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PROTOCOL FOR ASSESSING THE EFFECTIVENESS OF LUNG PRESERVATION SOLUTIONS IN DONATION AFTER CIRCULATORY DEATH DONORS

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Objective: to evaluate the effectiveness of lung preservation in donation after circulatory death (DCD) using a non-perfusion-based preservation method. **Materials and methods.** The study was conducted on eight healthy male Landrace pigs (weight range: 40–60 kg), divided into three groups based on the preservation solution used: Celsior group (n = 3), lungs preserved using a solution prepared according to the Celsior formulation.; NewSolution group (n = 3), lungs preserved using a custom-formulated solution developed in-house; NaCl group (n = 2), lungs preserved using saline (control group). **Results.** In the study groups (Celsior and NewSolution), the oxygenation index after reperfusion exceeded 350, while the control group (NaCl) exhibited an oxygenation index of less than 350. **Conclusions.** The method described for evaluating the effectiveness of new lung preservation solutions is technically simple and cost-effective, as it enables rapid experimentation with a sufficiently large number of observations. While this approach may not address all challenges in experimental transplantology, it provides a practical and efficient tool for preliminary screening of lung preservation strategies.

Keywords: lung transplantation model, non-perfusion method, lung preservation.

INTRODUCTION

Most end-stage chronic lung diseases are accompanied by progressive respiratory failure and systemic hypoxia. Lung diseases are among the leading causes of mortality worldwide [6, 11]. The COVID-19 pandemic, marked by pulmonary damage and high death rates, further underscored the urgent need for effective therapies and spurred global interest in advancing lung disease treatment methods.

In such a situation, lung transplantation (LT) represents a definitive treatment option. However, its widespread use is constrained by several factors, including the complexity and high cost of the procedure, shortage of donors, and the high vulnerability of donor lungs to injury [4]. Due to this vulnerability, only 15–20% of donor lungs are deemed suitable for transplantation – significantly lower than the acceptance rates for donor livers (69%) and kidneys (90%) [6]. Consequently, the mortality rate among patients on the transplant waiting list ranges from 20% to 40% within two years [6].

One promising direction to address this challenge is to develop a novel preservation solution with enhanced protective capabilities. Preclinical evaluation of such solutions requires the use of experimental animal models. While orthotopic LT in large animals (e.g., pigs, dogs, monkeys) offers the most compelling data, these experiments are technically demanding, resource-intensive, and expensive. The need to use a donor-recipient pair further increases logistical and financial burdens. Moreover, the postoperative care of recipient animals significantly adds to the cost.

Given these limitations, an alternative and more practical approach involves the preservation of donor lungs followed by *ex vivo* reperfusion with blood. This model allows for the assessment of graft viability by measuring the oxygenation capacity of blood collected from the pulmonary veins.

OBJECTIVE

To develop a model for preserving lungs from donation after circulatory death (DCD) donors, using *ex vivo*

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perfusion of porcine lungs to assess the efficacy of a new preservation solution in preclinical studies.

MATERIALS AND METHODS

The study was conducted on 8 male Landrace pigs (weighing 40–60 kg), divided into three experimental groups based on the preservation solution used: Celsior group (n = 3, lungs preserved using a solution prepared according to the standard Celsior formulation), NewSolution group (n = 3, lungs preserved using a custom-formulated solution developed in-house, and NaCl group (n = 2, lungs preserved using a 0.9% saline as a control. Our in-house solution was prepared with changes in the components and concentrations of a previously presented recipe [9]. The animals were randomly allocated to the respective groups. All pigs were kept under standardized laboratory conditions with free access to complete feed and water ad libitum. The study protocol was reviewed and approved by the institutional local ethics committee.

All procedures were performed under aseptic conditions, with the animals under general anesthesia and mechanical ventilation (MV) using an inspired oxygen fraction (FiO_2) of 21%. Cannulation of the femoral vein was performed, and 2 liters of venous blood were collected into CPDA-containing blood bags for subsequent use. Intravenous heparin was administered at a dose of 300 IU/kg. This was followed by the administration of 10 mL of 2% lidocaine intravenously to induce cardiac arrest. Asystole was confirmed by continuous electrocardiographic (ECG) monitoring.

A median sternotomy was performed, and the pericardium was incised. The pulmonary trunk was cannulated, and the left atrial appendage was opened. Lung perfusion was initiated using a gravity-driven fluid column with a pressure of 180 cm H_2O. A total of 2 liters of cold preservative solution was administered over approximately 15 minutes. Perfusion was stopped upon the appearance of clear effluent from the left atrial appendage. The lungs were then explanted as an intact block.

On a separate sterile table, the lungs were further perfused with an additional 1 liter of the preservative solution for 10 minutes under aseptic conditions. Subsequently, the pulmonary veins were cannulated, and retrograde perfusion of the lung block was performed with 1 liter of the same solution.

Upon completion of lung perfusion, the explanted lungs and the collected blood (stored in CPDA-containing bags) were placed in a refrigerator and maintained at +4 °C for 4 hours. Following this cold ischemic period, both the lungs and the stored blood were removed from refrigeration and allowed to equilibrate at room temperature for 15 minutes to facilitate preliminary warming. Subsequently MV was initiated with an inspired oxygen fraction (FiO_2) of 21%, and *ex vivo* perfusion of the lungs was commenced using the stored autologous blood. Perfusion was carried out through the pulmonary trunk under a hydrostatic pressure of 180 cm H_2O. Once blood flow through the pulmonary veins was established, blood samples were collected to assess acid-base balance, including lactate concentration, as well as the partial pressures of oxygen (pO_2) and carbon dioxide (pCO_2). These parameters were analyzed using an ABL800 blood gas analyzer (Radiometer, Denmark). The oxygenation index (OI) was calculated as the ratio of pO_2 to FiO_2.

Measurements were obtained at three time points: prior to initiation of perfusion (baseline, T0), and at 10 minutes (T10) and 20 minutes (T20) after the onset of blood perfusion. An OI exceeding 350 following reperfusion was considered indicative of effective lung preservation.

At the conclusion of the experiment, lung tissue samples were fixed in 10% neutral buffered formalin for histopathological analysis. All quantitative data were statistically analyzed using Statistica 12 (StatSoft) and R statistical software.

RESULTS

At baseline (T0), prior to initiation of lung reperfusion, pO_2 in the venous blood did not exceed 53 mmHg across all groups (Table, Fig., c). Ten minutes after the onset of *ex vivo* reperfusion with blood, a marked increase in pO_2 was observed in the groups preserved with either the Celsior or NewSolution preservation solutions. In both groups, pO_2 levels in the pulmonary venous effluent approached 100 mmHg, while the OI neared 500 (Table, Fig., a).

In the control group preserved with saline solution, the OI consistently remained below 350 throughout the entire reperfusion period.

In contrast, both preservation solution groups demonstrated an increases in the OI, pO_2, and blood lactate concentration (Fig., a, c, d). Concurrently, pCO_2 exhibited a decreasing trend (Fig., b).

DISCUSSION

The shortage of donor organs and the vulnerability of grafts to ischemia-reperfusion injury (IRI) remain major challenges in modern transplantology [12, 14]. According to the Global Observatory on Donation and Transplantation, 102,090 kidney transplants were performed worldwide in 2023, while 361,197 patients remained on the waiting list [13]. Thus, less than one-third of patients in need were able to receive assistance.

A similar situation is observed in Russia, where only 2,555 of 8,378 patients requiring organ transplantation underwent the procedure in the same period [13]. In clinical practice, the ideal donor is a brain-dead donor; however, the number of such donors is extremely limited. In recent years, international transplant programs have begun to explore new approaches, including the use of organs from patients who have undergone euthanasia [13]. According to the literature, 2,966 cases of euthanasia were performed in Belgium in 2022, and

82,963 cases were reported in the Netherlands between 2002 and 2021. The presented data underscore the critical severity of the organ shortage problem. Even with recent efforts to expand the donor pool through inclusion of organs from patients who have undergone euthanasia, the current supply remains insufficient to meet clinical needs. Consequently, research into the utilization of suboptimal donor organs and the development of strategies to improve their functional viability is highly relevant.

One promising approach involves the application of new preservation solutions and *ex vivo* perfusion techniques to restore or enhance the function of donor organs that would otherwise be considered unsuitable for transplantation [1]. Experimental studies in animal models provide a controlled platform for investigating these strategies. By refining preservation methods and improving the functional status of marginal organs during *ex vivo* assessment, it may be possible to expand the criteria for organ acceptance and increase the number of viable transplants [5, 6, 11].

The issues outlined above are particularly critical in the context of LT. Both in Russia and globally, the current limitations in LT emphasize the pivotal role of preclinical and experimental studies in addressing key challenges in transplantology. The use of organs from DCD donors represents could somewhat alleviate the organ shortage problem [8, 10].

This paper presents experimental findings using a DCD donor model, which is essential for exploring techniques to procure and evaluate the suitability of lungs from non–heart-beating donors for transplantation. Developing and validating methods for assessing the effectiveness of lung preservation in such experimental large-animal models can provide valuable insights that are directly translatable to clinical practice.

Several experimental LT models and functional assessment techniques have been described reported.

A well-established approach for studying IRI in the lungs involves *ex vivo* or *in situ* lung isolation with continuous perfusion using a synthetic medium and temperature control via a heat exchanger. This model is widely recognized as an effective compromise between complex large-animal transplantation experiments and small-rodent studies [3, 5–7, 11].

In the present study, mechanical perfusion was not used. Instead, a non-perfusion lung preservation strategy was used, comparing three groups: lungs preserved in a standard commercial solution, lungs preserved in the authors' experimental solution, and a saline-preserved control group. The goal was not to investigate lung reconditioning, but rather to evaluate baseline preservation efficacy.

During subsequent reperfusion with autologous blood, a gradual improvement in functional parameters was observed in the two preservation solution groups. In contrast, the saline-preserved control group exhibited the absence of effective gas exchange.

Interestingly, histopathological analysis of lung tissue after reperfusion revealed no significant differences between the groups. All samples demonstrated a morphology consistent with that of viable lung tissue, which did not correlate with the partial pressures of respiratory gases. These findings suggest that histology alone is not an informative marker of the functional status of preserved lungs.

The findings from this study, conducted using an isolated lung model from DCD donors, are consistent with previously published data indicating that a progressive increase in oxygenation during reperfusion is a key marker of effective lung preservation and viability [2, 6, 11]. In both preservation solution groups, the post-reperfusion OI exceeded 350, indicating effective preservation.

Importantly, the study demonstrated that the experimental preservative solution developed by the authors was comparable in efficacy to the commercially available Celsior solution.

Nevertheless, the study has several limitations. First, the sample size was small, with no more than three ob-

Table

Lung gas exchange parameters after preservation and blood reperfusion following 4 hours of cold storage

Time (min)	Preservation solution	IO	pCO_2	pO_2	Lac
0	Celsior	225,2 [225,2; 225,2]	95,20 [95,2; 95,2]	47,30 [47,3; 47,3]	3,80 [3,8; 3,8]
0	NaCl	250,5 [250,0; 250,0]	94,9 [94,9; 94,9]	52,6 [52,6; 52,6]	4,0 [4,0; 4,0]
0	NewSolution	184,3 [175,2; 221,0]	83,6 [66,3; 83,6]	38,7 [36,8; 46,4]	3,6 [1,4; 5,9]
10	Celsior	504,8 [504,8; 504,8]	18,7 [18,7; 18,70]	106,0 [106,0; 106,0]	6,6 [6,6; 6,6]
10	NaCl	266,67 [249,5; 283,8]	56,4 [32,8; 79,9]	56,0 [52,4; 59,6]	5,1 [1,5; 8,7]
10	NewSolution	466,4 [432,9; 500,0]	36,05 [25,4; 46,7]	97,9 [90,9; 105,0]	5,2 [3,2; 7,2]
20	Celsior	590,5 [590,5; 590,5]	19,4 [19,4; 19,4]	124,0 [124,0; 124,0]	7,1 [7,1; 7,1]
20	NaCl	252,9 [252,9; 252,9]	50,0 [50,0; 50,0]	53,1 [53,1; 53,1]	5,4 [5,4; 5,4]
20	NewSolution	625,2 [384,5; 954,8]	36,6 [21,3; 60,2]	131,3 [80,8; 200,5]	6,2 [2,8; 11,1]

Abbreviations and definitions. NewSolution, a preservation solution developed in-house; Celsior, a preservation solution prepared according to the Celsior formula; NaCl, saline; OI, oxygenation index (measured at 21% oxygen fraction); pO_2, partial pressure of oxygen (at 21% oxygen fraction) in mmHg; pCO_2, partial pressure of carbon dioxide in mmHg; Lac, lactate level in mmol/L.

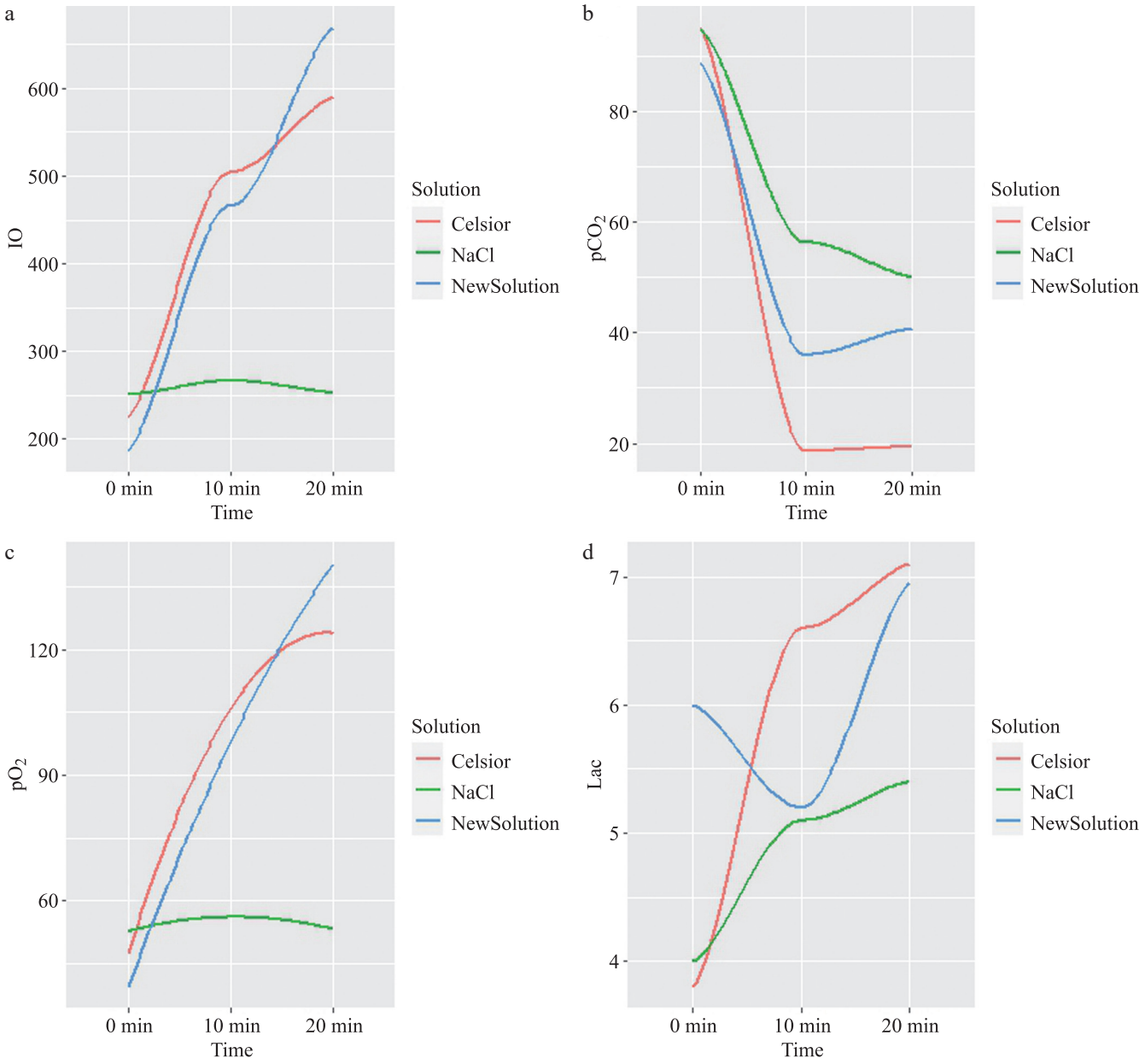

Fig. Lung function indicators after preservation during blood reperfusion after 4–10 hours of cold storage. Abbreviations and definitions: NewSolution, a preservation solution developed in-house; Celsior, a preservation solution prepared according to the Celsior formula; NaCl, saline; OI, oxygenation index (measured at 21% oxygen fraction); pO_2, partial pressure of oxygen (at 21% oxygen fraction) in mmHg; pCO_2, partial pressure of carbon dioxide in mmHg; Lac, lactate level in mmol/L

servations per group. Second, blood reperfusion was limited to 30 minutes. Finally, the preserved lungs were near-ideal donor organs, with minimal warm ischemic exposure, as explantation was performed immediately after circulatory arrest. These factors should be considered when extrapolating the results to clinical scenarios.

The experimental method described for evaluating the effectiveness of new lung preservation solutions is technically straightforward, cost-effective, and suitable for rapid implementation with a relatively large number of observations. While this approach does not fully address all the complexities of experimental transplantology, it can be used for the preliminary assessment of preservation strategies.

FINDINGS

1. The presented method for assessing the effectiveness of lung preservation in a DCD donor model using large animals – by measuring key gas exchange parameters during *ex vivo* blood perfusion – can serve as a first-stage approach in preclinical evaluation of novel preservation solutions. This model may be considered a practical alternative to orthotopic LT in long-term experimental studies.
2. For functional assessment of lungs in this experimental setting, pO_2 and pCO_2, along with OI, are the most informative indicators of preservation efficacy.

3. The histological picture of non-functioning lung tissue closely resembles that of functioning tissue. Therefore, histological examination alone is of limited diagnostic value in determining lung function following 4 hours of cold storage with isolated perfusion.

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The authors declare no conflict of interest.

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