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DEVELOPMENT OF AN EXPERIMENTAL TECHNIQUE FOR ORTHOTOPIC LEFT LUNG TRANSPLANTATION IN A RABBIT MODEL

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Objective: to develop, master and evaluate the efficiency of an isolated lung transplantation (LT) technique on a rabbit animal model using Perfadex Plus® solution for cold static storage. **Materials and methods.** Scottish Giant rabbits (n = 20) were used in this study and divided into two groups: donors and recipients. Donor lungs were preserved with Perfadex Plus® solution and stored for 6 hours at 4 °C. Recipient animals underwent unilateral orthotopic left LT. The postoperative follow-up period was 24 hours. Laboratory and instrumental control with assessment of blood gas composition, lactate level, ventilation parameters, and central hemodynamic parameters, was performed during the follow-up. Chest X-ray in direct projection was performed twice, and at the end of follow-up, material was taken for histologic examination. **Results.** We obtained a high oxygenation index in the post-transplant period (>350 at $p < 0.023$), as well as physiological indicators of lactate (3 ± 0.3 mmol/L at $p < 0.002$) and peak inspiratory pressure (15 ± 1 cmH_2O, $p < 0.001$). Radiological examination showed no radiological signs of severe primary graft dysfunction in all cases (mean RALE score 1), which was confirmed by histological studies. **Conclusion.** Left LT in rabbits is possible, the LT technique on a biological rabbit model using Perfadex Plus® solution is valid and efficient with the achievement of satisfactory gas exchange, ventilation and metabolism parameters.

Keywords: transplantology, lung transplantation, rabbit lung transplantation model, preservative solution, cold static storage.

INTRODUCTION

Currently, there are several unsolved issues about transplantation and perfusion of donor lungs. Some of them are ischemia-reperfusion injury (IRI), lack of highly effective antioxidant protection, and lack of a cheap and easily reproducible experimental model for scientific research, whose results can be extrapolated to humans [1–3]. Animals are the preferred experimental model for studying the respiratory system similar to that of humans. The choice of an optimal animal model is very important and should have the ability of the respiratory system to respond pathophysiologically to stressful conditions and the response should be close to how the human respiratory system responds to similar triggers. Consequently, the animal model should mimic human lung conditions in response to injury and surgical intervention at the clinical, biological, physiological and pathological levels [4–6].

Undoubtedly, an experimental model of large animals is preferable for simulating the pathophysiology of LT because their structural and functional features and anatomical characteristics are close to those of humans. However, the complexity of maintenance, high cost of a single study and species specificity of each large animal – dog, pig and sheep – dictate the need to search for an optimal animal. Based on international reports, rabbits are phylogenetically closest to large mammals [7, 8]. Immunological response to solid organ allotransplantation in these animals is close in specificity to similar manifestations in humans. An equally important aspect in choosing an animal LT model is the possibility of adequate monitoring of vital functions during the follow-up period, as well as the potential blood volume for hematological studies [9].

Therefore, we carried out work aimed at creating a reproducible orthotopic left LT model in rabbits. This study describes the peculiarities of anesthetic therapy and surgical technique both on the donor and the recipient, and it validates the technique as a whole.

Purpose of the present study: To develop and optimize orthotopic left-lung transplantation in an experimental rabbit model in order to assess the reproducibility of the animal model.

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MATERIALS AND METHODS

Male Scottish Giant rabbits weighing 4.5 kg to 5 kg were used in the study. The animals were divided into 2 groups: donors (N = 10) and recipients (N = 10). The experimental program was approved by the Committee on Biological Safety and Bioethics, Shumakov National Medical Research Center of Transplantology and Artificial Organs. The work was carried out in compliance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes and Directive 2010/63/EU.

The experimental design included removal of donor lung, static hypothermic preservation for 6 hours and orthotopic left LT procedure. The follow-up period to assess the severity of IRI and the effectiveness of the preservative properties of the experimental solution lasted for 24 hours. At the end of the experiment, the animal was removed from the experiment by exsanguination.

Donor lung removal

As part of the preoperative preparation, the animals were sedated with zolazepam (Zoletil 100, Virbac, France) subcutaneously at a dose of 50 mg. Under aseptic and antiseptic conditions, intravenous catheter Vasofix Certo 22G (BBraun, Germany) was inserted into the marginal ear vein, the catheter was fixed with a patch. Intravenous injection of atropine 0.3 mg and dexamethasone 2 mg was used as premedication. The donors were anesthetized with zolazepam 10 mg/kg, propofol (Fresenius Kabi, Germany) at a dose of 25 mg, followed by a combination of inhaled anesthetic Isoflurane (Baxter, USA) 1.5% vol. Tracheal intubation was performed by direct laryngoscopy using a size 4 endotracheal tube with an inflatable cuff. After correct intubation has been verified, rocuronium bromide solution (Fresenius Kabi, Germany) was administered at a calculated dose of 10 mg.

Artificial ventilation was performed using anesthesia machine WATO EX-65 Pro vet (Mindray, China) in volume controlled ventilation (VCV) mode with the following parameters: ventilation volume (V), 50 mL; respiratory rate (RR), 35/min; peak inspiratory pressure (P_{peak}), 17 cmH_2O; positive end-expiratory pressure (PEEP), 3 cmH_2O; inhalation/exhalation ratio (I:E), 1:1; fraction of inspired oxygen (FiO_2), 0.6; end-tidal carbon dioxide pressure ($EtCO_2$), 40 mmHg. Vital functions were monitored using the ePM 12M Vet device (Mindray, China) with an average heart rate of 170 beats/min, SpO_2 98, non-invasive blood pressure (NIBP) 90/45 mmHg. Tramadol (Tramvet, Russia) 25 mg intravenously was used for analgesia. Hemodynamic maintenance was provided by intravenous injection of potassium and magnesium aspartate (Panangin, Gedeon Richter, Hungary) 10 mL/hour and norepinephrine 100 ng/kg through a syringe dispenser.

Surgical access was performed via median sternotomy. After achieving hemostasis and the pulmonary artery trunk mobilized, heparin sodium 5000 U was injected intravenously with an exposure time of 3 minutes, followed by selective injection of alprostadil (Vasaprostane, IDT BIOLOGIKA, Germany) at 10 mcg dose. The pulmonary artery was cannulated with a 14 G intravenous catheter and antegrade pneumoplegia was initiated with Perfadex plus® at 40 °C and 60 mL volume through a syringe dispenser at a rate of 500 mL/hour and exposure time of 7–8 minutes. Mechanical ventilation (MV) parameters were varied: V, 25 ml; RR, 20/min; P_{peak}, 11 cmH_2O; PEEP, 5 cmH_2O; I:E, 1:1. Upon completion of perfusion of the preservative solution, the heart was removed first, and the pulmonary ligaments were crossed. The trachea was mobilized throughout its entire length and then tied with a silk ligature at the height of inspiration and crossed. Upon completion of organ removal, the lungs were placed in a sterile bag with subsequent static hypothermic preservation in an insulated container for 6 hours until it was implanted in the recipient.

Orthotopic left lung transplantation

Anesthetic management of the recipient animal differed from the donor stage in that an intravenous catheter Certofix Mono Paed S110 22G (BBraun, Germany) was placed in the marginal ear vein and a catheter CK-FLON 26G (India) was implanted in the middle artery of the ear to monitor invasive blood pressure. After the recipient has been anesthetized and vital functions monitored, the animal was laid on its right side, the surgical field was treated with an antiseptic and isolated with sterile surgical linen.

Surgical access was performed by left-sided thoracotomy in the fourth intercostal space with resection of the fifth rib. The wound edges were widened with a retractor, and after achieving hemostasis, we proceeded to mobilize left lung root elements. The pulmonary artery, main bronchus and separately the pulmonary veins were isolated from the surrounding tissues by blunt and sharp methods. After isolation of all vascular structures, the pulmonary artery, superior and inferior pulmonary veins were ligated. Last of all, the left main bronchus was ligated and crossed 0.5 cm from the tracheal bifurcation (Fig. 1).

Upon completion of pneumonectomy, 150 mg heparin sodium solution was administered, and single-lung ventilation was initiated with the following parameters: V, 25 mL; RR, 55/min; P_{peak}, 16 cmH_2O; PEEP, 5 cmH_2O; I:E, 1:1; FiO_2, 0.8; $EtCO_2$, 36 mmHg. Ninety minutes after the onset of anesthesia, 30 mg zolazepam and 25 mg tramadol were administered intravenously. Sedation throughout the operation was performed with isoflurane 1.0 vol.%. Hemodynamic maintenance was provided by intravenous injection of potassium and magnesium aspartate, 5–10 mL/hour, and norepinephrine

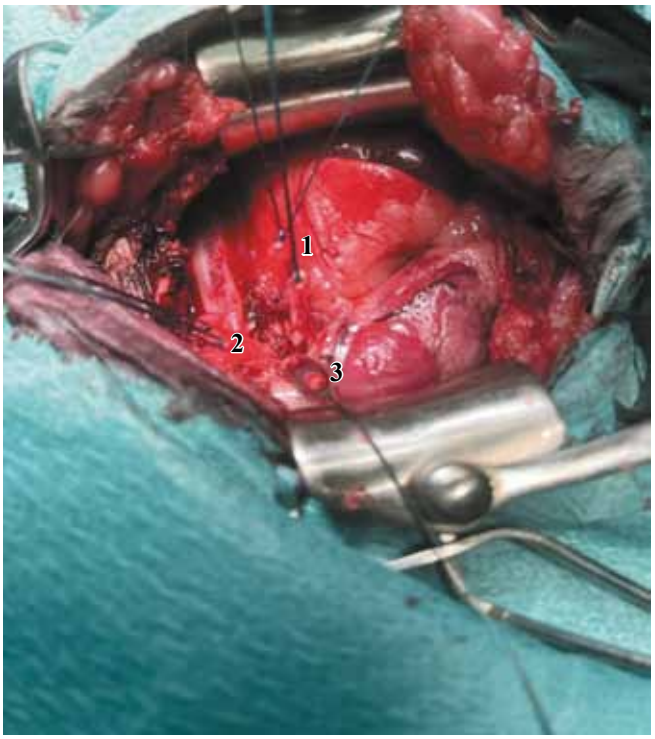

Fig. 1. Condition after pneumonectomy of the left lung. 1, pulmonary vein; 2, pulmonary artery; 3, main bronchus

100–460 ng/kg. Blood gas and electrolyte composition was studied every 30 minutes using an EDAN Blood Gas Analyzer (Edan Instruments, China); samples were taken from a catheter in the ear artery. Electrolyte and metabolic disorders were corrected symptomatically.

In order to conveniently apply a clamp to the left atrium, the pericardium was opened transversely from the mouths of pulmonary veins. Then, a vascular clamp was applied to the pulmonary artery and the left main bronchus. In order to avoid kinking of vascular anastomoses, a bronchial anastomosis was applied first. The bronchial anastomosis was formed by continuous wraparound suture using a PDS 6/0 thread. Anastomosis of the pulmonary artery was performed with continuous wraparound suture using a Prolene 8/0 thread. Atrial anastomosis was performed last. A Satinsky vascular clamp was applied to the pulmonary veins with maximal capture of the free wall of the left atrium. The pulmonary veins were crossed, and a single left atrial cuff was formed by angular vascular scissors, the anastomosis was performed with a Prolene 7/0 thread. After anastomosis formation was completed, graft reperfusion was initiated. After implantation of the donor lung, Methylprednisolone (Pfizer, Belgium) was administered intravenously at a dose of 50 mg before starting blood flow. Ventilation was resumed, the clamp was first removed from the pulmonary veins thereby initiating retrograde perfusion; after the graft was filled and blood appeared from the untied suture line on the pulmonary artery, the suture was tied. Next, the clamp was removed from the pulmonary artery, thereby completely resuming blood flow in the graft. The recruitment maneuver was performed automatically with P_{peak} at 30 cmH_2O.

Radiologic study

A straight chest x-ray was taken for a radiological examination. In order to assess IRI severity, the RALE (Radiographic Assessment of Lung Edema) scale was adopted [10]. The peculiarity of this technique for measuring the severity of lung lesions lies with its universality and simplicity, as well as validity of its application in animals. Since only the left lung was transplanted, the RALE score was assessed unilaterally. The lung was visually divided into two quadrants, and each quadrant was assigned a consolidation score from 0 to 4 to quantify the degree of alveolar opacities based on the percentage of the quadrant with opacities, and a density score from 1 to 3 to quantify the total density of alveolar opacities, except when the consolidation score for that quadrant was 0. Because consolidation is a process requiring more than 24 hours of follow-up and its assessment is difficult with short follow-up times, its value was conventionally taken as 1.

Left lung lesion was computed using the formula: Upper lobe consolidation score × upper lobe density score = Q1; Lower lobe consolidation score × lower lobe density score = Q2; Q1 + Q2 = total RALE score.

Statistical analysis was carried out using the StatTech v. 3.1.10 software (StatTech LLC, Russia). Quantitative indicators were evaluated for conformity to normal distribution using the Shapiro–Wilk Test (number of subjects less than 50). Quantitative indicators having normal distribution were described using arithmetic mean (M) and standard deviations (SD), 95% confidence interval (95% CI) limits. One-factor analysis of variance with repeated measures was used to compare three or more related groups for a normally distributed quantitative trait. Statistical significance of changes in an indicator in dynamics was assessed using Pillai's Trace (Pillai's Trace). Posterior analysis was performed using paired student's t-test with Holm correction. Results were considered statistically significant at $p < 0.05$.

RESULTS

Dynamics of blood gas composition and lactate levels, peak inspiratory pressure parameters in recipient animals

The main parameters studied during the left LT were peak inspiratory pressure in the recipient animal (Fig. 3), lactate levels (Fig. 4), as well as the calculated indicator – PaO_2/FiO_2 ratio for arterial blood from arterial catheter (Fig. 2). All investigated cases showed satisfactory graft function at control assessment of gas composition, peak airway pressure and lactate directly from the pulmonary vein.

When studying the oxygenation index, the indicators were found to have moderately decreased (345 ± 32, from 283 to 385) in all cases after implantation and at 1 hour. After graft reperfusion and at 24 hours of follow-up, oxygenation index values >350 were observed in five cases (N = 5). However, when assessing the statistical population, mean oxygenation index was found to be high at 3 hours of follow-up.

The dynamics of changes in peak inspiratory pressure was a significant indicator for assessing the functional status of the donor lung, taking into account the fact that MV parameters were selected individually. At the end of

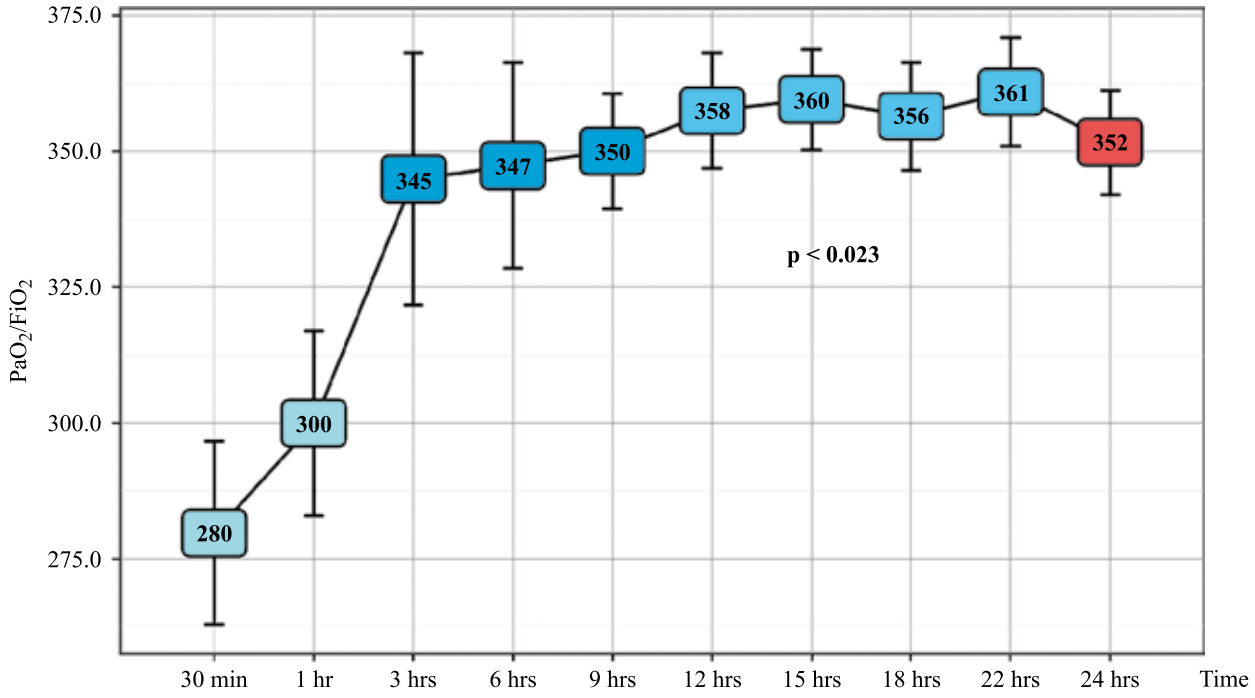

Fig. 2. Dynamics of oxygenation index after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

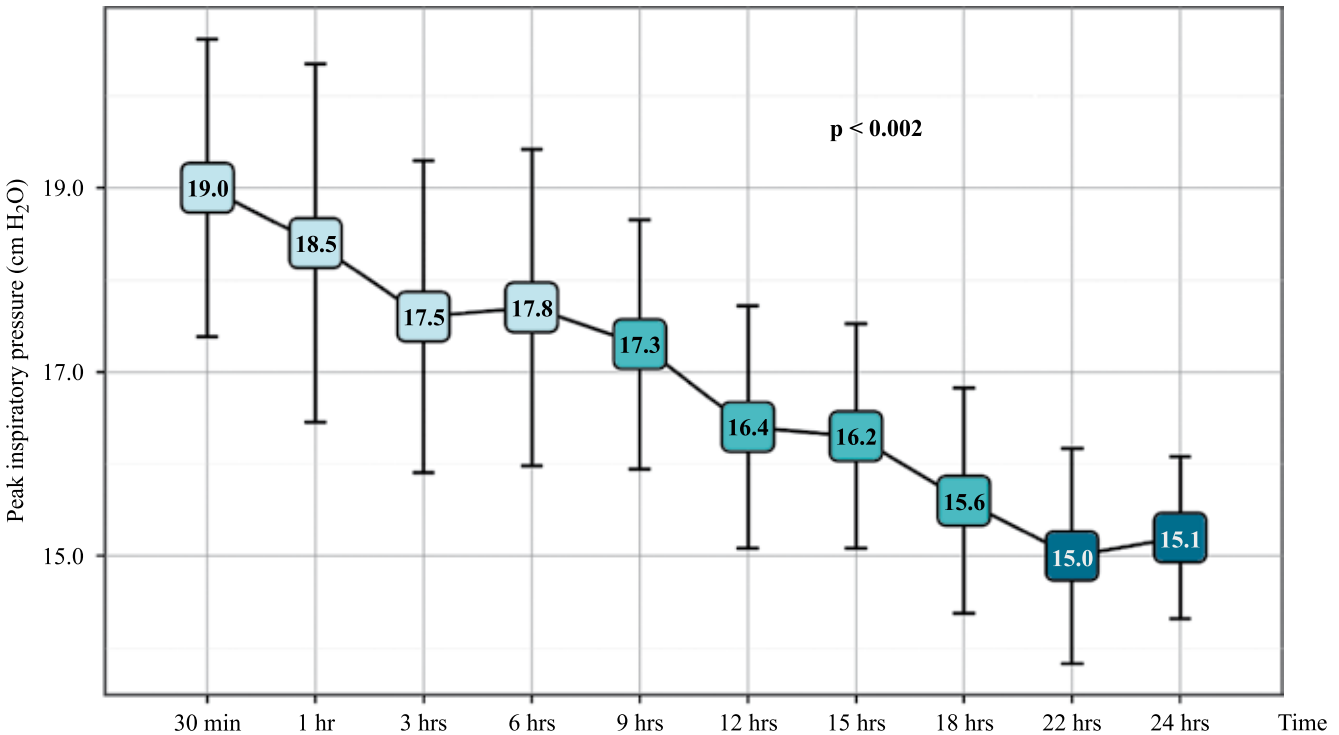

Fig. 3. Dynamics of changes in peak inspiratory pressure after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

the follow-up, peak inspiratory pressure was low during the statistical population assessment: 15 ± 1 (from 14 to 16) cmH_2O, which indicated preserved graft function and no severe interstitial edema.

The dynamics of lactate parameters reflected the severity of IRI after transplantation, as well as the correctness of MV and adequate warming of the animal. When evaluating the statistical population, it was noted that lactate levels exceeded 8 mmol/L in only two cases, but at the end of the follow-up period, the levels remained at the physiologic level, 3 ± 0.3 mmol/L.

Results of the study of gas parameters, peak inspiratory airway pressure and lactate levels after transplantation at 10 time points indicated effective gas transport and satisfactory functional status of the donor lungs in all cases.

Radiologic studies

Radiologic examination was performed twice during the follow-up period in all cases. The results are presented in Table.

Radiological studies showed that lung grafts had signs of primary graft dysfunction in all cases. However, at the end of the follow-up period, significant signs of IRI regressed in all cases.

It should be noted that, according to reports, this is the first experience of using the RALE scale to assess the severity of donor lung injury in an experiment in rabbits. This suggests that the significance of IRI assessment can be low when examining and describing radiographs.

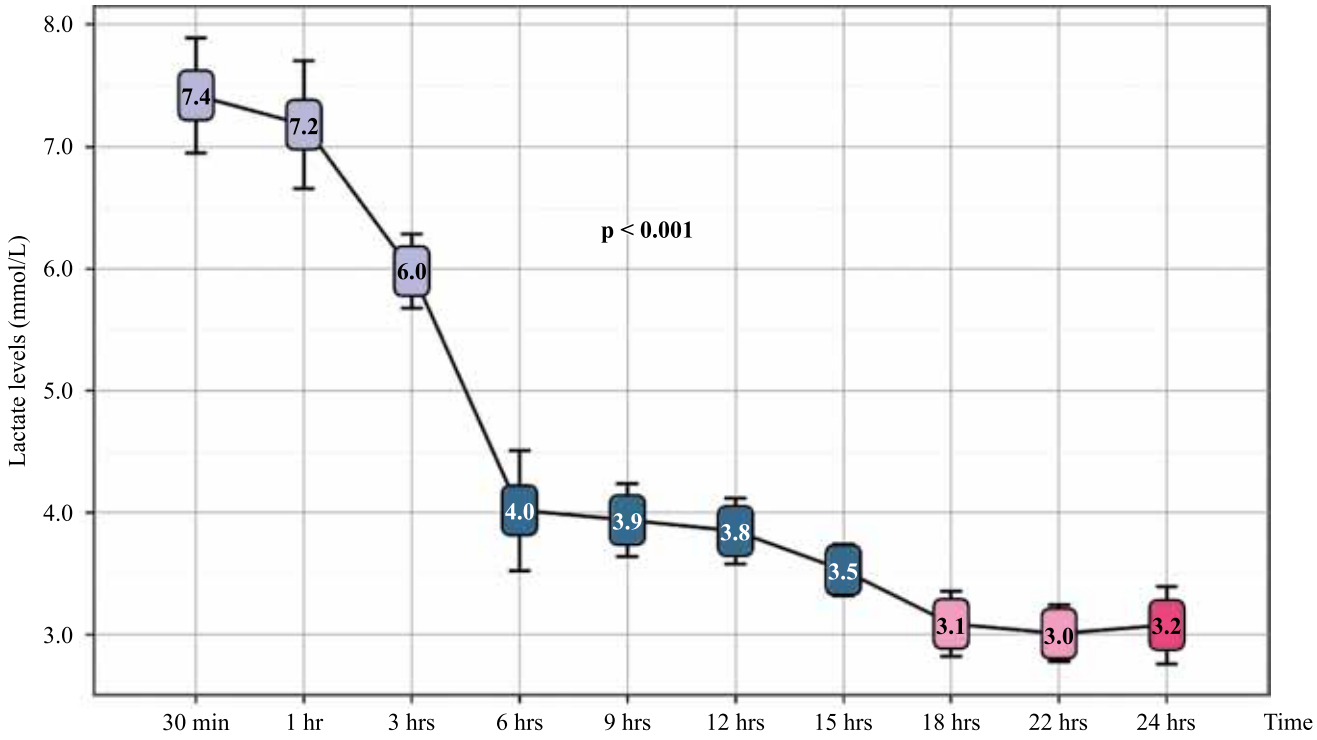

Fig. 4. Dynamics of changes in arterial blood lactate levels after left lung transplantation. The graph shows mean values, vertical lines indicate standard deviations, p is statistical significance

Table

Dynamics of the radiologic picture after left lung transplantation

	RALE Score after transplantation	RALE Score at 24 hours
Recipient 1	2	1
Recipient 2	1	1
Recipient 3	1	1
Recipient 4	2	2
Recipient 5	2	2
Recipient 6	1	1
Recipient 7	2	1
Recipient 8	2	1
Recipient 9	2	2
Recipient 10	1	1

Histological studies after transplantation

Histologic specimens were evaluated at 100× magnification (Fig. 5, a) and 200× magnification (Fig. 5, b) over the entire specimen area in each case.

A morphological study at 24 hours after implantation of the donor lung showed no difference in the microstructure of the lung parenchyma in the compared samples. Thus, in all cases, the pulmonary parenchyma architectonics were preserved, the alveolar-capillary barrier was intact, interstitial edema was moderate, functional structures were preserved, and there were no disseminated hemorrhage sites.

DISCUSSION

The LT procedure has a long history. Back in the last century, Soviet scientist Vladimir Demikhov attempted LT in an experiment on dogs. A significant contribution to the development of this area was made by French surgeon and biologist Alexis Carrel, who provided the formation of the basic principles of the technique. It is noteworthy that every scientist in world history has engaged in scientific research in experimental studies on animals with great respect [11]. Animal research facilitates the acquisition of experience and knowledge that would be implemented in clinical practice with great precision and outstanding results. There is still a need to find a universal animal model of LT. However, today humanity is closer than ever to perfection and professionalism, as well as to a deep understanding of solutions to combat IRI [12].

Although there are various experimental animal models of LT, each has its own peculiarities, strengths and weaknesses. The model of orthotopic left LT in rabbits is surgically and anesthesiologically challenging. The low weight of the animal requires a very competent anesthetic approach that is comparable to that of pediatric cardiac anesthesiologists. Constant monitoring of blood gas and electrolyte composition is necessary for early correction of metabolic disorders, and the possibilities of infusion therapy are severely limited due to the direct effect of infusion volume on postoperative interstitial edema and gas transport function of the blood. From a surgical point of view, the main challenges in working with the rabbit model are related to the need for microsurgical skills. Thin vascular walls, fragile tissue structures, and anatomical features present challenges for the operator.

Despite the abundance of biochemical, morphological and functional indicators for assessing the status of donor lungs in a clinical organ transplantation program, there is no more objective and statistically reliable criterion that characterizes the functional status of the graft than the oxygenation index. Thus, when studying this index within the framework of this work, high oxygenation index was obtained, which, together with the histological picture and the results of radiological studies, as well as ventilation parameters in the early postoperative period and the dynamics of changes in lactate levels, indicates the high efficiency of the technique of orthotopic left LT on a rabbit model at standard ischemic periods [13].

CONCLUSION

The experimental study showed that orthotopic left lung transplantation can be performed on an experimental rabbit model. The model has shown its efficiency and reproducibility. Certainly, the economic feasibility of this model looks more attractive in contrast to the use of large laboratory animals. The proposed experimental model will expand the arsenal of research teams that are dealing with LT problems.

The authors declare no conflict of interest.

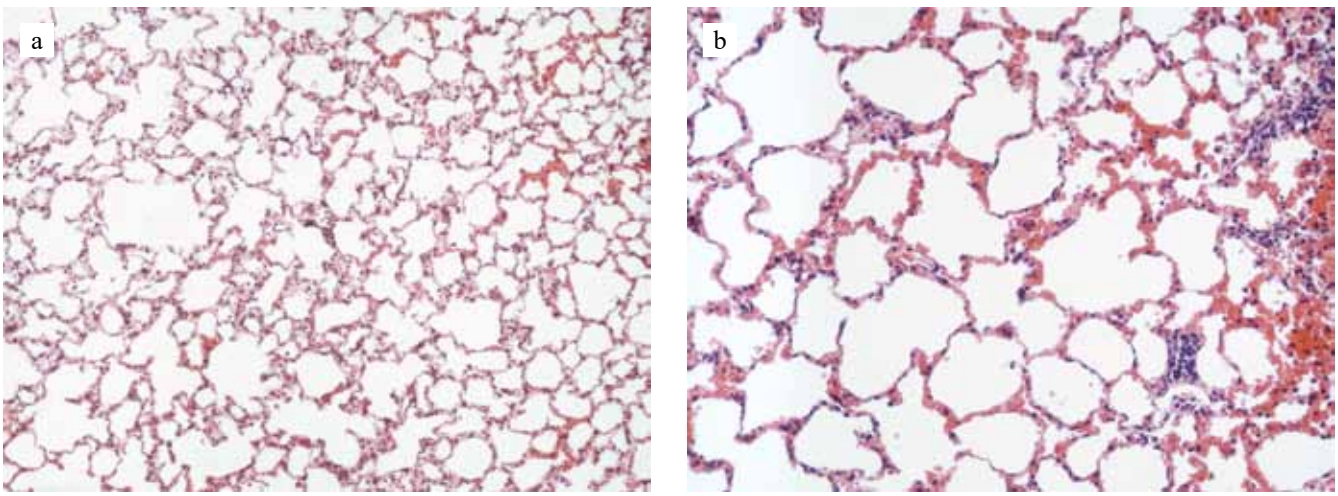

Fig. 5. Morphological study results: a, histological picture of donor left lung parenchyma at 24 hours after transplantation; 100× magnification; b, histological picture of the donor left lung parenchyma at 24 hours after transplantation; 200× magnification

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