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SURGICAL TECHNIQUE FOR EXPLANTATION OF A FUNCTIONING CARDIOPULMONARY COMPLEX IN AN EXPERIMENT

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Objective: to develop and approve the surgical technique for explantation of a functioning cardiopulmonary complex under normothermic autoperfusion. **Materials and methods.** Landrace pigs were used as the experimental model for a series of acute experiments (n = 10). During the experiment, invasive pressure in the cavities of the heart and main arteries, blood gas composition, and myocardial contractility were monitored. The functioning cardiopulmonary complex was explanted through a median sternotomy. The explanted complex was conditioned at 37–38 °C for 6 hours. **Results.** In the course of a series of experiments, it was shown that stable operation of the isolated heart-lung complex *ex vivo* for 6 hours was fundamentally possible provided that the parameters of the basic homeostasis constants are maintained. The technological solutions used made it possible to ensure safe hemodynamic and anatomical isolation of the working cardiopulmonary complex. **Conclusion.** The developed protocol for isolating a functioning cardiopulmonary complex allows to provide stable graft function for 6 hours under normothermic autoperfusion. Implementation of this concept in the development of transport systems would significantly facilitate their design and eliminate the use of expensive components. This would contribute to widespread introduction into clinical practice.

Keywords: chronic heart failure, heart transplantation, heart preservation, autoperfusion, donor organ preservation, ex vivo organ perfusion.

INTRODUCTION

Solid organ transplantation is undoubtedly one of the most significant achievements in medicine in the 20th century. However, many problems in this field still remain unresolved [1]. One of such problems is the development of technology for long-term conditioning of donor organs. The safe time limit of cold preservation of the heart remains the main constraint that does not allow expanding the geography of donor bases [2, 3]. Given the obvious advantages of normothermic conditioning of donor organs over static cold preservation, most current graft preservation strategies focus on maintaining blood flow and temperature control [4, 5]. Instead of cooling the organ to slow down metabolic processes, machine perfusion maintains normal metabolic activity under conditions close to the physiologic environment. This allows to significantly reduce cold ischemia time or abandon it altogether when implanting an organ into the recipient's body, as well as to perform extended screening of the morphofunctional status of the graft [4, 6–8]. However, widespread use of such perfusion platforms in many countries is limited by high cost [9–13]. In this regard, the development of an effective and cost-effective method of long-term conditioning of donor hearts is an urgent problem for modern transplantology.

Long-term studies of the physiological laws of cardiac autoregulation have traditionally been associated with the development of methods for long-term maintenance of effective cardiac activity *ex vivo* [14–16]. And although at that time, the ideas of experimental physiologists about transplantology were very far from the modern ones, today the realization of the concept of autonomous survival of donor organs under conditions of normothermic autoperfusion can become a solution to the problem of long-term conditioning of graft, significantly simplify the development of machine perfusion platforms and contribute to widespread introduction of these technologies into clinical practice.

MATERIALS AND METHODS

Female Landrace pigs, weighing 50 ± 5 kg, aged 4–5 months, were used as an experimental model for a series of acute experiments (n = 10). Care, mainte-

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nance of the experiment, observation and withdrawal of animals from it were performed in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes (Strasbourg, March 18, 1986) and were approved by the Bioethics Committee at Meshalkin National Medical Research Center (Protocol No. 2 of September 1, 2022).

On the day of the experiment, all animals were premedicated (Zoletil 100) on an empty stomach. The dose was selected individually, based on weight and height parameters. After the onset of sleep, the surgical field and the area of catheterization of neck vessels were prepared. Then the animal was transported to the operating table and fixed in a supine position for subsequent tracheal intubation, installation of central arterial and venous catheters into the external jugular and common femoral vein. The experiment was performed under endotracheal anesthesia with sevoflurane and myorelaxation (rocuronium bromide). Artificial ventilation (AV) was performed using an anesthetic breathing machine Fabius Plus (Draeger, FRG) with positive pressure on inhalation (20–30 cm of water column) and on exhalation (5–8 cm of water column), with a respiratory volume of 8 ml/kg, with a frequency of 12–14 breaths per minute. Vital parameters were recorded using an IntelliVue MP70 monitor (Philips, Netherlands).

During the experiments, we monitored invasive blood pressure (IBP) by catheterization of the right common carotid artery, central venous pressure (CVP) by catheterization of the right external jugular vein, and blood gasses. Blood analysis was performed using an automated hematology analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamics were investigated by catheterization of the right heart with a Swan–Ganz catheter, as well as using a portable multifunctional ultrasound system Philips CX50 (Philips Ultrasound, USA) with ECG synchronization.

Explantation of the functioning cardiopulmonary complex (fCPC) was performed through median sternotomy. Isolation of fCPC was initiated with mobilization of the superior vena cava (SVC) and ligation of the unpaired vein. Then the brachiocephalic trunk (BCT), both carotid arteries, and the left subclavian artery (LSCA) were isolated. The trachea was carefully separated from the esophagus using an electrocoagulator, achieving thorough hemostasis. Particular attention was paid to the release of the lower lung lobes, since the basal lung sections are extremely deep and mostly covered by the diaphragm dome, which makes visualization difficult and carries a high risk of surgical trauma to the parenchyma.

After administration of heparin (3 mg/kg body weight), LSCA was ligated and transected, avoiding rough traction. A 16–18 Fr arterial cannula was inserted through the right subclavian artery toward the heart and connected to a reservoir suspended 70 cm above the heart. The semi-unpaired vein draining blood in the animals directly into the coronary sinus was ligated and transected. Under IBP control, all brachiocephalic arteries were ligated, avoiding pressure increase of more than 130–140 mmHg in the aortic root due to dosed exfusion of blood into the reservoir. After clamping the descending thoracic aorta at the level of the isthmus, arterial blood was withdrawn into the reservoir until the blood level stabilized. After stabilization of blood level and arterial pressure, 1–1.5 liters of Ringer's solution was injected into the femoral vein. Then the vena cava was ligated and transected, the trachea was transected and reintubated with a cuffed tube, the fCPC was finally separated from the surrounding tissues, transferred to a container with warm saline (38°C) and observation continued.

Statistical data processing was performed using Statistica 10.0 software (StatSoft Inc., USA). Normality of distribution was checked using the Shapiro–Wilk test with subsequent assessment of equality of variances by Levene's test. In the case when distribution in the experimental groups was normal and there was intergroup equality of variance, further processing was carried out using parametric statistics, the Student's t test. When the distribution is different from the normal, nonparametric statistics were used. Differences between the parameters were considered reliable at $p < 0.05$.

RESULTS

In a series of acute experiments, 10 fCPC explantations were performed with 6-hour follow-up (Fig. 1).

Active reservation of the animals' own blood through an arterial cannula placed in the brachiocephalic trunk, and displacement of the maximum volume of blood from the lower half of the body by infusion of crystalloid solution into the femoral vein allowed to create the necessary blood supply (1500–2000 mL) to maintain normovolemia in fCPC during 6 hours of follow-up. An arterial blood reservoir, suspended at a height of 70 cm above the heart level, provided stable conditions for transfer of isolated fCPC into the transport container, acting either as a receiver for its own cardiac output or providing antegrade coronary perfusion according to the Langendorff technique (Fig. 2). After placement of fCPC in a container and installing measuring sensors, the arterial trunk was clamped (Fig. 2, b), then the blood volume in the fCPC was adjusted under pressure control in the heart cavities.

The main hemodynamic parameters were measured using a Philips CX50 ultrasound system (Philips Ultrasound, USA) with ECG synchronization, as well as direct tonometry in the heart cavities and main arteries (Table 1).

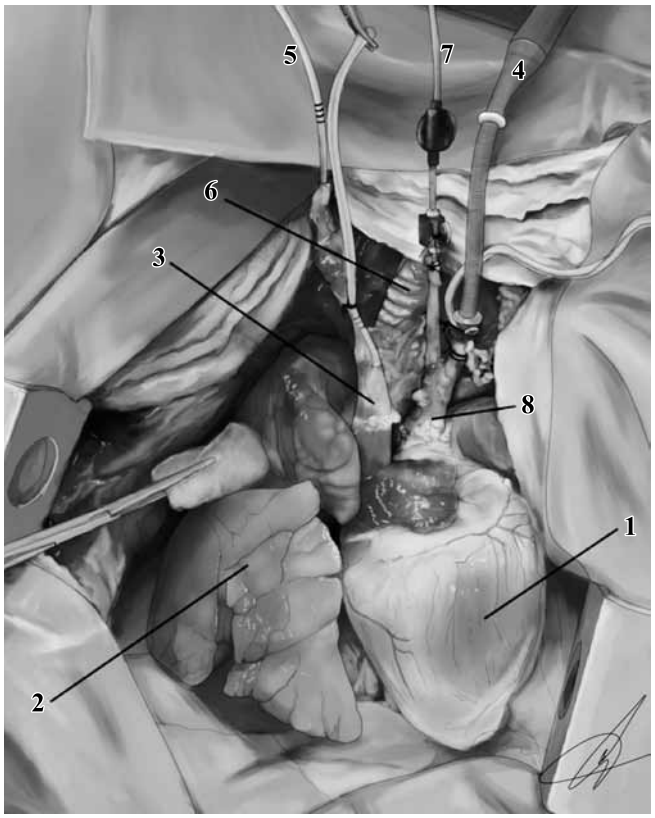

Fig. 1. General view of surgical wound; 1, heart; 2, right lung; 3, superior vena cava; 4, arterial cannula; 5, Swan–Ganz catheter; 6, trachea; 7, arterial catheter; 8, ascending aorta

To maintain the main homeostasiologic constants for 6 hours of normothermic autoperfusion, calcium chloride and glucose were infused into the right heart sections. fCPC ventilation was performed using anesthesia-breathing apparatus FabiusPlus (Draeger, FRG) with positive inspiratory pressure (20–30 cm of water column) and expiratory pressure (5–8 cm of water column) with respiratory volume of 8 mL/kg of body weight, frequency of 12–14 breaths per minute, FiO_2 – 70%. The main parameters of blood gas composition are shown in Table 2.

DISCUSSION

The need for research into the functioning of an isolated heart and the cardiopulmonary complex was fully recognized more than a century ago. In 1866, at the Carl Ludwig Physiological Institute in Leipzig, C. Elias described the effect of diastolic filling in an isolated perfused frog heart on cardiac output [15]. Later, a study describing the effect of filling pressure on contraction amplitude was published by C. Joseph in 1869 [16]. In 1881, H.N. Martin described the technique of preparing a hemodynamically isolated cardiopulmonary complex of a dog with an open chest using a resistor and a reservoir between the aorta and vena cava [17]. Thin-walled tubes, surrounded by a sealed cylinder (similar to modern hemodialysis columns), were used as a resistor. This

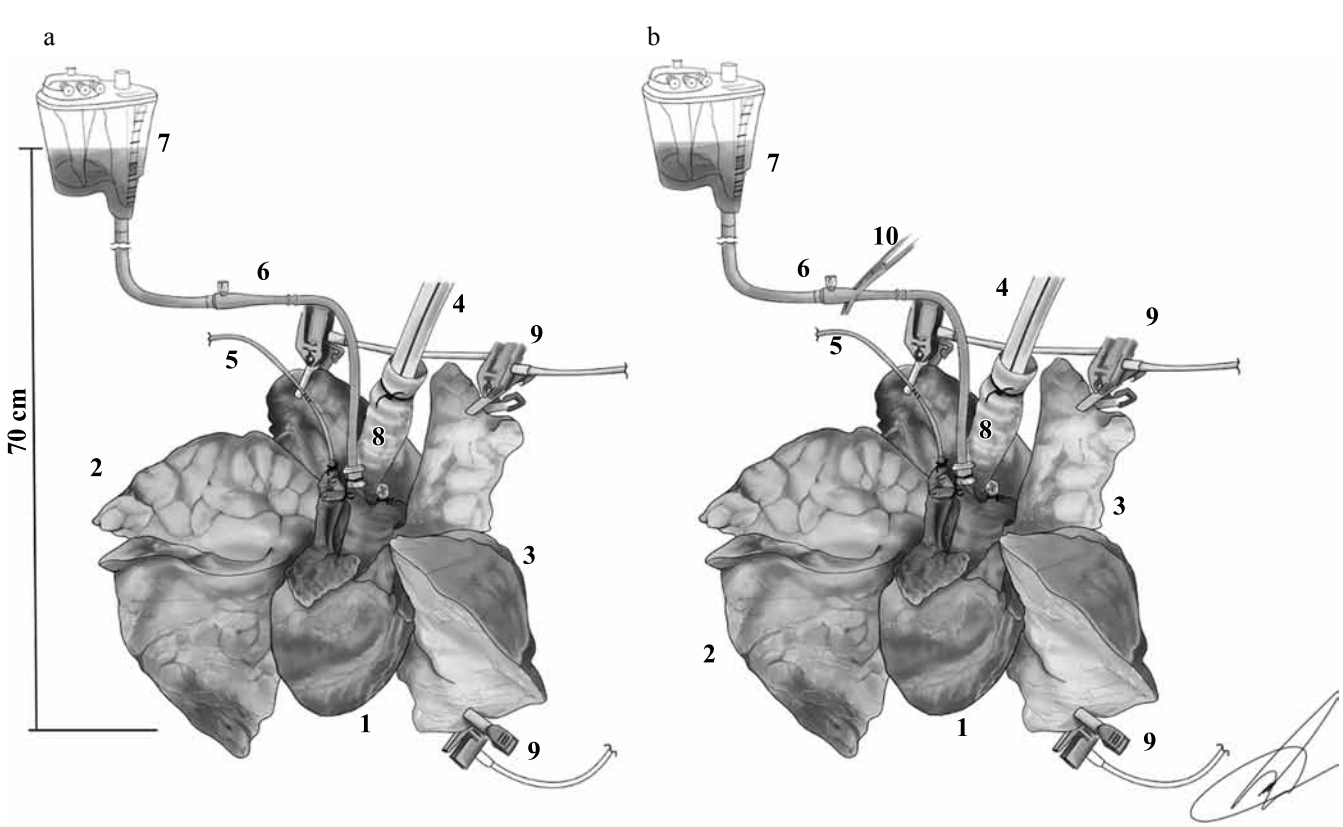

Fig. 2. Diagram of the isolated cardiopulmonary complex: a, stage of blood exfusion into the reservoir and preparation for transfer of the complex into the container; b, stage of final hemodynamic isolation of a working cardiopulmonary complex; 1, heart; 2, right lung; 3, left lung; 4, intubation tube; 5, Swan–Ganz catheter; 6, arterial cannula; 7, blood reservoir; 8, trachea; 9, electrocardiograph electrodes; 10, clamp

drug was used to study the contractile properties of the heart, cardiac metabolism, regulation of coronary blood flow and the effect of various pharmacological drugs [18–20]. Another widely known method of maintaining heart function after anatomical isolation was proposed in 1895 by O. Langendorff [21]. The developed method involved retrograde injection of saline into the aortic root. At the same time, it was proved that the heart could function for a long time thanks to the consumption of oxygen dissolved in saline solution. However, despite the absence of external work of the emptied left ventricle of the heart under retrograde Langendorff perfusion conditions, the use of saline significantly limited the survival time of the heart. Since meeting the myocardial oxygen demand required increased coronary flow of crystalloid solution, this inevitably led to myocardial edema and deterioration of its contractility. Enrichment of the solution by addition of washed red blood cells restored the oxygen content and viscosity of the perfusate, which helped to reduce the resistance of the capillary coronary bed.

Another modification that made the Langendorff preparation more practical was the scheme that included a reservoir filled with perfusate under constant pressure and connected both to the left atrium and aorta through a system of valves and artificial vascular resistance [22]. At the same time, blood flowing from the coronary sinus was reserved and excluded from recirculation. Thanks to this scheme, the left ventricle generated cardiac output by doing external work under controlled filling pressure conditions. A similar type of cardiac isolation was proposed by G. Elzinga [18].

In 1926, E.H. Starling and M.B. Visscher published the results of studies of an isolated heart, formulating the well-known law describing the relationship between diastolic heart volume and the force of heart contractions [14, 23]. At the same time, historically, Starling's cardiac preparation was not subjected to as many modifications as Langendorff's scheme.

A work by P.H. Huisman et al. presents a description of a modified Starling preparation, which was developed to study ventricular electrical activation and then adapted to study the valve apparatus and ventricular function and mechanics [24]. The P.H. Huisman method allowed to ensure a long period of stable mechanical work of

Table 1

Main hemodynamic parameters

Parameter	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
HR (bpm)	66 [51; 95]	94 [90; 100]	97 [87; 105]	93 [86; 97]	89 [87; 92]	89 [89; 94]
RAP (mmHg)	0	–1	5	4	3	1
mRVP (mmHg)	7 [3; 12]	8.3 [6.5; 11]	10.6 [8.7; 12]	6 [5; 7]	4.3 [3; 5.5]	5.5 [3; 7.5]
mPAP (mmHg)	6.5 [3.5; 10]	5.2 [3.5; 8.5]	10.5 [10; 11]	6.8 [4; 9]	5.2 [4; 6]	7.5 [4.7; 9.7]
PWP (mmHg)	1	0	6	2	1	4
IBP in aorta (mmHg)	67 [54; 74]	75 [65; 85]	93 [89; 101]	85 [70; 100]	73.5 [64.5; 85]	70.8 [62.5; 78]
CO (L/min)	903.0	846.0	1015.0	1089.0	1414.0	899.0

Note: HR, heart rate; RAP, Right Atrial Pressure; mRVP, Mean Right Ventricular Pressure; MPAP, Mean Pulmonary Artery Pressure; PWP, pulmonary wedge pressure; IBP, invasive blood pressure; CO, cardiac output. Data is presented as Me [Q1; Q3].

Table 2

Main parameters of blood gas composition

Parameter	Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Hematocrit (%)	24.5	29.8	28.5	26.0	27.0	27.6
Hemoglobin (g/L)	79	79	78	69	87	90
pH	7.9	7.8	7.8	7.8	7.7	7.7
Lactate (mmol/L)	6.3	8.4	5.3	1.5	2.1	3.6
Glucose (mmol/L)	7.7	11.1	8.7	3.5	5.9	3.3
Aortic PaO_2 (mmHg)	248	170	190	197	238	175
Right atrial PaO_2 (mmHg)	39.6	40.3	42.8	31.4	34.7	31.2
Left atrial PCO_2 (mmHg)	5.7	5.6	6.7	6.7	8.4	6.9
PaO_2/FiO_2	2.8	2.6	2.71	2.8	3.4	2.5
K^+ (mmol/L)	3.8	3.3	2.2	2.8	3.1	3.0
Na^+ (mmol/L)	143	147	152	155	160	163
Ca^{++} (mmol/L)	0.72	0.77	1.18	1.34	0.87	1.39

Note: PaO_2, arterial partial pressure of oxygen; PCO_2, partial pressure of carbon dioxide; FiO_2, fraction of inspired oxygen; K^+, potassium; Na^+, sodium; Ca^{++}, calcium.

the heart largely due to the use of whole, practically undiluted fresh blood as perfusate. Another important technique was the preservation of the anatomic integrity of the anastomosis between the pulmonary veins and the left atrium, which ensured normal filling of the left ventricle. The authors also corrected the weaknesses of the original Starling technique by performing complete denervation of the cardiopulmonary complex.

Fundamental knowledge gained during these studies formed the basis of modern technologies for prolongation of cardiac graft survival *ex vivo*. However, the principle of autonomous survival of an isolated cardiopulmonary complex remains unrealized in any of the existing models of donor organ transport modules [14, 24–26]. Previous experiments made it possible to identify several critical requirements necessary for successful isolation and long-term functioning of fCPC, among which are compliance with the principles of saving the donor blood volume for subsequent correction of the level of volemia and the possibility to effectively maintain the normothermic graft conditioning mode [27]. During the study, the effectiveness of using active exfusion of donor blood at the expense of the donor's own cardiac output was shown. The combination of methods of functional isolation of fCPC and Langendorff perfusion elements made it possible to obtain the maximum possible blood volume, to ensure stable hemodynamic parameters at all stages of fCPC explantation and dosed volume loading of the complex. Stability of hemodynamic parameters and self-regulation of coronary blood flow due to the height of the blood reservoir location allow for the most careful fCPC dissection with meticulous hemostasis.

An important feature of isolated fCPC functioning is the ability to maintain sufficient coronary blood flow under absolute hypovolemia of pulmonary circulation. If in the case of anatomical integrity, the right ventricles actually determine the left ventricular flow rate, in the case of fCPC isolation, the left sections are in optimal conditions of pressure and volume load with minimal shock work of the right ventricle. So, despite maintenance of average level of arterial pressure in the aortic root at 65–75 mmHg, cardiac output ranged from 846.0 to 1414.0 mL per minute, with the presence of a pulse curve in the right ventricular cavity corresponding in characteristics to that before fCPC explantation at all stages of the experiment, there was complete absence of a pulse curve in the pulmonary artery trunk.

Such sparing conditions for autonomous functioning of fCPC provide "rest" to the right heart, allowing it to generate sufficient stroke volume with minimal afterload. It is important to note that these fCPC functioning conditions have much in common with those observed during active cardiac perfusion. However, the development of the transport system based on normothermic autoperfusion principles allows us to significantly reduce the economic costs of prosthetic pumping function of the heart and oxygenating function of the lungs. This will facilitate introduction of long-term conditioning of donor organs *ex vivo* into clinical practice.

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The authors declare no conflict of interest.

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