preservation, during which the lung regeneration and assessment of their functions takes place [16].

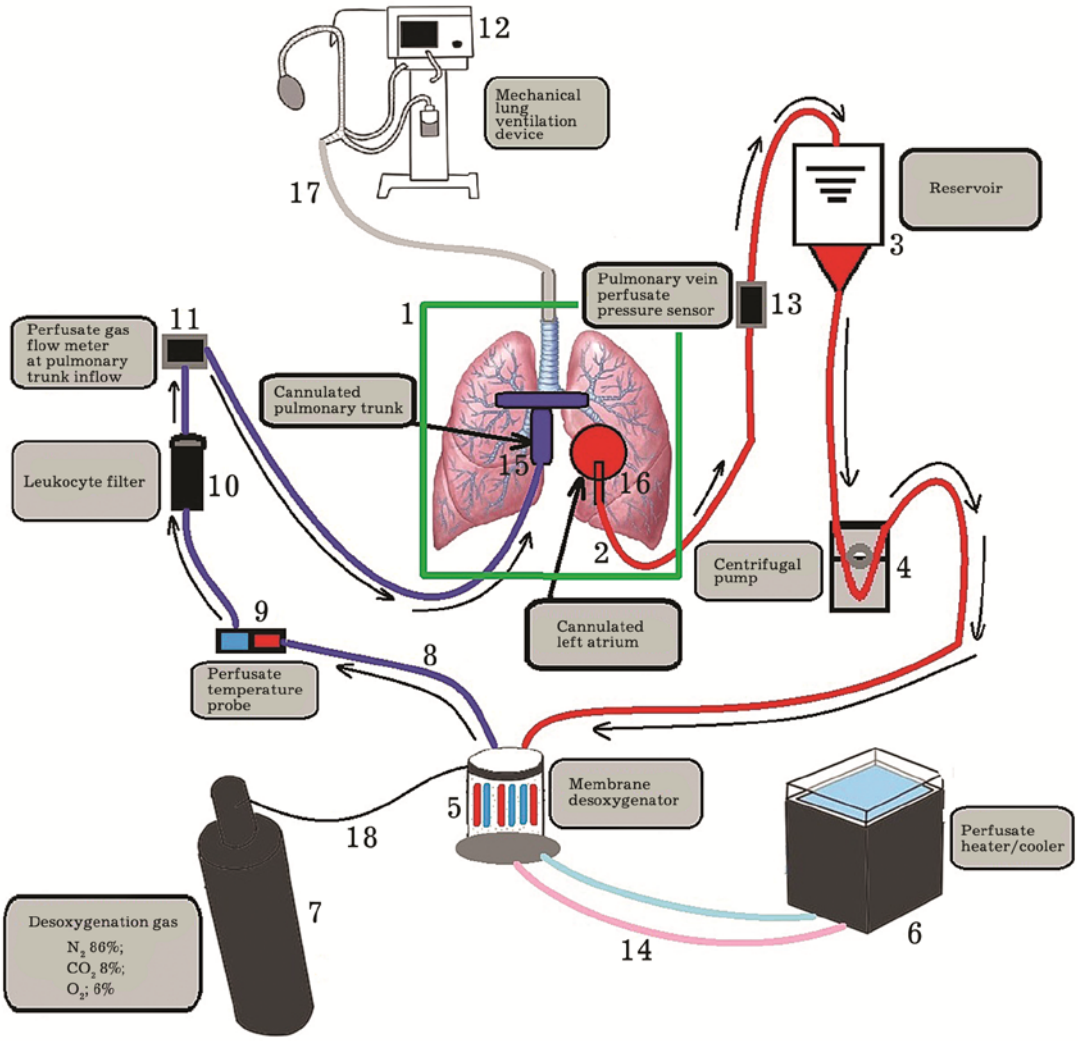

Fig. 1. Normothermic lung perfusion circuit.

The diagram shows a system for normothermic isolated lung perfusion, consisting of the following components: (1) open-type organ chamber; (2) arterial line; (3) perfusate reservoir; (4) centrifugal pump; (5) membrane desoxygenator with an integrated arterial filter; (6) heat exchanger; (7) reservoir with a gas mixture; (8) venous line; (9) perfusate temperature probe; (10) leukocyte filter; (11) venous line pressure sensor; (12) mechanical lung ventilation device; (13) arterial line pressure sensor; (14) heat exchanger line; (15) cannulated pulmonary trunk; (16) cannulated left atrium; (17) MLV device circuit; (18) gas mixture line

Commercial lung perfusion machines

Currently there are commercially available EVLP devices: OCS™ Lung (Transmedics), Vivoline® LS1 (Vivoline Medical, Lund, Sweden),

Lung Assist® (Organ Assist, Groningen, Netherlands), XPS™ (XVIVO Perfusion) (Fig. 2).

There are differences between all these devices in terms of technology and design, as well as in terms of clinical use; but the circuit lines of these systems are identical. The circuit includes: a ventilator, endotracheal tube, lung chamber perfusate reservoir, perfusate circulation pump, heat exchanger, membrane oxygenator, leukocyte filter, and cannula for connecting EVLP, circuit to the pulmonary artery and atrium [3].

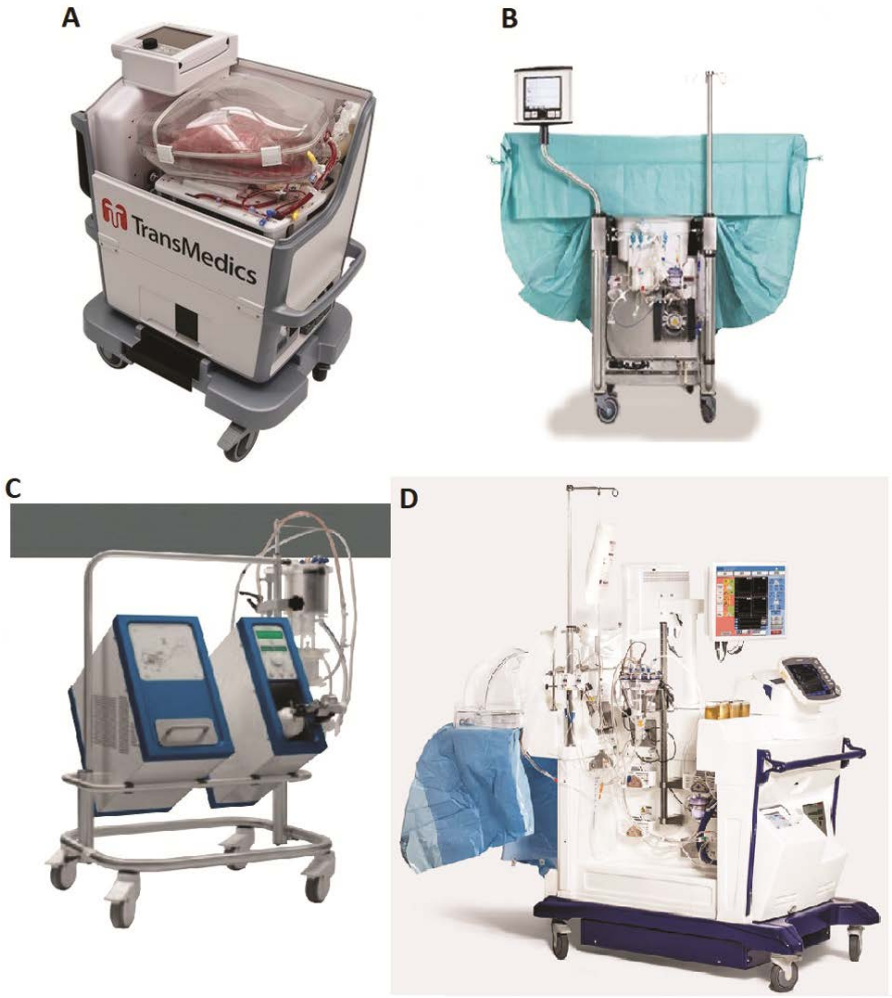

Fig. 2. Commercially available devices for ex vivo lung perfusion.

(A) OCSTM Lung (Transmedics), www.transmedics. com; (B) Vivoline LS1 (Vivoline Medical), www.vivoline.se; (C) Organ Assist, www.organ-assist.nl; (D) XPSTM (XVIVO Perfusion AB), www.xvivoperfusion.com [17]

Basic lung perfusion protocols used in clinical practice

In world clinical practice, three main EVLP protocols are used (Table 1). Toronto protocol, which is usually applied using ready-made components: ECMO, protective dome over the lungs, vascular cannulas (XVIVO Perfusion AB, Gothenburg, Sweden), cell-free perfusate STEEN SOLUTION (XVIVO Perfusion AB, Gothenburg, Sweden) [18–21]. The Lund protocol is used with the Vivoline system LS1 (Vivoline Medical AB, Lund, Sweden) [19]. Protocol OCSTM (Transmedics, Andover, Massachusetts, USA) is currently the only fully autonomous system. It is used immediately after explantation of the graft, without a period of cold preservation. It is used during transportation of the lungs to the recipient from the moment of organ explantation [20].

In both the Toronto and Lund protocols, the graft undergoes a period of cold preservation at 4° C before EVLP during the transportation to the recipient clinic, where in turn, in preparation for explantation, extracorporeal organ perfusion is performed.

The protocols differ in a number of respects: the left atrium is open in the Lund protocol as opposed to the Toronto protocol. The composition of the perfusate also differs: acellular solution Steen™ in the Toronto protocol and a cellular solution with a hematocrit of 15% in the Lund protocol. In the Lund protocol, the pressure in the pulmonary trunk is maintained at no more than 20 mm Hg, the perfusion begins at 15° C, the ventilation volume gradually increases to 100 mL/kg/min, and the respiratory rate reaches 15–20 per minute. In the Toronto protocol, the respiratory rate is 7 breaths per minute, positive end-expiratory pressure is 5 cm H_2O, the fraction of inspired oxygen (FiO_2) is 21%, pressure in the pulmonary trunk is 10–15 mm Hg, the pressure in the left atrium is 3–5 mm Hg, the perfusion begins at 25° C, and the flow rate of the perfusate

into the pulmonary artery is 7 mL/kg. The OCSTM protocol uses a cellular perfusion medium and an open left atrium [21].

During the first 30 minutes of perfusion, the perfusate flow was slowly increased to the target values of 2.0-2.5 L/min. The lungs are heated to 37° C, after which the ventilation begins with an inspiratory volume of 6 mL/kg/min, a positive end-expiratory pressure of 7 cm H_2O, and a respiratory rate of 10 breaths/min [20].

Table 1. Comparison of normothermic ex vivo lung perfusion protocols

Evaluated parameters	Protocol Toronto	Lund Protocol (Vivoline LS1)	OCS Protocol
Machine perfusion time	4–6 hours	2 hours	Transport time from donor to recipient
Perfusion solution	STEEN Solution	STEEN Solution + RBCS (Hct 14%)	OCS solution + RBCS (Hct 15–25%)
Flow characteristic	Centrifugal pump	Roller pump	Pulse pump
Target flow	40% CO	100% CO	2–2.5 L/ min
LA pressure (mmHg)	Atrium is closed, 3–5 mm H_2O	Atrium is open, 0	Atrium is open, 0
Ventilation	MLV device	MLV device	Bellows pump
Start temperature (°C)	25	15	32
Tidal volume (mL/kg)	7	5–7	6
Breaths per minute	7	20	10
FiO_2	0.21	0.50	0.21
PEEP (cm H_2O)	5	5	5

Notes: OCS, Organ Care System; RBCS, Red Blood Cells Suspension; Hct, hematocrit; FiO_2, fraction of inspired oxygen; CO, cardiac output; LA, left atrium; PEEP, positive end-expiratory pressure

Perfusion solution used in normothermic ex vivo lung perfusion

An integral component of the ex vivo lung perfusion technique is the perfusion solution. Most often, scientists mention the use of Steen's solution solution™ in clinical practice and experimental studies. It is used in the Lund and Toronto protocol. This is a balanced, buffered extracellular environment with optimal osmolarity, high dextran content, and antioxidant properties [13, 14, 22].

The main component of the solution is human albumin, which maintains optimal osmotic pressure. Dextran serves to protect the endothelium from excessive effect of leukocytes, thereby reducing endothelial damage, since leukocytes serve as the main source of reactive oxygen species during acute respiratory distress syndrome (ARDS) [23].

The solution also has physiological levels of electrolytes to stabilize the endothelium: a low level of potassium is required, since its high content depolarizes the membrane potential and increases the processes of peroxidation with the formation of reactive oxygen species, which has a negative effect on graft functioning [24]. In addition, the solution performs the functions of artificial blood, transporting oxygen, due to which metabolic processes take place in the transplant cells.

Many nowadays studies are focused on identifying the protective mechanisms of the solution, which are realized in the endothelium of the microvascular bed. The results of these studies demonstrate that the solution significantly reduces the ischemic reperfusion-induced endothelial inflammation and oxidative stress, as well as endothelial barrier dysfunction. Thus, data from a number of studies indicate that Steen Solution preserves pulmonary endothelial barrier function, promoting anti-inflammatory effects by regulating oxidative processes [14].

Surgical aspects of the process of explantation and connection of the graft to ex vivo normothermic lung perfusion

From a surgical point of view, the use of the EVLP technique occurs at the donor stage. The decision to evaluate the lungs by using EVLP, even if it was previously planned, is made after a diagnostic fiberoptic bronchoscopy, the analysis of the donor's arterial blood gas composition, and the assessment of the oxygenation index after brain death, thereby excluding pneumonia, the aspiration of gastric contents, and purulent endobronchitis. Visual inspection of donor lungs at explantation is also performed, with the exception of lungs from marginal donors, where evaluation of the graft is only possible using EVLP.

Technically, the removal of lungs from the donor's body is performed using the classical method, the only difference is that it is necessary to preserve a larger section of the trachea for subsequent convenient cannulation of the ex vivo pulmonary complex with an endotracheal tube [25]. If possible, at a cardioectomy previously performed by the cardiac surgical team, it is desirable to maintain the maximum length of the pulmonary trunk in order to comfortably cannulate the graft artery for perfusion. If it is not possible to maintain the sufficient length of the pulmonary trunk, a vascular graft is taken from the descending thoracic aorta or a section of the pericardium, after which an end-to-end anastomosis is performed with the pulmonary trunk. However, it is worth remembering that, guided by the perfusion protocol with a closed left atrium, the cardiac surgical team during cardioectomy needs to preserve a sufficient portion of the atrium walls for subsequent cannula fixation, and if the volume of the atrium walls is insufficient, the thoracic surgical team, as a rule, uses a pericardial patch [15].

Next, after pneumoplegia, anterograde (through the pulmonary trunk) and retrograde (through the pulmonary veins) perfusion with the

Perfadex® preservative solution, the lung graft is removed. At the next stage, the lungs are cooled with ice to +4ºC and transported to the recipient, where the EVLP is ready for launch [26]. The lungs in the clinic where the recipient is located are connected to a perfusion unit, and first the pulmonary trunk is cannulated. When using the closed left atrium technique, an anastomosis is applied to the platform of the left atrium with the ostia of the pulmonary veins between a special atrial cuff, which is the left atrium cannula connected to the chamber of the cardiotomy reservoir. The trachea is cannulated with a modified (shorter) endotracheal tube [27].

If the “open atrium technique” is used, the perfusate from the left atrium is collected in the organ chamber and collected by gravity into the cardiotomy reservoir through an opening at the bottom of the chamber. The temperature sensor is placed at the ostium of the pulmonary veins or the cone-shaped cuff of the left atrium cannula up to the Toronto protocol. After cannulation is completed, EVLP starts. The perfusion flow gradually increases, starting at 20% of cardiac output and subsequently reaching 100%. Ventilation starts when the required temperature of 30° C is reached. During EVLP, numerous biochemical and cytological tests of the perfusate are performed; even selective sampling from each pulmonary vein is possible to assess the lung function and separately the pulmonary lobes [28].

A comprehensive approach to the assessment of the lung graft, including a bronchoscopic control, the radiological, laboratory investigations, and histological evaluation allow making a final decision on the suitability of the lungs for explantation. It is also possible to resect part of the lungs by resorting to a lobectomy, which can be performed during the EVLP procedure per se [29]. The decision to reduce the size of the graft by resection may be made due to an inconsistency between the

size of the lungs and the size of the pleural cavity (if the grafts are too large compared to the recipient pleural cavity) or due to the presence of unresolved atelectasis as a result of the lung parenchyma damage[30].

At the end of ex vivo perfusion, it is possible to immediately begin implantation of the lungs into donor's body or to re-cool the organ ready for implantation.

Advantages of the normothermic ex vivo lung perfusion over the classical cold lung preservation

The main difference between the ex vivo lung perfusion technique from a classical organ storage at low temperatures is to maintain the lungs in a physiological state until transplantation. With classic cold storage of the lungs, oxidative processes and cell metabolism slow down, the need for oxygen and essential nutrients are reduced, which prevents damage to the organ.

The principle of normothermic lung perfusion is based on the creation of physiological storage conditions close to the human body. This allows the cells and tissues of the organ to remain metabolically active for several hours while waiting for the recipient [31–35]. This period of time allows for a long-term preservation of the lungs, and the functioning of the organ ensured by using perfusion and ventilation, provides for the continuity of metabolic processes. Maintaining physiological oncotic pressure of the perfusate and an optimal perfusion rate, recruiting lung tissue using various ventilation modes, as well as introducing drugs into the circuit altogether make it possible to evaluate and regenerate the lung grafts of unsatisfactory quality [32].

In interstitial pulmonary edema, the dehydration of the lung tissue is achieved by increasing the oncotic pressure of the perfusate. With the help of filters and membranes in the circuit, the cells and various

pathological structures are removed: microemboli in the vascular bed, neutrophils, leukocytes and pro-inflammatory cytokines formed as a result of the ischemia-reperfusion injury leading to a primary graft dysfunction in the early postoperative period [32, 33]. The possibility of the organ regeneration is being considered through recruiting various modes of ventilation, using an inhalation therapy, fibrobronchoscopy, introducing high doses of antibiotics and fibrinolytics into the circuit for the of lysis emboli in the branches of the pulmonary artery without a systemic effect on the body. A number of studies indicate a positive effect of gene therapy, the introduction of adenosine receptor agonists, and stem cell therapy on the graft condition [34]. The duration in practice does not exceed 12 hours [24]. It is likely that longer perfusion times (more than 12–24 hours) will soon become possible, which will allow comprehensively studying and regenerating damaged grafts with employing all possible therapeutic agents and methods, which effectiveness requires a longer time range [35]. Finally, EVLP represents a model for studying ways to precondition and protect the lung graft from subsequent inflammatory and immune insults after lung implantation into the recipient [40].

Possibility of treating isolated lungs using normothermic ex vivo lung perfusion

During perfusion, the donor lungs can undergo therapy and recover. A number of therapeutic approaches are currently being investigated.

I. Ruiz et al. In their study analyzed 210 clinical cases of lung transplantation with the following results: 197 patients discharged home and 13 deaths. Among 197 patients, 52% developed a bacterial or fungal infection. The study demonstrated that fibrobronchoscopy with

bronchoalveolar lavage of donor lungs during EVLP reduces the risk of developing such post-transplant complications. As a result of this treatment method, bacterial colonization in donor lungs was detected in only 25% of cases [36].

One of the studies stated that the incidence of pneumonia in donor lungs is directly related to the duration of mechanical ventilation in the donor's body [38]. The study demonstrated that a high-dose administration of broad-spectrum antibiotics to infected grafts during EVLP reduces bacterial load and inflammatory response. This allows transplantation of non-ideal lungs. The study examined 18 lung grafts with high bacterial colonization; they were subjected to normothermic EVLP with the introduction of high doses of broad-spectrum antibiotics, resulting in observing no bacterial growth in bronchoalveolar lavage washes of 13 pulmonary complexes, demonstrating a significant reduction in bacterial load [39, 40].

An integral phenomenon in the process of lung transplantation is the occurrence of ischemia-reperfusion injury, a pathological process that occurs when the blood flow is restored in the lung graft. The onset of perfusion plays a key role in the development of a primary graft dysfunction. The resumption of blood flow to the lungs in the recipient's body leads to endothelial dysfunction with the formation of reactive oxygen species: the aggregation processes intensify, pro-inflammatory cytokines and chemokines are produced in large quantities by the immune system and enter the bloodstream; cell death occurs through necrosis and apoptosis. M. Boffini et al. conducted a study of the EVLP effect on the development of graft dysfunction in the control group (ideal lungs) and the group in which previously rejected (not ideal) grafts were transplanted. The results revealed no statistical difference in the functioning of donor lungs, which indicates the protective properties of

the EVLP technique with respect to the graft dysfunction, which likelihood increases when using lungs from donors who died due to cardiac arrest, and non-ideal donors [40].

A number of studies have demonstrated the treatment for ischemia-reperfusion injury by administering steroids or N-acetylcysteine [41–46]. There is an option for direct cytokine removal using an adsorbent purification membrane or filter. A. Martens et al. studied the effect of corticosteroids on pulmonary ischemia. Each group included 6 pig lungs that were subjected to warm ischemia for 90 minutes; 500 mg of methylprednisolone was previously added in the study group, none of it in the control group. Lung function was assessed after 6 hours of EVLP: lung tissue elasticity was better preserved in the control group, but the study group had better parameters pertinent to lung tissue edema and density; and a reduced production of pro-inflammatory cytokines was also demonstrated. However, no significant difference in oxygenation was observed. The authors concluded that steroids have a positive effect on ischemic processes in donor lungs, and therefore recommend their use during EVLP [42]. In their study T. Kakishita et al. used an adsorption scavenging membrane to remove the excess cytokines from an injured donor lung. The heart-lung complex was extracted from pigs after electrically induced cardiac arrest and subjected to a 12-hour EVLP with an adsorbent membrane (n=5) and without an adsorbent membrane (n=6). In the control group perfusate (no membrane), tumor necrosis factor-α and interleukin-8 levels were increased 2 hours after perfusion. However, there was no significant difference in oxygenation, pulmonary vascular resistance, pulmonary edema formation, or myeloperoxidase activity between the two groups [43]. I. Iskender et al. in their study obtained favourable results when removing cytokines with filters. Donor pig lungs (n=5 in both groups) were stored for 24 hours at 4° C followed by 12

hours of EVLP. During perfusion in the experimental group, the solution was continuously passed through a CytoSorb filter device. The cytokine removal significantly reduced airway pressure and lung tissue dynamic compliance over the 12-hour perfusion period. Chest radiographs obtained at the end of perfusion showed a better pronounced pulmonary patterns in the control group. An electrolyte imbalance manifested by increased concentrations of hydrogen, potassium and calcium ions in the perfusate, was worse in the control group. Cytokine expression profiles, the tissue myeloperoxidase activity, and microscopic lung damage were significantly lower in the filtration study group. The authors claim that continuous filtration of perfusate through sorbent granules is effective and safe during a long-term EVLP, and a timely removal of cytokines reduces the development of pulmonary edema and normalizes electrolyte imbalance by suppressing the anaerobic glycolysis and activation of neutrophils in such conditions [44].

Regenerating lungs by gene therapy using an adenoviral vector encoding interleukin 10

In their study M. Cypel et al. described the effect of IL-10 therapy. The study was first conducted on pig lungs and then on human lungs, which were imperfect and not initially suitable for donation. During the study, IL -10 was administered regularly throughout the extended 12-hour EVLP. According to the study results in control grafts, the lung tissue after EVLP with IL-10 restored the alveolar structure and increased production of anti-inflammatory cytokines compared to those of the control group where gene therapy was not used [45].

Efficacy of treatment with fibrinolytics in a model of isolated lungs from donors who died as a result of cardiac arrest

I. Inci et al. tested the use of urokinase in the lung vessels of domestic pigs. Animals were distributed into three groups (n=5 in each group). The control group used heart-beating donors (HBDs). The lungs were washed, explanted, and stored in cold (4°C), low potassium dextran solution for 4 hours. Pigs in the other two study groups were non-heart-beating donors (NHBDs); their lungs were locally cooled for 1 hour in a closed chest after 3 hours of warm ischemia. Urokinase (100,000 IU) was added to the perfusate during reperfusion in one of the NHBD groups, forming the NHBD-UROK group. There was a statistically significant difference between the NHBD-UROK and NHBD groups. In the group with urokinase, a decrease in a pulmonary vascular resistance, an increase in the oxygenation index, and a decrease in pulmonary tissue edema were seen. Pulmonary vascular resistance did not differ between the HBD and NHBD-UROK groups. Thus, the authors indicate that urokinase administration during EVLP reduces pulmonary vascular resistance and improves oxygenation in a preclinical model of lungs from marginal donors [46]. I. Inci et al. by adding 100,000 IU of urokinase to the perfusate were also able to improve the performance of the donor lungs affected by acute pulmonary embolism. Such organs are usually discarded and are considered an absolute contraindication for transplantation. The result was a decrease in pulmonary vascular resistance and an increase in the elasticity of lung tissue in the experimental group [47].

However, in the study by A. Liersch-Nordqvist et al., alteplase infusion in a marginal donor lung model showed no statistically significant improvements in gas exchange, pulmonary vascular resistance, or lung compliance [48]. Twelve pigs were randomized into two groups

and all animals underwent electrical ventricular fibrillation and were then left intact for 1 hour postmortem. The removed lungs were washed with Perfadex solution, after which the organs were stored at 8°C for 4 hours. In the experimental group, alteplase was added to the Perfadex solution, but no alteplase in the control group. As a result, there were no significant differences between the groups in oxygenation index, compliance, or pulmonary vascular resistance at any stage of the study at different oxygen contents in the FiO_2 respiratory mixture.

Many studies mentioned the positive effect of the use of inhaled drugs containing β2-adrenergic receptors and adenosine A2A receptors during ventilation, before and during EVLP. T. Kondo et al. in their study demonstrated the administration of β2-adrenergic receptor and adenosine A2A receptor agonists into dog lungs. The hypothesis was that this inhalation therapy reduces the ischemia-reperfusion injury. Beagles that died from a cardiac arrest were left at room temperature for 210 minutes, then the lungs were removed and subjected to EVLP for 240 minutes. The animals were divided into two groups: an experimental group (received an aerosol β2-adrenergic receptor agonist, 350 μg of procaterol, 20 minutes after the start of EVLP; n=7) and a control group (received an aerosol 0.9% saline solution; n=6). Physiological parameters were assessed during EVLP. As a result, the experimental group had significantly lower peak airway pressure and pulmonary artery pressure than the control group. The dynamic lung compliance was higher, pulmonary vascular resistance was lower, and pulmonary edema was lower in the experimental group than in the control group. Focusing on the levels of cyclic adenosine monophosphate (cAMP) and total adenosine nucleotide in lung tissue after EVLP, the values were higher in the experimental group than in the control group. The expression of the cystic fibrosis transmembrane conductance regulator (CFTR) gene was

also increased in the procaterol group. The conclusion of the study was that inhalation of a β2-adrenergic receptor agonist during EVLP reduced an acute lung injury and improved the lung function [49].

D.P. Mulloy et al. reported an improvement in the oxygenation index (PO_2:FiO_2) and oxygen saturation of donor blood in an animal model of porcine lungs as a result of adding ATL-1223 adenosine A2A receptor agonist to the perfusate [50]. The pigs had been subjected to hypoxic cardiac arrest followed by 60 minutes of warm ischemia. The lungs were removed and washed with Perfadex® solution at 4°C. Three groups (n=5 in each group) were stratified according to the preservation method: a gold standard of cold static preservation (CSP: 4 hours at 4°C), immediate EVLP (I-EVLP: 4 hours of EVLP at 37°C) and delayed EVLP (D-EVLP: 4 hours cold storage and 4 hours EVLP). The EVLP groups received Steen solution during perfusion with the addition of heparin, methylprednisolone, cefazolin, and adenosine A2A receptor agonist. Then the lungs underwent implantation and 4 hours of reperfusion in the recipient before assessing the graft for the ischemia-reperfusion injury. As a result, the reported oxygenation index before euthanasia did not differ between groups. Oxygen saturation after transplantation was significantly higher in the D-EVLP group compared to the I-EVLP or CSP groups. The values of airway pressure, pulmonary artery pressure, IL-8, IL-1β, and TNF-α expression were significantly lower in the D-EVLP group than in two other groups. It is important to note that oxygenation after transplantation was below acceptable clinical levels only in the D-EVLP lung groups.

Efficacy of hygiene fiberoptic bronchoscopy for the clearance of aspirated gastric contents

T. Khalifé- Hocquemiller et al. conducted a study on the effect of hygiene fiberoptic bronchoscopy during EVLP to improve donor lung function. Twenty pigs were randomly assigned to four groups. In the first and second groups, an aspiration injury to the lungs was induced by injecting 1 ml/kg of gastric juice into the left lower lobe (LLL) under bronchoscopic guidance. After 24 hours, the lungs of the first group (LI group) were examined, the lungs of the second group were reconditioned for 4 hours using the EVLP technique (LI-EVLP group), after which they were also examined. The lungs of the third and fourth groups were not subjected to gastric aspiration, but the lungs of the fourth group underwent EVLP for 4 hours followed by their evaluation. The following parameters were recorded: the changes in anatomy, hemodynamics, gas exchange, the airway ventilation capacity. The numbers of bacteria in bronchoalveolar lavage, in animal blood and perfusate were determined. The LLL condition was assessed considering the pulmonary tissue edema; histological changes (using a blinded semi-quantitative assessment of severity); myeloperoxidase activity; apoptotic cell death; levels of IL-1, IL-6, IL-8, IL-10, and TNF-α. Animals from the aspiration groups, compared with the non-exposed groups, had irreversible atelectasis, a high level of infection, a higher percentage of neutrophils in the BAL fluid, a low oxygenation index, high IL-1 and levels of S135 and IL-8, a high percentage of apoptotic cells, and worse parameter of histology examination severity. In the LI-EVLP group, these altered values did not improve when compared with the non-aspiration groups. Therefore, at present, the lungs affected by the aspiration of gastric contents are not considered for transplantation; and the classical EVLP

technique with hygiene fiberoptic bronchoscopy is ineffective in this situation [51].

However, T. Khalifé-Hocquemiller and colleagues conducted a repeat study with the introduction of exogenous surfactant through the bronchoscope channel during EVLP with the aim of possibly improving the function of the lungs subjected to gastric aspiration [51].

The lungs of pigs of the first group were examined 24 hours after lung tissue injury induced with the gastric juice. And the lungs of the second group underwent EVLP (for 4 hours) with the hygiene FBS with a surfactant performed immediately before the EVLP; the lungs of animals in the third group were examined after 24 hours of the lung hygiene flush with sterile 0.9% saline followed by EVLP. The assessment of the lungs was performed by the authors according to the method described above. The animals aspirated without surfactant had irreversible atelectasis, high rates of pulmonary infection, and a high percentage of neutrophils in BAL fluid, a lower oxygenation index, high levels of IL-1 and IL-8, and a higher percentage of apoptotic cells. It is worth saying that after EVLP the results did not improve, but the hygiene bronchoscopy performed with the introduction of surfactant before perfusion normalized the oxygenation index, reduced the pulmonary vascular resistance and the percentage of apoptotic cells. The performance of the experimental group with aspiration approached the group of animals where the EVLP was used without the aspiration of gastric contents.

The recent literature reposts describe a number of gas mixtures that have anti-inflammatory, anti-apoptotic and antioxidant properties [51]. S. Haam et al. investigated the effect of hydrogen on the lung function during ex vivo perfusion. Ten pigs were randomized into control (n=5) and experimental groups (n=5). After electrical fibrillation, the lungs were kept in the donor's body for 1 hour for thermal ischemic injury.

Next, the organs underwent a 4-hour EVLP. Ventilation was carried out with the atmospheric air in the control group, and the air with the addition of 2% hydrogen gas in the experimental group. The oxygenation index of the lung group receiving the experimental gas mixture was higher than that of the control group, but the difference was not statistically significant. The pulmonary vascular resistance, peak airway pressure, and the pulmonary edema severity were recorded shoeing a marked decrease in the hydrogen group. Compared to the control group, the experimental group demonstrated a statistically significant decrease in the expression of IL-1β, IL-6, IL-8 and TNF-α. S. Haam et al. claimed that the use of hydrogen gas in the respiratory mixture during ventilation improved the lung function, and subsequently this could also be used in clinical practice [52–58].

Other gases – carbon monoxide, nitric oxide, and hydrogen sulfide – showed favourable results, but xenon and argon did not improve the lung function. The effect of carbon monoxide was studied by B. Dong et al. an hour after the death of the rats: the authors ventilated the lungs of the animals for another hour with 60% oxygen in the control group (n=6) and 500 ppm CO in 60% oxygen in the experimental group (n=6). The lung ventilation with carbon monoxide resulted in a decrease in pulmonary edema after perfusion. The results reported in the study demonstrated better oxygenation, high levels of tissue cyclic guanosine monophosphate (cGMP), high expression of heme oxidase-1, p38 phosphorylation, a decreased phosphorylation of N-terminal kinase and a decreased expression of the messenger RNA, interleukin-6, interleukin-1β in the experimental group. Therefore, the authors came to the conclusion that the introduction of carbon monoxide into the breathing circuit of the donor and the lungs of the marginal donor reduces ischemia-reperfusion injury to the lungs [53].

The same group of researchers studied the effects of nitric oxide (NO) on a lung graft. The Lungs of cardiac arrest rats were ventilated with NO during EVLP after warm ischemia. One hour after the rats died, their lungs were ventilated for an hour with either 60% O_2, or 60% O_2 with 40 ppm NO. With NO ventilation, the authors noted a decrease in pulmonary edema, an increase in the oxygenation index, a decrease in the pulmonary vascular resistance, an increase in the cGMP level in lung tissue, and the maintenance of endothelial NOS (eNOS), a decreased growth of tumor necrosis factor-alpha (TNF-α). The ventilation with nitrogen monoxide had no effect on MAP-kinase or NF-κB activation [54].

T.J. George et al. studied the effect of hydrogen sulfide on the lung function. Rabbits were ventilated for 2 hours before explantation of the heart-lung complex. The experimental group (n=5) was ventilated with atmospheric air (21% O_2) with the addition of 150 ppm H_2 S, while the control group (n=5) was ventilated only with the atmospheric air. After removal of the pulmonary complexes, they were stored in a refrigerated low-potassium dextran solution for 18 hours. After the cold storage, the organ was perfused with donated rabbit blood in an EVLP machine. During perfusion, the lungs ventilated with hydrogen sulfide showed better oxygenation, better elasticity, a decrease in the pulmonary artery pressure, a decrease in reactive oxygen species, which in turn had a favourable effect on the lung graft functioning. In addition, before the perfusion, the lungs from the experimental group showed a high preservation and the activity of mitochondrial cytochrome-c-oxidase. Thus, it is possible to note an improvement in the functioning of the graft after perfusion of lungs ventilated with H_2 S [55].

A. Martens et al. in their study showed ex vivo lung perfusion with xenon (Xe) and argon (Ar) ventilation to evaluate the effects of gases on donor lungs. Domestic pigs were divided into four groups (n=5 in each

group). In the negative control group, the lungs were immediately flushed, whereas in the positive control group (PC) and experimental (Ar, Xe) groups, the lungs were flushed after a warm ischemic interval of 2 hours in the donor body. All grafts were subjected to normothermic EVLP for 6 hours. The lungs were ventilated with atmospheric air of 70%N_2/30% O_2 in the control groups, and of 70% Ar/30% O_2 and 70% Xe/30% O_2 in the experimental groups. As a result, there was a significant difference between the negative and positive control groups, which were compared with regard to the timing of irrigation. In the positive control group, a decrease in the pulmonary vascular resistance, a decrease in peak airway pressure, PaO_2/FiO_2, a decrease in pulmonary tissue edema, and a more preserved histological structure of the lung tissue were noted. But the main result of the study implied no significant differences found between the experimental groups where ventilation with xenon and argon was carried out, that is, the ventilation with argon or xenon did not improve the function of lung grafts [56].

Stem cell therapy is the latest and most promising method in lung transplantation. Mesenchymal stem cells and multipotent adult progenitor cells can regenerate damaged lung tissue and secrete paracrine factors that regulate epithelial and endothelial permeability, thereby enhancing alveolar fluid clearance and attenuating the immune response in damaged lungs. S. Gennai with the working group studied the effect of human mesenchymal stem cells (hMSCs) on restoring the function of the lungs unsuitable for transplantation. Using EVLP and microvesicles derived from hMSCs, scientists were able to increase alveolar fluid clearance in a dose-dependent manner, as well as improve airway conductivity and pulmonary hemodynamics. Microvesicles derived from normal human lung fibroblasts as a control had no effect. Co-administration of microvesicles with anti-CD44 antibody attenuated these effects,

suggesting a key role for the CD44 receptor in mediating the microvesicle effect in damaged cells. Thus, microvesicles obtained from hMSCs were as effective in the rehabilitation of marginal donor lungs as the mesenchymal stem cells of the donor [57]. S. La Francesca et al. studied the effect of multipotent adult progenitor cells (MAPCs) on the graft dysfunction. Four donor lungs not used for transplantation were subjected to 8 hours of cold storage at 4°C. After warming for 30 minutes, allogeneic MAPCs (1×10^7 MAPCs/lung) were introduced into the LLL using a bronchoscope, and in the control group, 0.9% sterile saline solution was injected into the right lower lobe (RLL). LND consistently demonstrated a significant reduction in the lung tissue inflammation histologically, and also demonstrated a reduction in the signs of inflammation at cytological examination of bronchoalveolar lavage washes when compared with RLL lung tissue treated with saline. The authors suggested that the use of MAPCs during the processing of donor lungs may reduce the markers of lung injury caused by cold ischemia [58].

Conclusion

Lung perfusion technique ex vivo is a promising and effective procedure for the assessment, reconditioning, and regeneration of the lungs during transplantation. Constantly improving technical aspects of extracorporeal normothermic lung perfusion and rapidly developing promising therapeutic options make it possible to increase the number of lungs suitable for transplantation, thereby significantly reducing the number of post-transplant complications. Together, this makes it possible to reduce mortality on the waiting list and improve the results of transplantation of such a complex and important organ as the lungs.

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