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EXPERIMENTAL STUDY OF A NEW DEXTRAN-40-BASED COMBINED SOLUTION ON A SMALL LABORATORY ANIMAL MODEL

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Background. Organ shortage remains an unsolved issue in the field of transplantology. It is particularly severe in such a progressive area as lung transplantation. The creation of extracorporeal systems for rehabilitation of donor organs has been made possible by perfusion techniques; however, the search for the best perfusion and preservation solutions remains important. **Objective:** to evaluate the efficacy of the developed solution for preservation and normothermic *ex vivo* lung perfusion (EVLP), as well as to conduct a comparative analysis with the standard perfusion solution for EVLP. **Materials and methods.** Experimental studies on small animal models were conducted. All animals were divided into 2 groups – control and experimental. The study stages consisted of: procurement of donor lungs, static cold storage, EVLP and orthotopic left lung transplantation. In the experimental group, the lungs were preserved using an experimental solution, while in the control group, they were preserved in PERFADEX® Plus (XVIVO, Sweden). Static cold storage lasted for 10 hours. Orthotopic left lung transplantation was performed after EVLP. The follow-up period was 2 hours, after which blood samples and sections of the transplanted lung were taken for morphological examination. Upon completion of the experiment, the animal was removed from the experiment by exsanguination. **Results.** Respiratory index at the end of perfusion was statistically significantly higher in the experimental group (434 mmHg) than that of the control group (394 mmHg). Pulmonary vascular resistance (PVR) in both groups had a downward trend, which is a good prognostic sign of the efficacy of perfusion agents. PVR was lower in the experimental group compared to the control group – 36 versus 89 dynes/sec/cm^{-5}. **Conclusion.** The developed combined dextran-40-based solution showed its effectiveness as a preservation agent for static cold storage and as a perfusion solution for EVLP.

Keywords: lung transplantation, ex vivo lung perfusion, preservative solutions, perfusion solution.

INTRODUCTION

Lung transplantation (LT) has become a highly effective therapeutic option for end-stage lung disease, but access is limited by the insufficient number of donor organs available [1, 2]. Expanding donor selection criteria allows for more organs to be transplanted, but this also increases the risk of primary graft dysfunction with the use of "marginal" or compromised organs [3, 4].

Normothermic *ex vivo* lung perfusion (EVLP) allows for objective assessment of compromised lungs that were previously deemed unsuitable for transplantation. EVLP not only enables evaluation of the organ but also extends preservation times, offering logistical advantages. Moreover, recent developments have highlighted the potential of EVLP as a therapeutic platform for reconditioning donor lungs. Clinical trials have demonstrated the safety and feasibility of transplanting organs assessed through EVLP, with survival rates comparable to those of organs preserved using traditional cold static storage methods [5–7].

The success of EVLP is largely attributed to the pioneering work of Professor Stig Steen, who performed the first human LT following *ex vivo* assessment [8]. A key factor in the success of this perfusion technique was Steen's human albumin-based perfusion solution, known as the Steen Solution™. Human serum albumin, a primary component of the solution, maintains physiologically relevant colloid osmotic pressure, minimizing lung damage [9]. Additionally, the presence of dextran 40 in the solution helps reduce the negative impact of leukocytes on the vascular endothelium [10].

Despite the positive properties of the solution, lungs are still susceptible to ischemia-reperfusion injury (IRI) during EVLP. IRI is characterized by an acute inflammatory response and increased oxidative stress, both of which contribute to primary graft dysfunction in the early

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postoperative period [11–13]. It is well established that proinflammatory cytokines in the perfusate and tissue increase significantly over time, even during successful perfusions [14, 15]. The duration of perfusion directly correlates with the degree and severity of LT injury [16, 17].

There is still incomplete understanding of the pathophysiological events occurring during EVLP, and as a result, existing perfusion solutions continue to evolve in terms of composition and addition of new adjuvants. In this context, a combined solution for both preservation and normothermic EVLP has been developed. Unlike the original Steen Solution™, this new solution features dextran 40 and a modified electrolyte composition as its base.

This study aimed to evaluate the efficacy of this experimental solution in comparison to the original Steen Solution™, which is widely regarded as the gold standard for EVLP in clinical practice worldwide.

MATERIALS AND METHODS

Experiments were conducted using small animal models, specifically male Wistar rats weighing 250–300 g. The following stages were carried out in the series of experiments:

– Lung procurement;
– Static hypothermic storage;
– *Ex vivo* lung perfusion;
– Orthotopic left lung transplantation.

In the experimental group, donor lungs were preserved using the experimental solution, while in the control group, Perfadex Plus was used as the preserving agent. In all cases, static hypothermic storage was maintained for 10 hours.

The animals were divided into two equal groups: donors (n = 30) and recipients (n = 30). The donor group was further divided into two subgroups:

Group 1 – EVLP using the experimental solution (n = 15);

Group 2 – EVLP with Steen Solution™ solution (n = 15).

After *ex vivo* perfusion, orthotopic left lung transplantation (OLLT) was performed. A follow-up period of two hours was observed, after which blood samples and tissue sections of the transplanted lung were collected for morphological analysis.

Donor lung procurement procedure

The donor animal was placed in a specialized anesthesia induction chamber, where sedation was induced using an isoflurane vaporizer (RWD R5835, China) at a flow rate of 1 L/min and a concentration of 5 vol/%. The depth of anesthesia was monitored by assessing the animal's response to pain stimuli and respiratory rate. Tracheal intubation was performed using a 14 G IV catheter, and the intubation tube was connected to the SAR-830/AP Ventilator (CWE, USA) circuit. Mechanical ventilation (MV) was initiated with 100% oxygen and the following parameters: respiratory rate (RR) 85/min, respiratory volume (V_{RV}) 1.2 mL, flow volume (V_{FV}) 700 mL/min, peak pressure (P_{peak}) 8 cmH_2O, positive end-expiratory pressure (PEEP) 3 cmH_2O, and isoflurane flow at 3.5 vol/%. A median sternotomy was performed, and after dissection of lung tissues and hilar structures, 500 units of heparin were injected through a puncture of the right ventricular apex. After a 3-minute exposure, 12 mL of whole donor blood was drawn into a heparinized syringe. A 2.0/2.5 mm cannula was then inserted into the right ventricle and advanced into the pulmonary artery, while a 2 mm diameter metal angular cannula was placed into the left ventricle for adequate perfusate drainage.

The donor lungs were preserved by antegrade perfusion with Perfadex Plus solution at 4 °C, using a syringe pipette to deliver 20 mL of solution at a rate of 200 mL/h (3.3 mL/min) for an exposure time of 6 minutes [18].

During preservation, the MV parameters were adjusted with atmospheric air as follows: respiratory rate (RR) 40/min, V_{RV} 1.5 mL, V_{FV} 300 mL/min, P_{peak} 6 cmH_2O, and PEEP 3 cmH_2O. After graft preservation, the diaphragm, superior vena cava, pulmonary ligaments, and pleura were dissected. The trachea was separated from the esophagus. Once the lungs were fully mobilized, a 14 G plastic cannula was inserted into the tracheal lumen for subsequent ventilation under *ex vivo* lung perfusion (EVLP) conditions. Following the procurement process, the lung graft was placed in a sterile container filled with 30 mL of Perfadex Plus solution for static hypothermic storage, where it was preserved for 10 hours.

Normothermic *ex vivo* lung perfusion procedure

In the experimental group, the perfusion circuit was filled with 10 mL of the dextran 40-based experimental solution and 12 mL of whole donor blood. In the control group, the extracorporeal circuit was filled with 20 mL of Steen Solution. The following adjuvants were added to the solution in all groups: Glucose 40% (4 U), $NaHCO_3$ 4.8% (2 U), Vasaprostane (10 μg), Insulin P (3 U), Methylprednisolone (20 mg), Cefazolin (0.5 mg/mL), and Heparin (300 U).

Once the solution temperature reached 25 °C during continuous recirculation, the perfusate was analyzed for acid-base and electrolyte parameters, as well as glucose concentration.

For deaeration, the graft was retrogradely filled at a rate of 1 mL/min through the left atrium cannula, passi-

vely with a water column, until the solution appeared in the pulmonary artery cannula. After this, the pump was stopped, and the perfusion line was connected to the pulmonary artery cannula. Initial volumetric perfusion rate was set at 1.2 mL/min, which represents 15% of the target perfusion rate. The required 100% perfusion rate was calculated based on the estimated mass of the lung graft, as determined by the following formula (1):

$$V = 0.0053 \times m - 0.48, \quad (1)$$

where m is animal weight in grams.

The estimated lung graft mass was calculated based on a perfusion rate of 6 mL/min/gram [18, 19]. MV of the lung graft was initiated 15 minutes after the onset of normothermic machine perfusion, upon reaching a temperature of 33 °C. During this period, perfusion parameters were recorded, and pulmonary vascular resistance was calculated.

Gas and electrolyte composition of the perfusate was analyzed before the start of graft perfusion and then at 15-minute intervals. Samples were taken simultaneously from two points in the perfusion circuit: the outflow perfusate from the left atrium and the circulating perfusate sampled after the oxygenator. Comparing oxygen and carbon dioxide levels from these two sampling points enabled evaluation of perfusion efficiency and assessment of the graft's functional status.

At 120 minutes, a final analysis of gas and electrolyte composition was performed. Machine perfusion was then discontinued, and MV was continued. For further preservation, 20 mL of the dextran 40-based experimental solution cooled to 4 °C was infused into the pulmonary artery via the perfusion system at a rate of 200 mL/hour.

Orthotopic left lung transplantation

Lung implantation was performed using the cuff technique to minimize warm ischemia time and reduce variability due to surgical technique [20]. The principle of this method involves using intravenous catheter segments as cuffs to secure the graft vessels and facilitate implantation into the recipient's corresponding vessels. Specifically, 14 G catheters were used for bronchial implantation, 16 G for the pulmonary artery, and 14–16 G for the pulmonary vein depending on vessel diameter [21, 22].

To minimize warm ischemia and provide local cooling during cuff placement, the graft was irrigated with dextran 40-based preservation solution at 4 °C. The graft was suspended by the lung root and stabilized using a flexible holder. Donor lung vessels were passed through their respective cuffs, with the vascular edges folded over the cuff body and secured using a 7/0 Prolene ligature. The bronchial cuff was prepared and implanted in a similar fashion. This procedure took an average of 30 minutes.

Following anesthesia induction and initiation of MV, the recipient animal was positioned in right lateral decubitus on the operating table. A thoracotomy was performed through the 5th intercostal space, with resection of the 4th rib [23, 24]. The native lung's vascular structures were mobilized, and a vascular clamp was applied to the lung root before removal of the left lung. To prevent twisting of vascular anastomoses, the left main bronchus was implanted first. For ease of cuff placement, the pulmonary artery and veins were incised transversely, and the corresponding cuffs were inserted and secured with ligatures. Upon completion of all anastomoses, the vascular clamp was released to initiate graft reperfusion.

The follow-up period was 2 hours, after which blood was selectively collected from the pulmonary artery and pulmonary veins for gas analysis.

Morphological study

Following perfusion, samples of the right lung parenchyma were fixed in 10% neutral buffered formaldehyde (pH 7.4) for 24 hours. Similarly, 2 hours after transplantation, samples of the left lung parenchyma were collected and fixed in 10% formaldehyde under identical conditions. For paraffin embedding, the tissue specimens were dehydrated using isopropyl alcohol and cleared with petroleum ether. The samples were then embedded in paraffin blocks and sectioned at a thickness of 5 μm.

Histological sections were stained with hematoxylin and eosin (H&E) for microscopic examination. Microscopic analysis was conducted using a Leica DM 750 light microscope (Leica, Germany), equipped with a 10× eyepiece and objective lenses of 4×, 10×, 40×, and 100× magnification. Digital images of the histological sections were captured using an ICC50 camera (Leica, Germany).

Samples were assessed for vascular thrombosis, hemorrhage, interstitial and alveolar edema, and cellular infiltration.

Statistical data processing methods

Statistical analysis was conducted using the licensed SAS Enterprise Guide 9.4 software. All variables were tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. For normally distributed data, parametric statistical methods were applied; in the case of non-normally distributed data, non-parametric methods were used. Group comparisons for variables such as oxygenation index, pulmonary vascular resistance, pulmonary arterial pressure, lactate, glucose, buffer bases, and peak inspiratory pressure were performed using the Kruskal–Wallis test. A p-value <0.05 was considered statistically significant. Box-and-whisker plots were generated using SAS Enterprise Guide 9.4.

RESULTS

The experimental study comprised two main phases: EVLP and OLLT in recipient animals. During the EVLP procedure, key indicators reflecting the functional status of the donor lungs were continuously monitored and recorded in both groups. These indicators included oxygenation index (OI) (Fig. 1), pulmonary artery pressure (PAP), and pulmonary vascular resistance (PVR). Comparative analysis of these parameters between the groups was performed using the Kruskal–Wallis test, with p-values <0.05 considered statistically significant.

OI is a key measure of gas transport during lung perfusion, with a lower acceptable value typically considered at 350. The study observed high OI values in both groups. At the beginning of the procedure, median OI in the control group (Steen Solution) was 498.5 [460; 537], and in the experimental group, it was 518 [483; 553]. Statistical analysis revealed no significant differences between the groups ($p > 0.05$). Throughout the *ex vivo* procedure, the PaO_2/FiO_2 ratio remained comparable between the two groups; however, a significant increase in OI was noted in the experimental group in the final analysis. Specifically, median OI in the Steen Solution group was 394.4 [373; 416], while in the experimental group, it increased to 434.7 [422; 447], with the difference reaching statistical significance ($p < 0.0001$).

While the volumetric perfusion rates were identical in both groups during EVLP, differences in PAP were observed (Fig. 2).

In the control group, initial PAP values were within the acceptable threshold of 15 mmHg, with a median of 9.07 [7.7; 10.4] mmHg. Throughout the EVLP procedure, PAP fluctuations in this group were minimal, with a final median value of 8.47 [7.2; 9.8] mmHg. In contrast, the experimental group demonstrated a consistently lower PAP, showing a downward trend from an initial median of 4.45 [3.3; 5.6] mmHg to 3.4 [2.9; 3.9] mmHg by the end of perfusion. This notable difference in PAP dynamics played a crucial role in calculating PVR values, which served as an objective indicator of vascular compliance in donor lungs during EVLP (Fig. 3).

Median PVR in the control group was 604.3 [515; 693] Dynes/sec/cm^{-5}, whereas in the experimental group, it did not exceed 297.8 [223; 373] Dynes/sec/cm^{-5}. These differences were statistically significant. Although both groups exhibited a marked downward trend in PVR during the EVLP procedure, by the end, the group using the experimental dextran 40-based solution demonstrated significantly lower vascular resistance compared to the Steen Solution™ group, with median values of 35.8 [31; 41] *vs.* 89.1 [75; 103] Dynes/sec/cm^{-5}, respectively ($p < 0.0001$).

Lactate level dynamics were monitored throughout the perfusion period (Fig. 4).

Lactate dynamics had a general upward trend throughout the EVLP procedure, as expected due to the absence of metabolic pathways for lactate clearance in the *ex vivo* setting. While no statistically significant differences were noted between the groups at the 60- and 90-minute

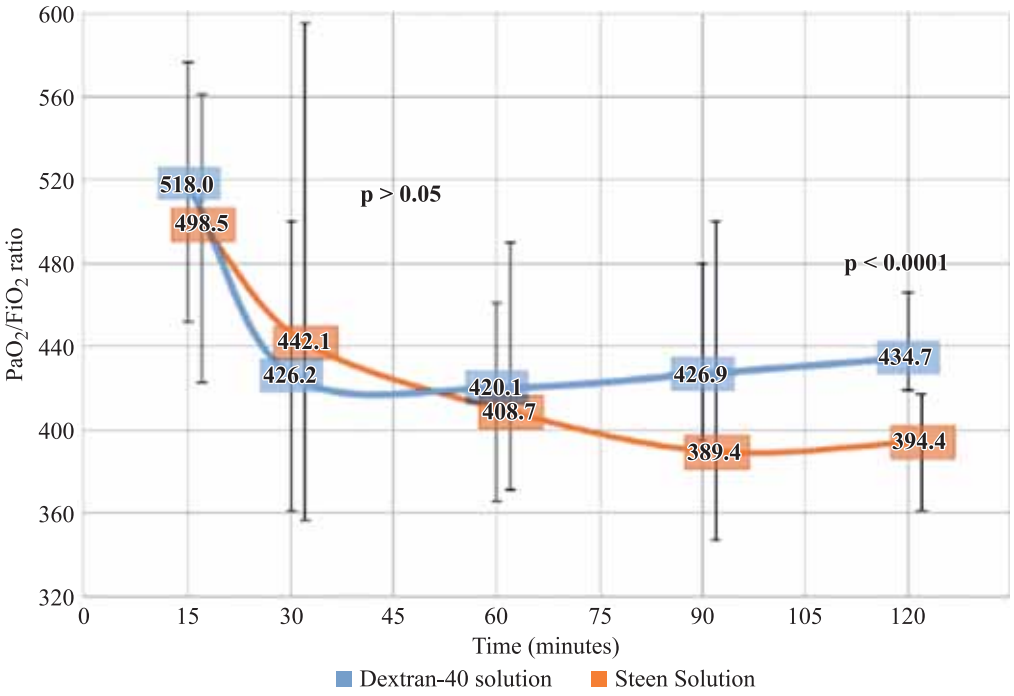

Fig. 1. Dynamics of oxygenation index during EVLP. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

marks, the experimental solution group demonstrated narrower fluctuation ranges in median lactate levels. Importantly, at the final measurement point, the maximum lactate values were significantly lower in the experimental group – 7.5 [7.2; 7.6] mmol/L compared to 7.87 [7.8; 8.5] mmol/L in the Steen Solution™ group.

After EVLP, OLLT was performed. To assess the functional integrity of the graft post-transplant, OI (Fig. 5) and lactate levels (Fig. 6) were measured twice during the 120-minute post-transplant follow-up period.

After implantation of donor lung, OI values in the group perfused with the experimental dextran 40-based

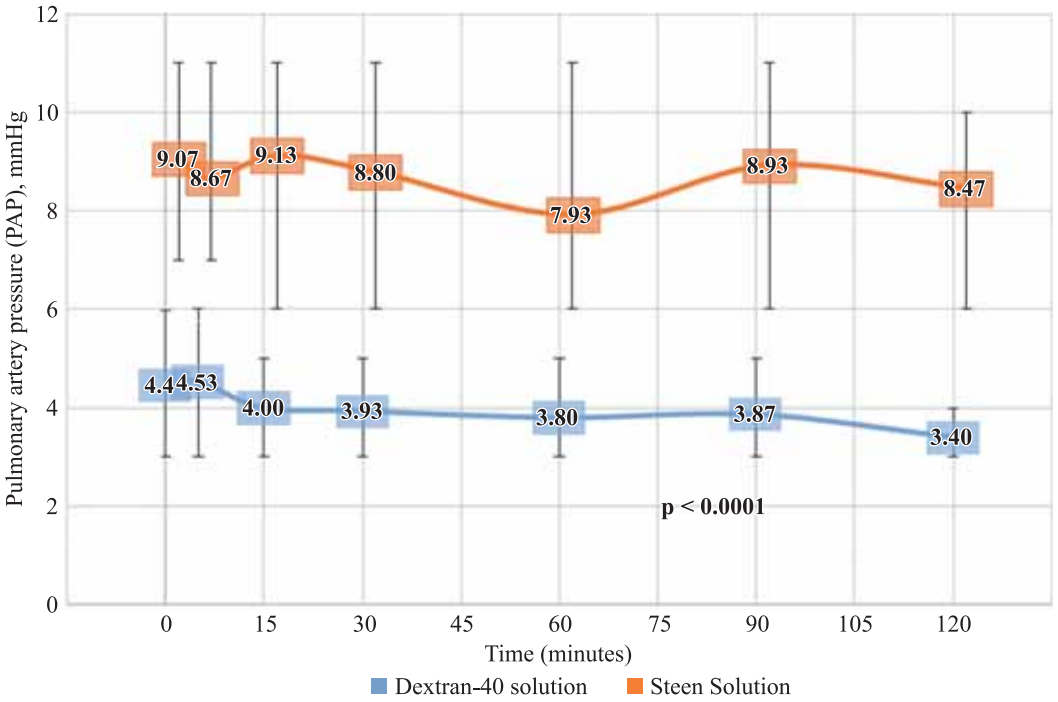

Fig. 2. Dynamics of pulmonary artery pressure during EVLP. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

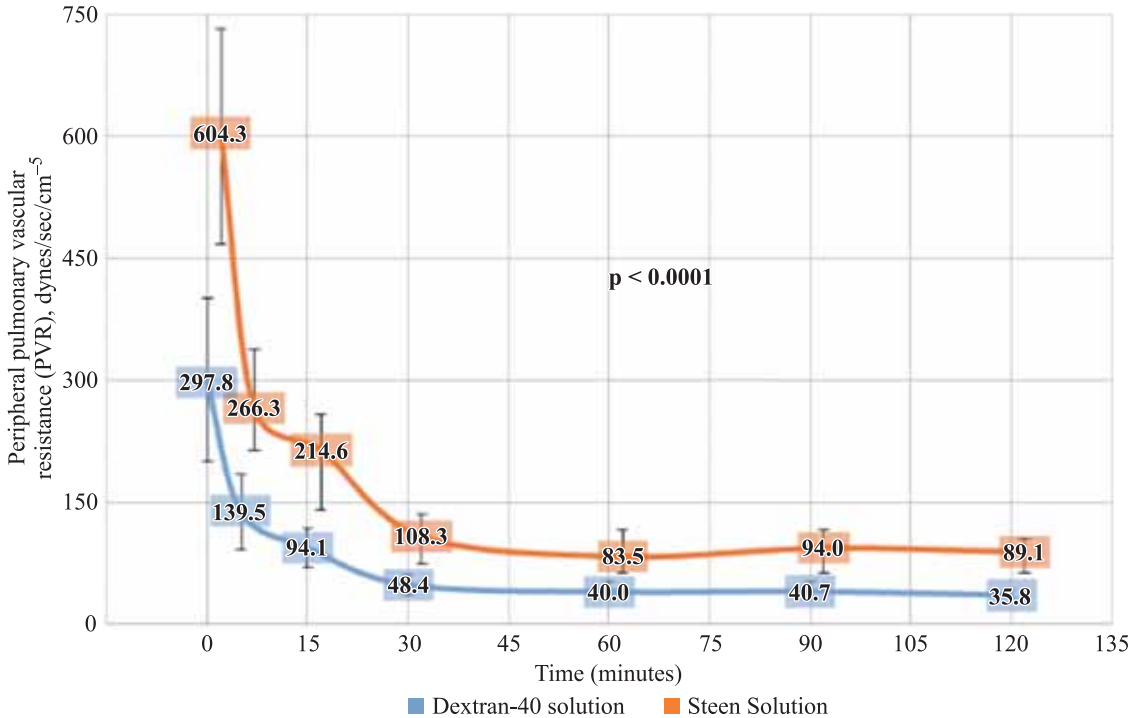

Fig. 3. Dynamics of peripheral vascular resistance during EVLP. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

solution remained significantly elevated, consistently exceeding the critical threshold of 350. The Steen Solution™ group exhibited borderline OI values during EVLP, and after two hours of post-transplant monitoring, median OI had declined to 122 [113; 131]. Meanwhile, in the experimental group, median OI remained at 364 [353; 375] (p = 0.000).

Lactate levels, serving as an indirect marker of IRI, remained within the permissible range (below 10 mmol/L) in both groups. However, they were significantly elevated in the group where EVLP was performed using Steen Solution™. After 120 minutes of follow-up, the median lactate level in the control group was 8 [7; 9] mmol/L, compared to 6 [5; 6] mmol/L in the experimental group. This, alongside the OI, indicates a reduced functional

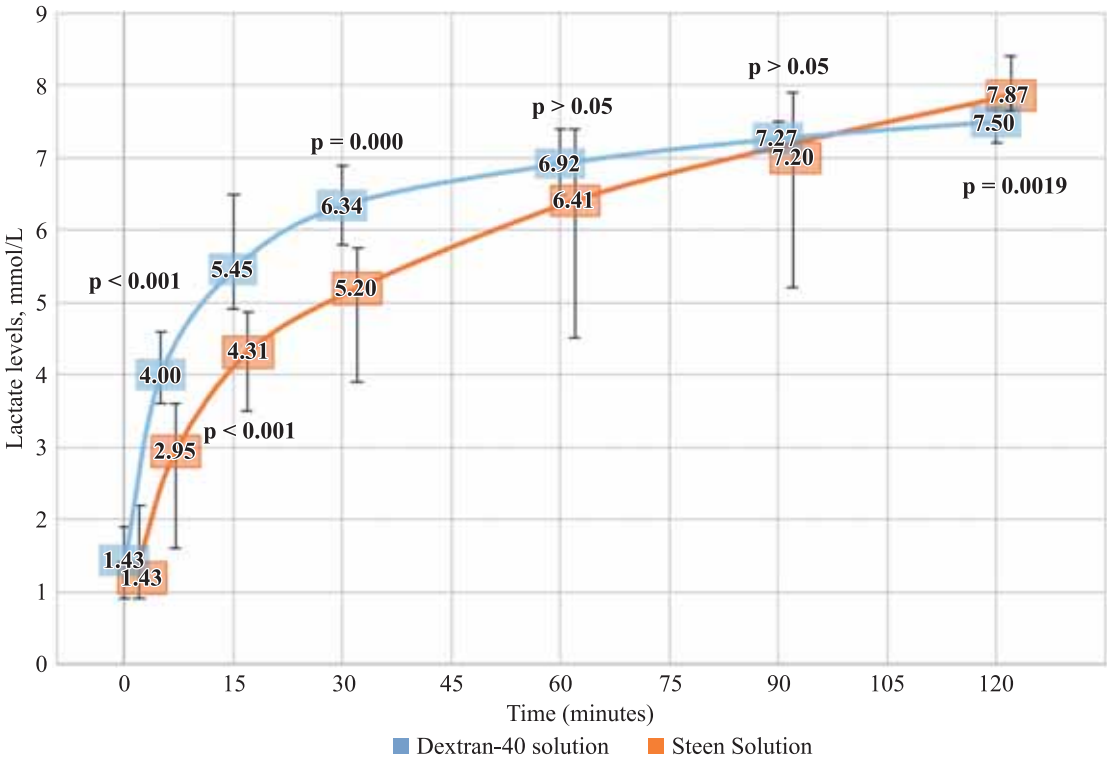

Fig. 4. Dynamics of changes in lactate levels during EVLP. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

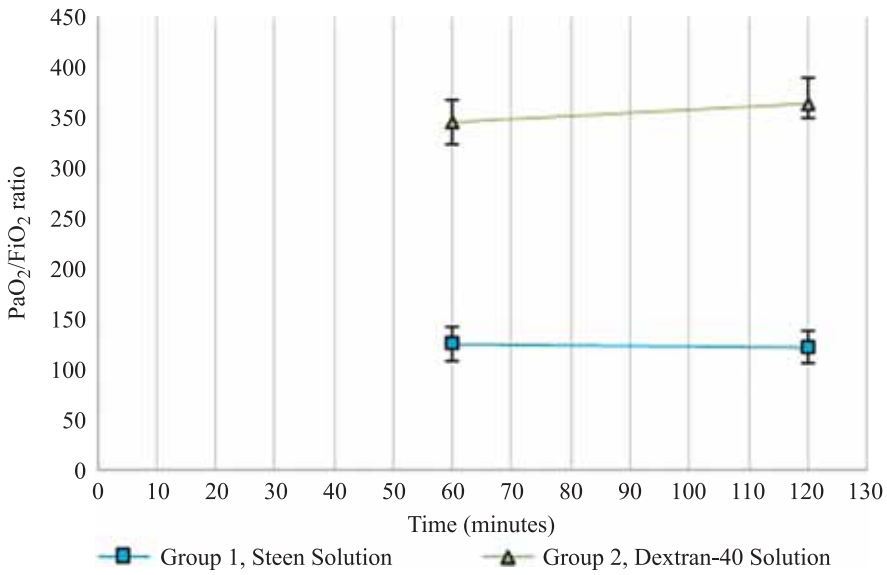

Fig. 5. Dynamics of oxygenation index after transplantation. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

status of the donor lung. The differences were statistically significant (p = 0.043).

Histopathological evaluation post-EVLP

Microscopic examination of lung specimens was done at 100× magnification (Fig. 7, a) and 200× magnification (Fig. 7, b). Each sample was assessed across the entire tissue section.

Upon completion of the 120-minute perfusion procedure, lung tissue samples were collected for histological analysis. Microscopic examination revealed occasional focal disruptions of the alveolar-capillary membrane, although the overall integrity of the lung parenchyma was preserved. The alveolar spaces appeared distended, but no signs of edema were observed. Mild thickening was noted in the alveolar septa and peribronchovascular regions.

Histopathological evaluation post-transplant

Histological evaluation of the transplanted lung specimens was performed at 100× magnification (Fig. 8, a) and 200× magnification (Fig. 8, b), across the entire tissue section in each case.

After OLLT, histological examination of the lung tissue was conducted. Most sections demonstrated preserved architecture of the lung parenchyma with well-expanded alveoli and no evident structural defects. Occasional microatelectasis was observed in isolated lung

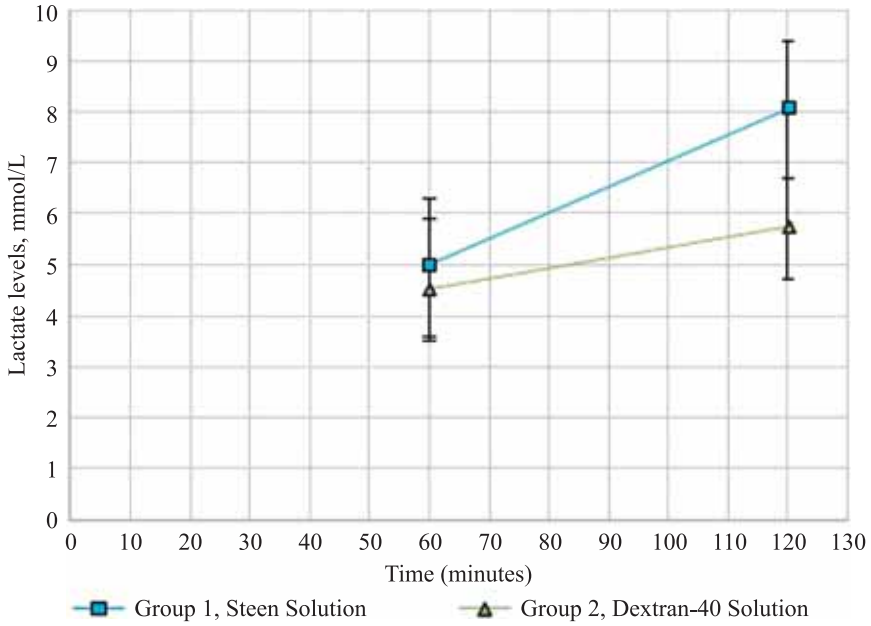

Fig. 6. Dynamics of changes in lactate levels after transplantation. The indices are presented as median, vertical lines indicate interquartile range, p is statistical significance

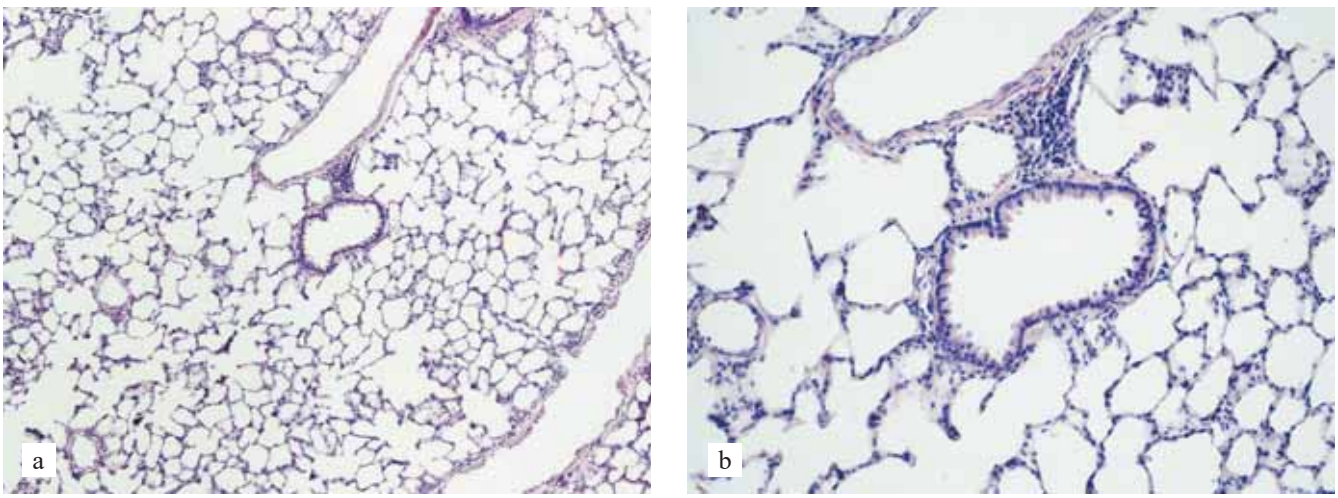

Fig. 7. Results of morphologic studies: a, histologic picture of donor right lung parenchyma after 120 minutes of EVLP, 100× magnification; b, histologic picture of donor right lung parenchyma after 120 minutes of EVLP, 200× magnification

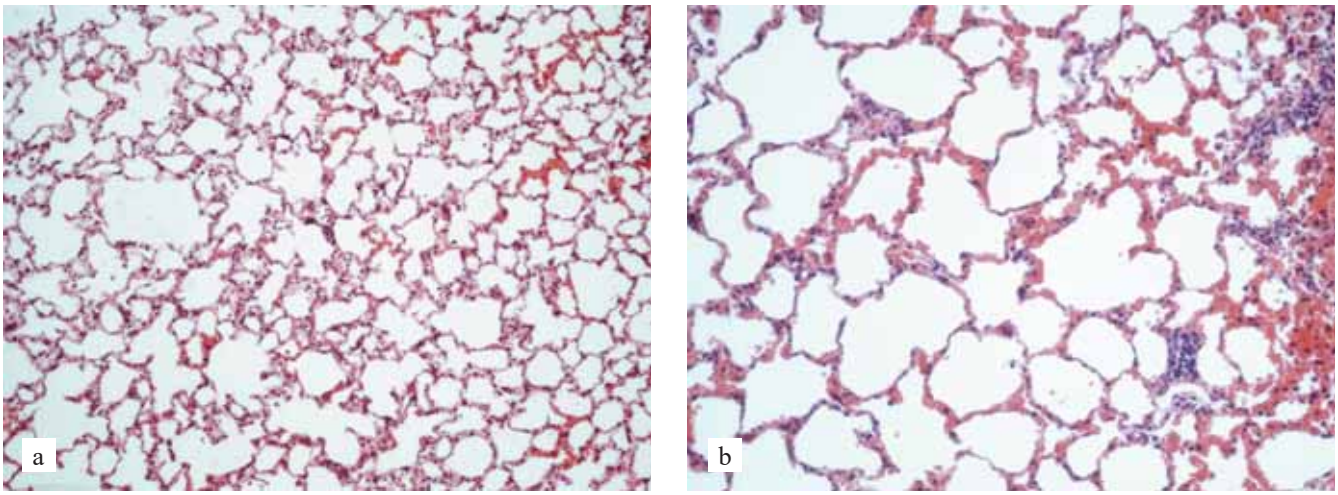

Fig. 8. Results of morphologic studies: a, histologic picture of donor left lung parenchyma 24 hours after transplantation, 100× magnification; b, histologic picture of donor left lung parenchyma 24 hours after transplantation, 200× magnification

segments. Mild thickening was noted in the alveolar spaces and peribronchovascular regions. Vascular congestion within the microcirculatory bed was present, along with sporadic foci of minor intraalveolar hemorrhage. Slight interalveolar septal edema was identified. The observed morphological picture in both groups is consistent with physiological changes following EVLP and subsequent transplantation and are not indicative of pathological alterations.

DISCUSSION

EVLP has become a crucial component of LT programs globally. While it has primarily been used for quality assessment of suboptimal donor lungs, its potential for active treatment and functional restoration of donor organs is even greater. One key element of the EVLP procedure is the perfusion solution, which enables the perfusion of isolated lungs without causing edema. Currently, the human albumin-based buffer solution known as Steen Solution™ is commercially available. Clinical studies have demonstrated its high efficacy in EVLP, employing various protocols and perfusion durations. Notably, Steen Solution can be used with or without the addition of donor blood. However, several studies have raised both positive and negative aspects regarding the addition of erythrocyte mass [25]. Despite the widespread clinical use of Steen Solution™, many research teams are developing alternative perfusion solutions.

The development of new solutions is driven by the need to identify the most optimal formulation for lung perfusion. A key factor in this search is the high cost of Steen Solution™ and, consequently, the financial limitations it imposes on the EVLP procedure. The high cost has significantly hindered the broader use of EVLP for both evaluation and rehabilitation of donor lungs. This study demonstrated the efficacy of a novel dextran 40-based combination solution. One of the main advantages of this experimental solution is its versatility, as it can be used both as a preservation agent for static hypothermic storage and as a perfusion solution during EVLP.

The study evaluated the efficacy of this experimental solution in a rat EVLP model, followed by single-lung transplantation. A static hypothermic storage period of 12 hours was chosen, as it is considered appropriate for clinical practice and models expanded criteria donation. In most translational *ex vivo* perfusion studies, the perfusion is typically carried out in pig models [26].

Experimental models using large animals are often associated with high maintenance costs and complex logistics. One potential solution to this issue is the use of small laboratory animals as experimental models. While such studies are economically advantageous, they present technical challenges in perfusion. To date, only one EVLP system designed specifically for rats, developed by Harvard Apparatus, is commercially available. Many research teams, however, have opted to design their own benches tailored to specific lung perfusion research needs, aiming to reduce the cost of consumables [27]. In our study, we used a custom-designed low-volume bench with a filling volume of just 25 mL, compared to foreign systems where the primary filling volume typically ranges from 150 mL [28–31]. This compact bench setup enabled a thorough analysis of the properties of the experimental solution, especially as the addition of donor blood was essential as the primary adjuvant. In contrast, experimental platforms with circuit filling volumes over 50 mL complicate the use of donor blood, significantly limiting their utility.

As a result of the study, the respiratory index (RI) at the end of perfusion was statistically significantly higher in the experimental group compared to the control group – 434 mmHg versus 394 mmHg, respectively. Des-

pite the higher RI in the experimental group, both groups surpassed the minimum threshold value of 350 mmHg, indicating that the perfusion was effective. PVR decreased in both groups, which is a positive prognostic indicator of perfusion efficacy. However, PVR in the experimental group was significantly lower than in the control group – 36 *vs.* 89 Dynes/sec/cm^{-5}, respectively. Morphological analysis showed that lung parenchyma architecture was preserved, with isolated areas of neutrophilic infiltration observed. Some sections displayed areas of alveolar-capillary membrane rupture. Slight thickening of alveolar air spaces and peribronchovascular connective tissue was noted in both groups. These findings highlight the positive attributes of the developed solution compared to the original Steen Solution™. The possibility of using the experimental solution for both preservation and EVLP provides clear advantages over the foreign counterpart. The study demonstrates the recovery of lung function after prolonged hypothermic storage, as evidenced by the increase in RI and decrease in PVR during perfusion.

CONCLUSION

The dextran 40-based combined solution showed its effectiveness both as a preservative agent for static hypothermic storage and as a perfusion solution for EVLP. The use of a low-volume bench for experimental studies in a rat model enhanced the efficiency of lung graft function analysis while reducing consumable costs. Donor lungs preserved and perfused with the experimental solution exhibited better RI and lower PVR compared to the original Steen Solution™, highlighting its efficacy. Recovery of lung function after prolonged hypothermic storage was confirmed by an increase in RI and a decrease in PVR during perfusion, indicating safe and adequate preservation of the graft. Therefore, the developed dextran 40-based solution presents a promising and effective alternative for preservation and *ex vivo* perfusion of donor lungs when compared to existing foreign solutions.

The authors declare no conflict of interest.

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