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NORMOTHERMIC *EX VIVO* PERFUSION OF ISOLATED LUNGS IN AN EXPERIMENT USING A RUSSIAN-MADE PERFUSION SYSTEM

S.V. Gautier[1, 2], O.M. Tsirulnikova[1, 2], I.V. Pashkov[1], D.O. Oleshkevich[1], I.A. Filatov[5], V.K. Bogdanov[5], D.M. Bondarenko[1], N.P. Mozheiko[1], A.A. Karpov[3], N.S. Bunenkov[3, 4], N.V. Grudinin[1]

[1] Shumakov National Medical Research Center of Transplantology and Artificial Organs, Moscow, Russian Federation
[2] Sechenov University, Moscow, Russian Federation
[3] Almazov National Medical Research Centre, St. Petersburg, Russian Federation
[4] Pavlov University, St. Petersburg, Russian Federation
[5] Biosoft-M, Moscow, Russian Federation

According to global health statistics, respiratory diseases, together with infectious complications and hereditary lung diseases, rank as the third leading cause of death. Today, lung transplantation (LTx) is a well-recognized modality of treatment for end-stage chronic lung disease. However, the number of LTx surgeries performed is much lower than other solid organs. This is due to the high requirements for the potential donor and characteristics of the lung graft, reflecting the efficiency of gas exchange function. Non-compliance with the selection criteria leads to deselection of donors, which, according to various estimates, occurs in 80–85% of cases. One of the ways to increase the number of lung transplant surgeries is to restore them to the level of optimal gas exchange parameters, which can be achieved and objectively assessed during normothermic ex vivo lung perfusion (EVLP). EVLP is becoming increasingly common at leading transplantation centers in Europe and North America. This has significantly increased the number of transplant surgeries as a result of using lungs procured from suboptimal donors and rehabilitated via EVLP. In our pilot study, the developed Russian-made mechanical circulatory support system showed that performing normothermic EVLP for isolated lungs under experimental conditions is feasible. Basic and optimized perfusion protocols have fully shown that they are reliable and efficient.

Keywords: lung transplantation, donation, ex-vivo perfusion.

INTRODUCTION

Respiratory diseases, including those with complications, as well as hereditary lung diseases, are a socially significant problem all over the world as they lead to end-stage pulmonary disease [1]. Lung transplantation (LTx) is currently the most effective radical way of treating patients with severe respiratory disease resulting from lung diseases of various etiologies [1–3]. However, the use of LTx is limited by the extremely low percentage of donor organs suitable for transplantation [4]. Donor lungs are extremely susceptible to complications of brain death. It is accompanied by a high incidence of non-specific changes, such as neurogenic edema, which makes lungs unsuitable for transplantation and, as a consequence, leads to deselection of organs from LTx [5]. A combination of factors affecting the quality of a lung graft decreases the number of suitable donor lungs to 15–20%, while liver and kidney transplants, on average, are used in 69% and 90% of cases, respectively [3, 6, 7, 8]. The shortage of effective lung donors increases the 1- and 2-year waitlist mortality rates by 20–40% [9].

One of the ways to solve the problem of donor organ shortage is the use of expanded criteria donors (ECDs) and the use of ex-vivo graft perfusion in order to restore the functional activity (rehabilitation) of the donor organ [8].

The relatively recent beginning of application of the above method in world practice, as well as the limited number of studies, make urgent and necessary the development of this direction in national programs in order to improve the effectiveness of transplantation care. This necessitates experimental work on the development and improvement of the ex vivo lung perfusion (EVLP) technique.

The objective of this study is to perform and evaluate the effectiveness of a pilot isolated closed-circuit ex vivo perfusion using a proprietary extracorporeal perfusion complex under experimental conditions.

Corresponding author: Nikita Grudinin. Addreess: 1, Shchukinskaya str., Moscow, 123182, Russian Federation. Phone: (903) 805-63-58. E-mail: Zbignev.religa@mail.ru

MATERIALS AND METHODS

Isolated lungs obtained from a 45 kg Romanov sheep were used in the experimental study. The experimental work program was approved by the Biosafety and Bioethics Committee. The work was carried out in compliance with the rules of the European Convention for the treatment of laboratory animals and the 2010/63/EU Directive [14, 15].

The experiment included stages of anesthesia of the experimental animal, lung explantation, static hypothermic storage, initiation of ex vivo lung perfusion with fixation of main parameters.

Donor anesthesia stage

On the day of the experiment, 60 minutes before surgery, the animals were sedated in the pen with a zolazepam solution at 15 mg/kg dose. During sedation, the animal was taken to the operating room, the operating field was shaved, which corresponded to the anatomical landmarks between the cricoid on the neck of the experimental animal and the anterior projection of the Tuffier's line, and vascular access points were determined. The external ear vein was catheterized with a 20 G catheter.

The animal was positioned on the operating table in the supine position. Under aseptic conditions, a 7 Fr double-lumen central venous catheter was placed into the left external jugular vein at the level of the cricoid.

Then, under aseptic conditions, the common carotid artery was catheterized with a 5 Fr catheter for invasive blood pressure (BP) monitoring. Monitoring was done via the Efficia CM Philips™ monitoring system.

Premedication was performed under invasive monitoring of BP, central venous pressure (CVP) and ECG: lornoxicam 8 mg, metoclopramide 10 mg, chloropyramine 20 mg. Injection anesthesia included intravenous atropine 1 mg, methylprednisolone 500 mg, and zolazepam 10 mg/kg.

Tracheal intubation was performed by direct laryngoscopy. Mechanical ventilation was performed using an anaesthetic machine in volume control mode at 8–10 mL/kg, peak inspiratory pressure did not exceed 25 cmH_2O, positive end-expiratory pressure (PEEP) did not exceed 5 cmH_2O, respiratory rate (RR) was 25 breaths per minute. Anaesthetic depth was controlled using an isoflurane vaporizer.

Optimal anesthesia for explantation surgery was achieved at 2.52.5 vaporizer level – 3% vol. Optimal hemodynamic parameters were: BP 110/80 mmHg, SpO_2 99–100, heart rate 90 bpm.

Donor lung procurement procedure

Surgical access was achieved through median sternotomy. The pericardium was opened longitudinally, and the aorta and pulmonary artery were divided bluntly. After administering sodium heparin at 300 units/kg dose, the aorta and the pulmonary artery were sutured. The aorta was cannulated with a 7 Fr catheter to collect donor blood. The pulmonary artery was cannulated with a 20 Fr straight cannula. The first step was autologous blood harvesting in a hemocontainer with citrate preservative. After blood harvesting was completed, prostaglandin E1 solution (alprostadil) 20 μg was injected into the pulmonary artery. In order to decompress the left heart, the left auricle was incised longitudinally. Then Celsior™ solution (Ganzyme, France) was injected through the pulmonary artery at 4 °C temperature in 2 liters. Upon completion of the perfusion of the preservative solution, we proceeded to lung explantation. For the convenience of procurement, the lungs were taken together with the heart as a single complex, and a machine suture was applied to the trachea 5–6 cm from the bifurcation. On completion of the explantation, the lungs were placed in a sterile bag, followed by static hypothermic preservation in a thermocontainer for 2 hours.

Assembly of the EVLP perfusion circuit

In the experiment, the perfusion circuit had a closed structure. The circuit consisted of a cardiotomy reservoir and membrane oxygenator. A hydrocirculatory heat exchanger element, a deoxygenating mixture consisting of N_2 86%, CO_2 8%, O_2 6%, and an oxygen-air mixture were connected to the oxygenator. A centrifugal blood pump "Biosoft-M LLC" with a hydrophilic head was installed in the trunk system between the cardiotomy reservoir and oxygenator. The line after the oxygenator was connected to a cannula installed in the pulmonary artery. Outflow into the oxygenator was performed actively through a cannula placed in the left atrium. The pressure in the trunk system was measured by placing three invasive sensors: the first was placed after the oxygenator to measure pressure in the proximal perfusion circuit, the second was placed directly in the pulmonary artery cannula to measure perfusion pressure in the pulmonary artery, the third sensor measured pressure in the cannula placed in the left atrium. The graft was positioned in a sterile container communicating with the cardiotomy reservoir. The graft was perfused through the pulmonary artery; perfusate was drained actively through a cannula inserted into the left atrium through the left ventricular wall. The schematic design of the circuit is shown in Fig. 1.

We used our own perfusion solution based on human albumin as perfusate. The perfusate was 1.5 liters in volume. Erythrocyte mass was prepared by centrifugation of whole deleukocyted blood for 15 minutes at 3,500 rpm. Meropenem 1000 mg, methylprednisolone 1000 mg, short-acting insulin 4 U, 40% glucose solution 5 mL were all added to the perfusate. Target hematocrit level was 15%.

The experimental protocol was developed on the basis of the ex vivo lung perfusion protocol proposed by M. Cypel et al. (Toronto, Canada) in 2009 [20]. Ex vivo perfusion lasted for 360 minutes.

EVLP initiation

The initial perfusion temperature was 20 °C, the target pulmonary artery pressure should not exceed 15 mmHg. The perfusion rate was adjusted based on pulmonary artery and left atrial pressures at the start of perfusion and was 150–200 ml/min. Left atrial pressure was regulated by the cardiotomy reservoir positioning height, the optimal range was 3–5 mm Hg. Gas-air mixture flow, where FiO_2 <0.5, was set corresponding to the target minimum pO_2 values >100 mmHg. A deoxygenating mixture with a 1 : 1 flow rate to the perfusion rate was required to achieve a pCO_2 of 40 to 50 mmHg.

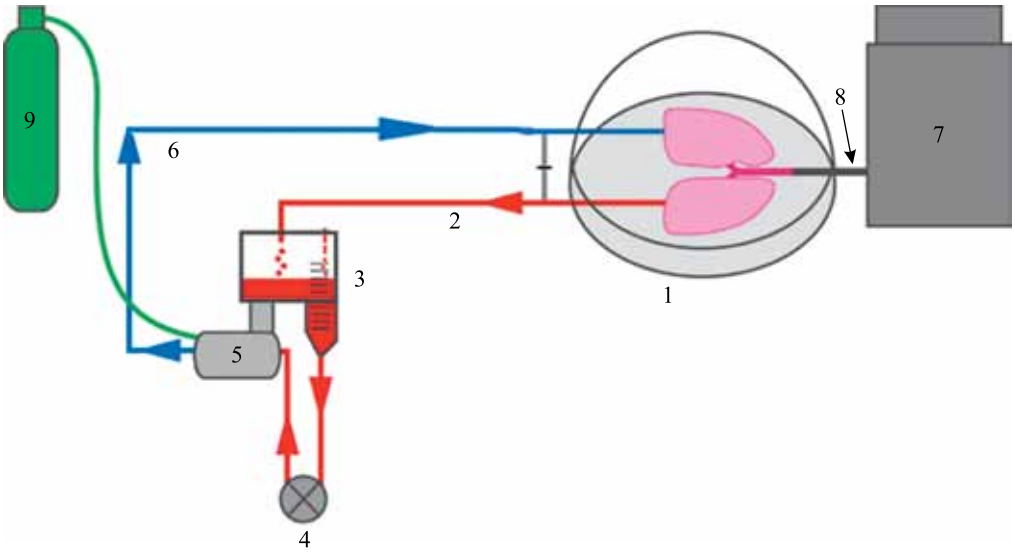

Fig. 1. Schematic layout of perfusion circuit. 1, Open organ chamber; 2, left atrial line; 3, cardiotomy reservoir; 4, centrifugal pump; 5, oxygenator; 6, venous line to the pulmonary artery; 7, ventilator; 8, air line; 9, balloon with deoxygenating mixture connected to oxygenator

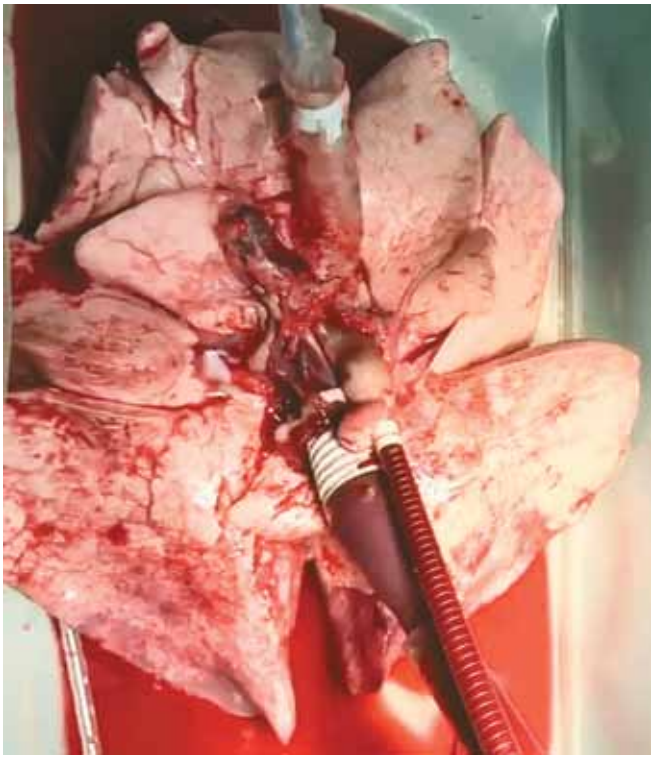

Fig. 2. View of the lung graft during perfusion

The ionic and gas composition of the perfusion solution was monitored using an ABL 800 gas analyzer. Target perfusion volume was 40% of the calculated cardiac output. When all parameters were stabilized, perfusion rate was increased to 800 mL/min for 20 minutes, the perfusate was warmed to 32 °C.

Upon reaching the target temperature of 34 °C, mechanical ventilation was initiated. The ventilation parameters were composed of an inspiratory volume of 7 mL/kg, PEEP 5 cmH_2O, RR 10 breaths per minute. Fraction of inspired oxygen was FiO_2 <0.5. The gas composition of the perfusate was monitored. Over the next 20 minutes, the target temperature of 37 °C was reached, the volumetric perfusion rate increased by 40% of cardiac output (based on 70 ml/kg of experimental animal weight) to 1200 mL/min. Perfusion lasted for 360 minutes. General view of the lung graft at the time of perfusion is shown in Fig. 2.

Assessment of graft function during EVLP procedure

After warming the graft to 37 °C and stabilizing the gas and ionic composition parameters of the perfus-

ate, with an oxygen fraction at 50% inspiration (FiO_2 = 0.50), we performed instrumental, manual and laboratory evaluations. The surgeon palpated and visually assessed the homogeneity of lung parenchyma and the absence of infiltrative changes in it, and atelectasis was resolved. Throughout the entire period of perfusion, pulmonary artery and left atrium pressures were measured directly. The data were displayed on the monitor in real time and recorded every 30 minutes. The main parameters were pulmonary artery pressure (PAP, mm Hg) and pulmonary vascular resistance (PVR, Wood units/m^2), which was calculated using the formula.

$$PVR = \frac{PAP - LAP}{PaF},$$

were PVR is pulmonary vascular resistance, PAP – pulmonary artery pressure (mmHg), LAP – left atrial pressure (mmHg), PaF – perfusion volume (L/min).

PVR was expressed in Wood units; to calculate in dyn·s/cm^5 units, the result of the equation was multiplied by 80.

In order to analyze pulmonary oxygenation function, two blood portions were taken from the venous cannula (pulmonary artery) and arterial cannula (left atrium). The samples were analyzed on an ABL 800 blood gas analyzer (Radiometer Medical ApS, Denmark). We used the PaO_2/FiO_2 equation (ratio of arterial oxygen partial pressure to fraction of inspired oxygen) to calculate oxygenation index. Obtained data were plotted against time points corresponding to the graft assessment periods.

Upon completion of perfusion, lung parenchyma fragments were fixed in 10% neutral buffered formalin solution (pH 7.4) for at least 24 hours. Isopropyl alcohol and petroleum ether were used to fix the material into paraffin blocks. 5 μm-thick paraffin sections were stained with hematoxylin and eosin. Microscopic analysis was performed using a light microscope with a 10× eyepiece lens and objective lenses 4, 10, 40, and 100. Photographs were taken with a digital camera.

The obtained sections were examined by a pathologist for vascular thrombosis, hemorrhages, interstitial and alveolar edema, and cellular infiltration.

RESULTS

The PaO_2/FiO_2 ratio prior to donor lung explantation was 240 mmHg. Throughout the entire ex vivo perfusion procedure, the respiratory index had positive growth dynamics. After 360 minutes of perfusion, the oxygenation index was 430 mm Hg, which is a good indicator of lung respiratory function recovery (Fig. 3).

Pulmonary vascular resistance

Throughout the ex vivo perfusion procedure, PVR witnessed a steady decrease. At the beginning, PVR was 800 dyn·s/cm^5; however, at the end of perfusion, PVR was 320 dyn·s/cm^5; the dynamics of PVR changes are shown in Fig. 4.

Dynamic compliance

Changes in the dynamic compliance index from 20 to 46 mL/cmH_2O at the end of perfusion indicates its adequacy and is an indirect criterion for the absence of pulmonary parenchymal edema. It is this index that objectively represents lung recruitability and reflects airiness and extensibility of pulmonary parenchyma. These changes in the parameters of dynamic compliance of the lung transplant are not an unambiguous criterion of donor organ's suitability, but they indicate preservation of lung parenchyma (Fig. 5).

Morphological study data

Histological examination of lung samples after perfusion showed structural integrity of the tissue and no signs of edema. In most sections, the alveoli were well inflated. The pulmonary alveolus and the peribronchovascular interstitium were slightly thickened (Fig. 6).

The lung fragments had a preserved structure. The lung parenchyma was not pathologically altered in all

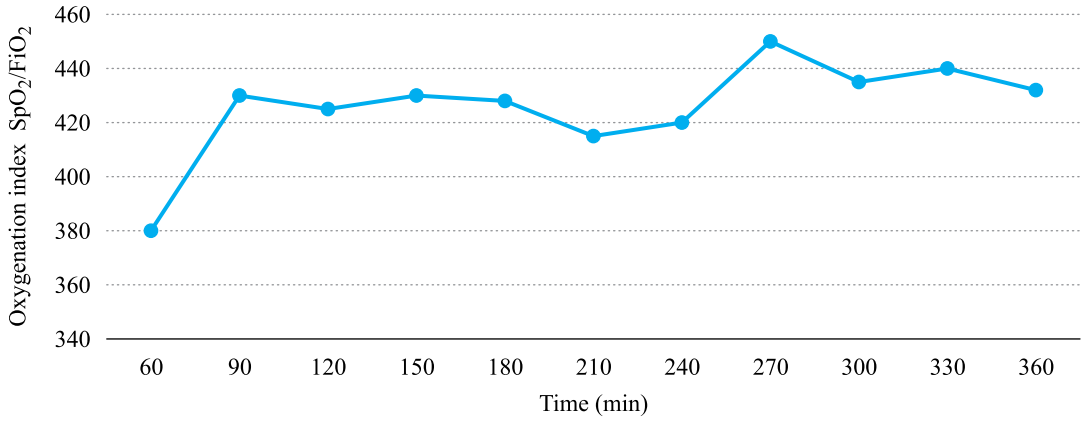

Fig. 3. Oxygenation index dynamics

groups of specimens; well-inflated alveoli were noted in most sections. Microatelectasis was heterogeneously distributed in both groups and occurred only in separate sections. The pulmonary alveolus and the peribronchovascular interstitium were slightly thickened.

DISCUSSION

Pathophysiological processes resulting from brain death cause intracorporeal organ damage, making it largely difficult to objectively assess the true functional

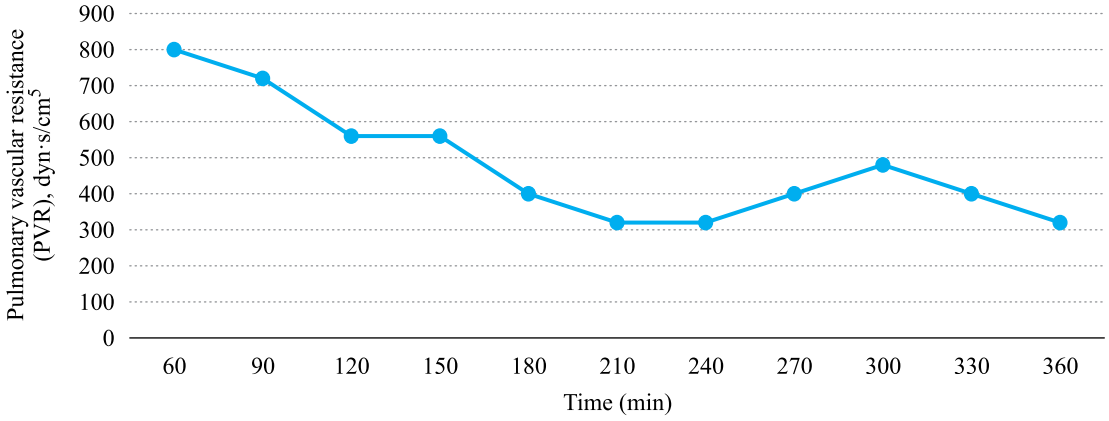

Fig. 4. Pulmonary vascular resistance dynamics

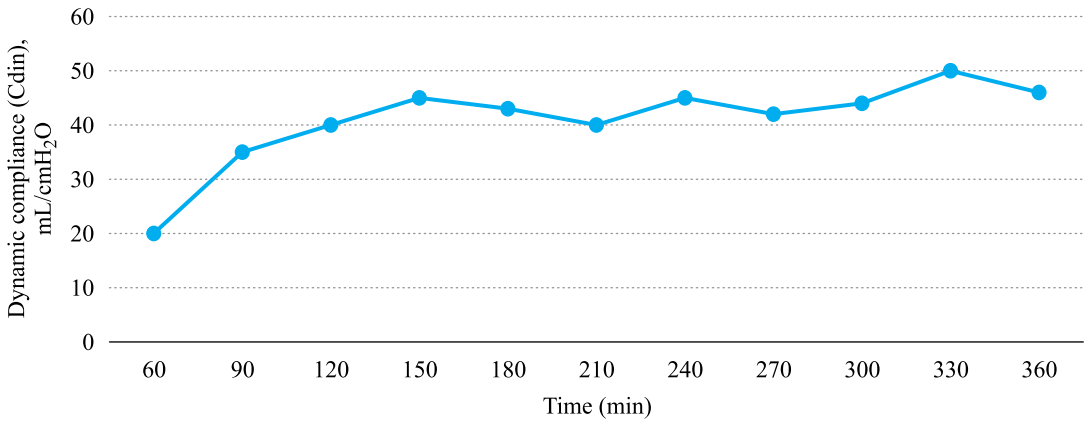

Fig. 5. Dynamic compliance dynamics

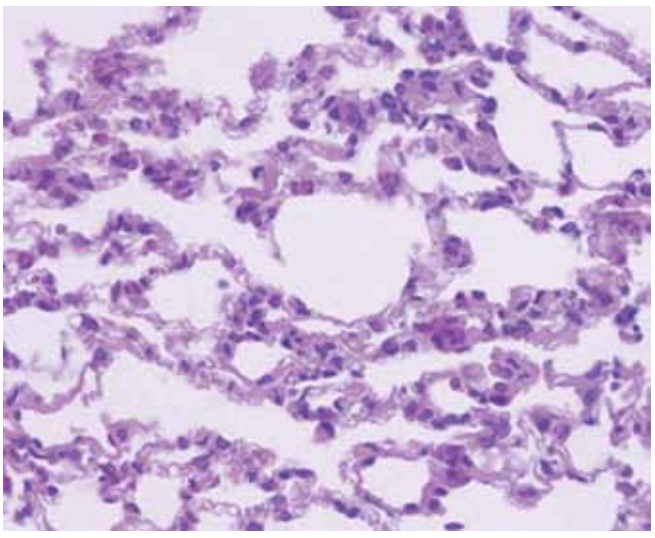

Fig. 6. Histological view of a lung specimen taken at the end of perfusion

state of donor lungs. Some reasons for organ deselection before transplantation (pulmonary edema, low gas exchange rates, presence of a large amount of purulent bronchial secretion, etc.) can be corrected and reassessed using extracorporeal lung perfusion techniques [15, 17, 18].

In order to restore and evaluate compromised lungs obtained from suboptimal donors, normothermic EVLP is used in clinical practice of the leading world transplant centers [15]. This technique allows mitigating the effects of damaging pathophysiological factors of the donor on the graft, rehabilitating and reassessing the lung graft [5, 16–20].

The emergence of EVLP has opened new horizons in lung transplantation worldwide. In 2006, a research team of Stig Steen et al. (Lund University Hospital, Sweden) reported the first results of successful transplantation

of one lung after EVLP procedure [23]. In 2009, Cypel et al. (Toronto, Canada) presented their own protocol, which later became the most widespread due to the best results and due to the possibility of a prolonged perfusion, lasting for more than 12 hours [6]. The EVLP procedure allowed for major expansion of the donor lung pool, thereby increasing the number of transplantations. The study was based on a perfusion protocol developed by Cypel et al. for lung evaluation and rehabilitation. The study was able to demonstrate the effectiveness of EVLP procedure on the first compact domestically manufactured circulatory assist device in an experiment on a ram model. The experimental conditions were as close to clinical practice as possible.

As of today, there are no registered normothermic organ perfusion devices in the Russian Federation. Developing a domestic perfusion equipment is a priority amid the current donor lung shortage. The blood circulation auxiliary device developed by Biosoft-M is small in size, has high functionality and ease of operation compared with its foreign counterparts. The closed-type perfusion protocol we have chosen is optimal and promising, allowing for prolonged perfusion of donor lungs. Prolonged perfusion gives a better chance of recovery of the function of expanded criteria donor lungs [6, 21]. According to the Toronto protocol, the time required to adequately assess the recovery of lung transplant function is from 4 to 12 hours [6, 22]. In the experiment, we were able to achieve a satisfactory oxygenation index, which was 430 mmHg at the end of perfusion, and PVR reduction, which indicates adequate perfusion. The absence of pulmonary parenchymal edema and pathological changes, according to histological study results, indicates the effectiveness and safety of the technique [19, 20].

CONCLUSION

Normothermic EVLP can be performed using a device developed for auxiliary blood circulation and adapted for normothermic EVLP purposes. The presented protocol was shown to be effective and has good prospects for further use and improvement.

The authors declare no conflict of interest.

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