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NORMOTHERMIC EX VIVO HEART AND LUNG AUTOPERFUSION: ASSESSMENT OF FUNCTIONAL STATUS AND METABOLISM

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Objective: to carry out a comparative study of the efficacy of a 6-hour normothermic *ex vivo* heart and lung autoperfusion and cold cardioplegia using Bretschneider's solution (Custodiol®, Germany). **Materials and methods.** Landrace pigs weighing 50 ± 5 kg at the age of 4–5 months (n = 10) were used as a model for a series of acute experiments. In the experimental group (n = 5), the cardiopulmonary complex was conditioned by autoperfusion for 6 hours. In the control group, the heart pumping function was restored after 6-hour cold cardioplegia using Bretschneider's solution. The efficiency of graft preservation was assessed by measuring hemodynamic parameters, myocardial contractile function, and myocardial oxygen consumption. **Results.** After reperfusion and repeated isolation of the working cardiopulmonary complex, cardiac output was 0.63 [0.37; 0.8] L/min and 0.37 [0.23; 0.37] L/min in the experimental and control groups, respectively ($p < 0.05$). Indicators – global left ventricular stroke work index and preload recruitable stroke work – were significantly higher in the experimental group ($p < 0.05$). **Conclusion.** Normothermic autoperfusion is significantly more effective in preserving the morphofunctional status of a donor heart than static cold storage with Bretschneider solution for 6 hours.

Keywords: cold cardioplegia, cardiac transplantation, heart preservation, autoperfusion, normothermic perfusion, ischemia-reperfusion injury.

INTRODUCTION

Ischemia-reperfusion injury (IRI) is the main adverse outcome of the restoration of blood flow in a donor heart. IRI is also the main cause of early graft dysfunction. In most cases, donor heart harvesting begins with termination of blood supply and in situ washing with a cold preservative solution, followed by explantation and storage on ice [1]. Meanwhile, despite several negative consequences, this cold cardioplegia method remains the gold standard for donor organ preservation. Cooling of the organ to 4 °C slows down cellular metabolism, thereby reducing oxygen demand, but anaerobic metabolism and other cellular metabolic processes continue at lower temperatures [2–4].

Even though the IRI cascade is activated in all donor grafts, its reversibility as well as the limits of plasticity of compensatory mechanisms of endothelial autoregulation, are mainly determined by graft ischemic time. Prolonged ischemia is especially dangerous for cardiac grafts because of extreme sensitivity of the myocardium to hypoxia [5]. Using the cold cardioplegia preservation method, heart grafts can be safely preserved for 4–6 hours; further extension of the ischemic time leads to higher risk of early graft dysfunction [1, 6]. Machine perfusion technologies allow avoiding complications characteristic of static cold preservation methods. However, the search for the optimal scheme and mode of ex vivo coronary perfusion of the heart graft remains a subject of research. Despite the proven safety of ex vivo perfusion of donor heart at the stage of transportation, the problem of assessing graft contractility remains unresolved. The device used in clinical practice – OCS™ Heart Transmedics® system (Andover, MA, USA) – is based on the principle described by Oskar Langendorff in the late 1800s [7]. In this system, oxygenated perfusate is injected retrogradely into the aortic root, forcing the aortic valve to close, while venous blood, flowing from the coronary sinus, is drained actively, or due to the right ventricle's ejection into the reservoir [8, 9].

Coronary perfusion according to Langendorff is an effective way to meet the metabolic needs of the myocardium. However, since the left ventricle remains unloaded in this perfusion scheme, it is difficult to assess the pumping function of the heart and, therefore, to predict the functional outcome of transplantation. Described in 1926 by Ernest Starling and Maurice Visscher, experiments with hemodynamic isolation of the cardiopulmonary complex became widely known due to the pattern discovered by the authors describing the relationship

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between diastolic heart volume and the strength of heart contractions [10, 11]. At the same time, the use of the technique of isolating the autoperfused cardiopulmonary complex can provide not only a long and effective existence of the graft ex situ, but also a dynamic assessment of contractility using ultrasound diagnostic methods [12].

The inability to effectively assess the quality of a donor organ often leads to errors in predicting the consequences of its use, and the "better safe than sorry" idea leads to a significant number of organ rejections despite the fact that they may have been suitable for transplantation [13]. In addition to the evaluation of laboratory parameters of donor organ functional quality, normothermic autoperfusion will allow for more sophisticated diagnostic procedures such as ex vivo echocardiography or coronary angiography [14]. The technique of functional echocardiographic evaluation of the donor heart in experiment was described in detail in a recently published paper by Ruggeri et al. This study derived comparable results of ex vivo evaluation of the heart under the condition of volume loading with those of standard transesophageal or transthoracic cardiac echocardiography [15].

Thus, the possibility of replacing the cold asystole period with normothermic autoperfusion has the potential of eliminating the negative consequences of prolonged ischemia. This may allow long-distance delivery of donor organs, increase access to transplantation in remote regions, and ensure the selection of an optimal donor and recipient regardless of their geographic distance [16].

Objective: to carry out a comparative assessment of the functional and metabolic status of the cardiac graft after a 6-hour normothermic autoperfusion and cold cardioplegia using Bretschneider's solution (Custodiol®, Germany).

MATERIALS AND METHODS

Preparing animals for the experiment

Female Landrace pigs, weighing 50 ± 5 kg at the age of 4–5 months (n = 10) were used as a model for a series of experiments. Care, provision 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, 03/18/1986). In the experimental group (n = 5), cardiac conditioning was performed under 6-hour normothermic autoperfusion of the cardiopulmonary complex ex vivo, followed by cold cardioplegia using Bretschneider's solution at 4 °C for 1 hour, followed by reperfusion with a cardiopulmonary bypass machine. As a control group (n = 5), there were hearts preserved for 6 hours according to the clinically accepted protocol of cold cardioplegia using Bretschneider's solution.

On the day of the experiment, all animals were premedicated (zoletil-100) on an empty stomach. The dose was selected individually, according to 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. The experiment was performed under endotracheal anesthesia with sevoflurane and muscle relaxation (rocuronium bromide). Mechanical ventilation was performed using FabiusPlus anesthesia-breathing apparatus (Draeger, Germany) with positive pressure on inhalation (20–30 cm water column) and exhalation (5–8 cm water column) with a respiratory volume of 8 ml/kg at a rate of 12–14 breaths per minute. Vital parameters were recorded using an IntelliVue MP70 monitor (Philips, Netherlands).

During the experiments, invasive blood pressure in the heart cavities and main vessels, cardiac arrhythmias (electrocardiography), and temperature of the organ complex were monitored. Blood analysis was performed using an automatic hematological analyzer XT-4000i (Sysmex, Germany) according to the manufacturer's guidelines. Central hemodynamic parameters were investigated by right heart catheterization with a Swan–Ganz catheter, as well as with the help of a portable multifunctional ultrasound system Philips CX50 (Philips Ultrasound, USA) with ECG synchronization using an S5-1 sector array probe. The ex vivo position of the probe was along the long axis of the left ventricle and in the apical four-chamber position. Left ventricular (LV) diastolic function was assessed by calculating the rate of change of LV pressure during isovolumic relaxation (–dP/dt). In the absence of mitral regurgitation, –dP/dt was calculated by an alternative non-invasive assessment method using the formula:

$$-dP/dt = (DBP - LVEDP) = IVRT$$

where DBP is diastolic blood pressure, LVEDP is left ventricular end-diastolic pressure.

LVEDP was estimated based on the ratio of mitral inflow peak velocity E/A: LVEDP = 10 mmHg if E/A 1.6 and 20 mmHg if E/A >1.6. IVRT is isovolumic relaxation time, which is calculated by subtracting T1 (time from the onset of QRS to the end of blood flow in the LV outflow tract) from T2 (time from the onset of QRS to the beginning of flow through the mitral valve) according to the methodology described in detail in the work by Parekh et al. [17]. Cardiac function was evaluated by calculating cardiac output (CO); global left ventricular stroke work index (LVSWI) by the formula SW = SV × (ESP – EDP), where SV is stroke volume, ESP is end-systolic pressure, and EDP is end-diastolic pressure; preload recruitable stroke work (PRSW) as a ratio of LVSWI and EDP.

To assess the efficiency of respiratory function of autologous lungs, the gas composition of blood samples taken from the left atrium was monitored using a Radiometer ABL800 FLEX analyzer (Denmark). Myocardial oxygen consumption was calculated by the formula:

$$\text{LV } O_2 \text{ cons} = \frac{([O_2]a) - ([O_2]cs) \times CAF}{\text{LV mass}},$$

$$\text{ml-}O_2\text{/min/100 g},$$

where $[O_2]_a$ is the arterial blood oxygen content, $[O_2]_{cs}$ is coronary sinus blood oxygen content, CAF is coronary blood flow, LV mass is left ventricular myocardial mass.

Blood oxygen content was calculated by the formula:

$$O_2 =$$

$$= \frac{\%O_2\text{Sat} \times [\text{Hb}] \times O_2\text{capacity of Hb (1.34 ml} - O_2\text{/g)}}{100},$$

$$\text{ml-}O_2\text{/dl}.$$

Coronary vascular resistance (CVR) was calculated by the formula:

$$\text{CVR} = \frac{\left(\text{iARP(m)} - \text{iRAP(m)}\right)}{\text{CBF} \times 100 \text{ g}}$$

where iARP(m) is the mean invasive aortic root pressure, iRAP(m) is the mean invasive right atrial pressure, CBF is coronary blood flow, mL/min).

Surgical technique of the experiment

Explantation of the working cardiopulmonary complex (CPC) was performed through a midline sternotomy. Isolation of the CPC began with the removal of the pericardium and mobilization of the superior vena cava (SVC), then the brachiocephalic trunk (BCS), left subclavian artery (LSA), and inferior vena cava (IVC) were isolated. The trachea was carefully separated from the esophagus using an electrocoagulator, achieving hemostasis. After administration of heparin (3 mg/kg body weight), the LSA was ligated as distally as possible, and an introducer was placed through the arterial stump to measure the invasive blood pressure (iBP) in the aortic root and to guide diagnostic catheters. Then the BCS was ligated and crossed, and an 18 Fr arterial cannula was inserted into the arterial stump, which was connected to the arterial reservoir fixed at a height of 1 meter above the heart level. After clamping the descending thoracic aorta at the level of the isthmus, the arterial trunk was opened, and arterial blood was drawn into the reservoir. After stabilization of blood level and arterial pressure, 1–1.5 liters of Ringer's solution was injected into the femoral vein. After that, the vena cava was ligated and crossed, the trachea was crossed and reintubated with a cuffed tube. The functioning CPC was finally separated from the surrounding tissues, transferred to a container with warm saline solution (38 °C), the arterial trunk was clamped, and observation was continued for 6 hours. Throughout autoperfusion, a continuous infusion of 5% calcium chloride solution (3–5 ml/hour) and 10% glucose (5–10 ml/hour) was performed to maintain blood levels in the reference range.

After 6 hours of normothermic autoperfusion of CPC, cold cardioplegia was performed by injecting 2 liters of Bretschneider's solution (Custodiol®, Germany, HTK) into the aortic root. The CPC was then stored in Bretschneider's solution at 4 °C for 1 hour. After this time, the heart was perfused for 15–20 minutes using a heart-lung machine filled with the animal's own blood. If necessary, electrical defibrillation was performed. After warming and recovery of cardiac activity, the CPC was filled with blood, isolated and cardiac ultrasound was performed.

Tissue samples for histological examination were excised from the apical part of the left ventricle of the heart and the middle lobes of the left and right lungs, fixed in 10% neutral formalin, after fixation, dehydrated in alcohols of increasing strength and embedded in paraffin using a dispenser with heating and cooling plates. Histological sections, 4–5 μm thick, were prepared from paraffin blocks on a Microm HM 550 microtome. Before staining, the sections were deparaffinized for 10–15 minutes in two portions of pure xylene, followed by its removal in three portions of alcohol of decreasing strength (absolute 70°) to distilled water. Histological sections were stained according to standard methods: hematoxylin and eosin, Van Gieson with combined dyeing of elastic fibers with orsein, and PAS reaction was performed. Polarization microscopic examination of the myocardium was performed using an Axio Scope. A1 microscope (Zeiss, Germany) equipped with an analyzer and polarizer, AxioCam HRm and AxioCam HRc cameras (Zeiss, Germany) and ZEN blue software (Zeiss, Germany).

Statistical processing was performed using Statistica 10.0 software (StatSoft Inc., USA). The normality of distribution was checked using the Shapiro–Wilk test with subsequent assessment of equality of variance by Levene's test. The significance of differences between the comparison groups and the groups (p) for continuous data was calculated using the Mann–Whitney U nonparametric test in independent groups and Wilcoxon test in dependent groups. The level of significance between the comparison groups and the groups was considered reliable at $p < 0.05$, which corresponds to the criteria accepted in biomedical research.

RESULTS

In all experiments, 0.3–0.5 ml of 0.01% epinephrine solution was administered to compensate for adrenergic stimulation at the reperfusion stage. However, despite this, the hearts of the control group were not able to maintain aortic root blood pressure at the 60-mmHg level, and in most experiments, after 5–10 minutes of

independent functioning of the CPC, bradycardia and pronounced dilation of heart chambers were observed, requiring urgent drainage of chambers and reperfusion using a heart-lung machine.

Moreover, in the experimental group the reperfusion time required to wean the CPC from artificial circulation with the ability to independently maintain blood pressure in the aortic root at a level not lower than 60 mmHg was 87 [67; 102] minutes and 19 [17.5; 22.5] minutes ($p < 0.05$) in the control and experimental groups, respectively. At the same time, in all experiments of the control group, restoration of heart rhythm required multiple electrical defibrillation (up to 10 shocks) followed by electrical cardiac stimulation. The main hemodynamic parameters are presented in Table 1.

Cardiac pumping function was evaluated at baseline and within an hour after the end of reperfusion and repeated hemodynamic isolation of the CPC. The main parameters of cardiac ultrasound examination are summarized in Table 2.

Diastolic function was assessed at three points: 1) baseline, 2) immediately after reperfusion and restoration of autoperfusion, and 3) one hour after weaning from artificial circulation. When estimating the rate of change in LV pressure during isovolumic relaxation (–dP/dt), a significant decrease in –dP/dt was observed in the control group, which indicates a deterioration in the diastolic function of the heart, while in the experimental group this index changed insignificantly over time (Fig. 1).

At histological examination in myocardial samples taken after reperfusion in the control group, the phenomena of karyorrhexis and disappearance of nuclei were observed in cardiomyocytes. Despite the presence of free lumen of blood vessels, moderate perivascular edema was observed around them, spreading to the interstitium in which single leukocytes were found (Fig. 2).

In the experimental group, in contrast to the control group, the severity of intracellular and intercellular edema was significantly less, and the phenomena of leukodiapedesis in the interstitium were more pronounced. Preservation of transverse striation of cardiomyocytes with zones of increased anisotropy and subsegmental contractures was observed (Fig. 3).

DISCUSSION

Normothermic ex vivo perfusion is a promising method of donor heart conditioning. It can shorten cold ischemia period significantly, and allows for a wide screening of metabolic and functional parameters [9, 18, 19]. Moreover, the use of ex situ cardiac perfusion techniques has been proven to restore the function of the organ of borderline status and thereby increase the number and quality of grafts available for transplantation [20]. However, despite several studies, the choice of the optimal mode, as well as the sensitivity of diagnostic

Table 1

Basic hemodynamic parameters

Parameter \ Group	Control (n = 5)		Experimental (n = 5)	
	Before conservation	After conservation	Before conservation	After conservation
HR (bpm)	91.2 ± 14.8	100 (pacemaker)	93.3 ± 11.7	100 (pacemaker)
$iRAP_{(m)}$ (mmHg)	4.0 ± 1.4	11.4 ± 1.9*	7.4 ± 1.5	7.0 ± 2.3#
$iLAP_{(m)}$ (mmHg)	4.0 ± 1.9	10.3 ± 1.6*	3.4 ± 1.8	9.4 ± 1.6*
$iAP_{(m)}$ (mmHg)	70.6 ± 12.5	63.0 ± 15.8	67.2 ± 9.7	63 ± 7.5
$iPAP_{(m)}$ (mmHg)	15.5 ± 3.2	16.4 ± 3.7	15.3 ± 4.2	13.3 ± 3.3
CVR (mmHg)* min/mL/100 g	8.03 ± 1.5	13.9 ± 4.3*	7.1 ± 1.3	8.8 ± 1.1*#

Note. Data are presented as M ± SD; *, $p < 0.05$ compared with baseline values; #, $p < 0.05$ compared with control group; HR, heart rate; $iRAP_{(m)}$, mean invasive right atrial pressure; $iLAP_{(m)}$, mean invasive left atrial pressure; $iAP_{(m)}$, mean invasive aortic pressure; $iPAP_{(m)}$, mean invasive pulmonary artery pressure; CVR, coronary vascular resistance.

Table 2

Basic parameters of myocardial contractile function

Parameter \ Group	Control (n = 5)		Experimental (n = 5)	
	Before conservation	After conservation	Before conservation	After conservation
CO, l/min	0.83 [0.74; 1.86]	0.37* [0.23; 0.37]	0.84 [0.78; 0.94]	0.63# [0.37; 0.8]
LVSWI, ml·mmHg	26.09 [5.25; 48.25]	12.67* [1.9; 27.4]	33.89 [21.4; 47.9]	21.06# [15.28; 27.25]
PRSW, ml	0.63 [0.06; 1.2]	0.25* [0.03; 0.57]	0.65 [0.42; 0.88]	0.57# [0.34; 0.76]

Note. Data are presented as Me [Q1; Q3]. *, $p < 0.05$ compared with baseline values; #, $p < 0.05$ compared with the control group.

markers in predicting the functional outcome of transplantation is still a matter of debate.

Autoregulation of coronary blood flow is one of the most important properties, its preservation largely determines the compensatory plasticity of the graft in the resolution of IRI. In its turn, the preservation of this coronary bed property is largely determined by the coronary flow characteristics. The TransMedics Organ Care System (OCS), used today successfully, provides coronary flow in the range from 650 to 900 mL/min, with perfusion pressure from 60 to 80 mmHg [21, 22]. However, Hatami et al., in their studies, concluded that myocardial energy reserves can be preserved at aortic perfusion pressures as low as 40 mmHg [23]. According to Repse et al., vasomotor regulation of the coronary channel changes significantly with increasing duration of machine normothermic perfusion, which leads to excess coronary blood flow over time [24]. Controlled coronary perfusion under low perfusion pressure can potentially limit myocardial and endothelial injury [25]. In this regard, normothermic autoperfusion of the graft as a method of

Table 3

Myocardial oxygen consumption ($mL\text{-}O_2/min/100\ g$)

Group	Before conservation	After conservation
Control (n = 5)	12.44 [7.9; 18.5]	8.52 [4.25; 12.65]
Experimental (n = 5)	15.44 [8.7; 22.4]	117*# [106.5; 131]

Note. Data are presented as Me [Q1; Q3]; *, $p < 0.05$ compared to baseline values; #, $p < 0.05$ compared to the control group.

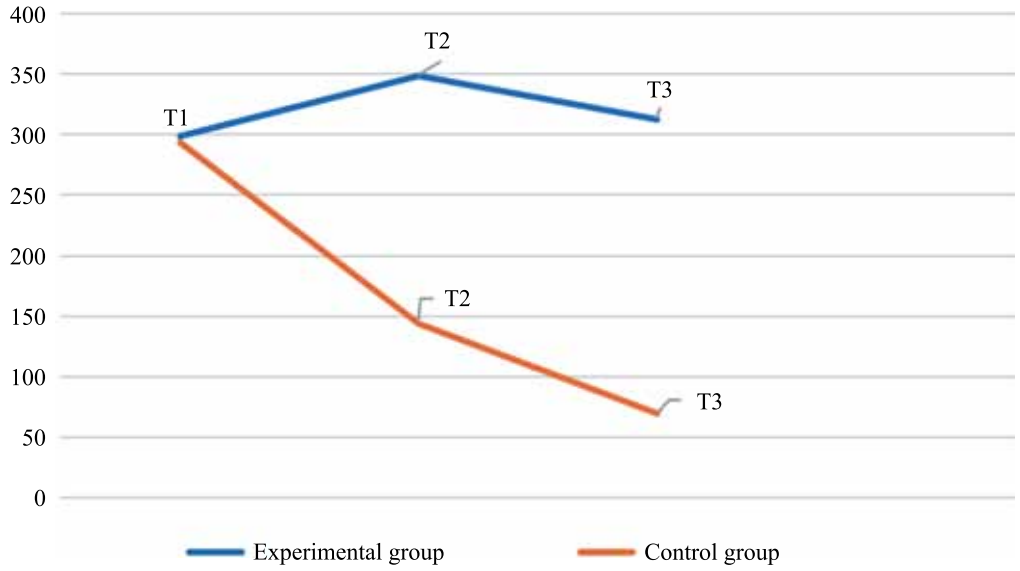

Fig. 1. Dynamics of –dP/dt changes during the experiment. T1, baseline; T2, immediately after reperfusion and restoration of autoperfusion; T3, one hour after weaning from cardiopulmonary bypass

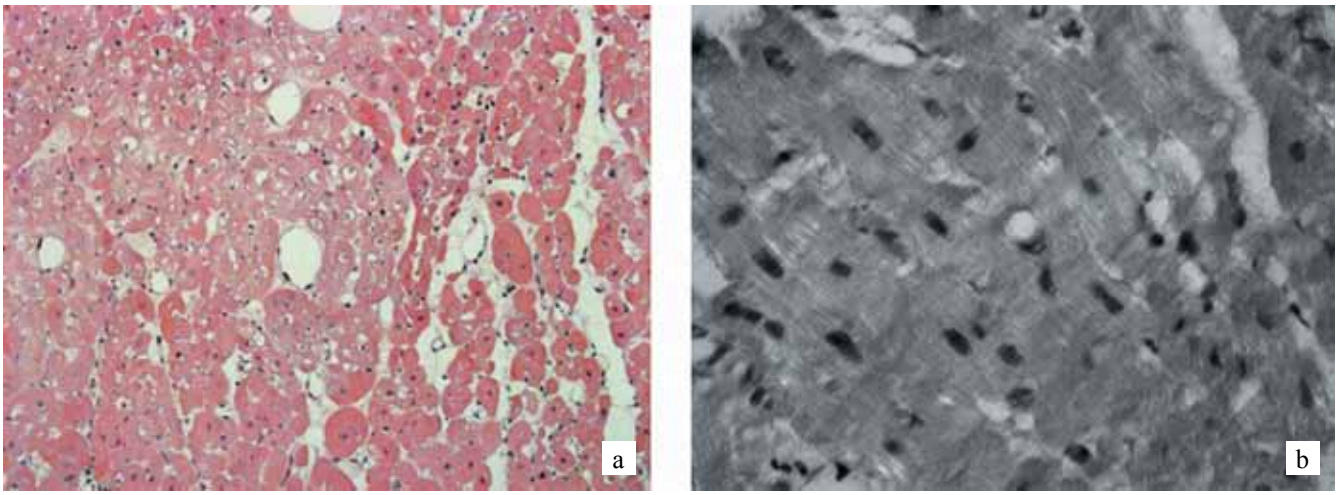

Fig. 2. Left ventricular myocardium after reperfusion, control group: a, H&E stain, magnification 400×; b, polarized light microscopy, magnification 630×

prolonged conditioning is of great interest, since in this case, hemodynamic parameters of the functioning of the complex are determined by the pumping function of the graft itself, taking into account its own metabolic needs.

The results of the conducted study prove that it is possible to provide effective coronary blood flow in an autoperfused cardiopulmonary complex. Thus, the previously established ability of the coronary arteries to vasodilate in response to increased myocardial oxygen consumption was confirmed in a series of experiments [26]. According to data obtained by Duncker et al., coronary resistance in the control group after reperfusion was statistically significantly higher than in the autoperfusion group. This fact indicates the preservation of regional vasomotor autoregulation in order to maintain adequate but not excessive oxygen supply to the myocardium.

The applied scheme of complete anatomical isolation of the autoperfused cardiopulmonary complex made it possible to create the necessary conditions for the effective functioning of the graft ex situ, as well as for the assessment of function and metabolism. However, along with the obvious advantages of autoperfusion technology over static cold preservation, the issue of preserving the structure and gas exchange function of autologous lungs remains open. Leukocyte sequestration observed during autoperfusion was described in earlier studies [27, 28]. However, this phenomenon was observed even when leukocyte-depleted perfusate was used. Taking into account the fact that the degree and rate of leukocyte sequestration in the lungs is inversely proportional to the rate of pulmonary blood flow, complete modeling of the initial cardiac output is the main condition for successful and long-term functioning of autologous lungs. Preservation of the initial hemodynamic parameters is important for predicting the functional outcome of transplantation and to evaluate the transplant from the standpoint of possibility to effectively provide cardiac output in the recipient's body.

Despite a number of successful trials of OCS, the validity of using lactate profiles as assessment markers has been questioned by multiple groups of researchers [29–31]. Also, it has been suggested that left ventricular contractility parameters can more accurately predict graft behavior after transplantation in contrast to metabolic markers, including lactate trend [32, 33].

According to Gellner et al., the working mode of perfusion with passive afterload, allows a more detailed prediction of post-transplant cardiac function [34]. In their works, White et al. and Xin et al. tried to load the left and right atria, facilitating ventricular ejection during active aortic root perfusion. In a series of experiments, the authors used perfusate injection both antegradely into the left atrium and retrogradely into the aorta [32, 35]. In this case, in systole, the left ventricle overcame aortic counterpressure, ejecting perfusate into the reservoir connected to the brachycephalic arteries, and in diastole, aortic counterpressure facilitated coronary perfusion. Also, a passive afterload perfusion regimen has been proposed as an alternative [28]. Instead of using retrograde flow to maintain coronary perfusion during diastole, an afterload module based on the Windkessel principle was used [36]. However, the only perfusion platform currently available for clinical use assumes "idle" operation of the left ventricle.

In our opinion, the use of a preserved thoracic aortic fragment as a Windkessel receiver is extremely insufficient and, in case of volume overload of the CPC, threatens serious endothelial damage. The intrinsic elastic properties of the aortic wall do not allow effective damping of cardiac output exceeding 1000–1500 mL/min preventing coronary hyperperfusion, while ensuring physiological blood pressure profile.

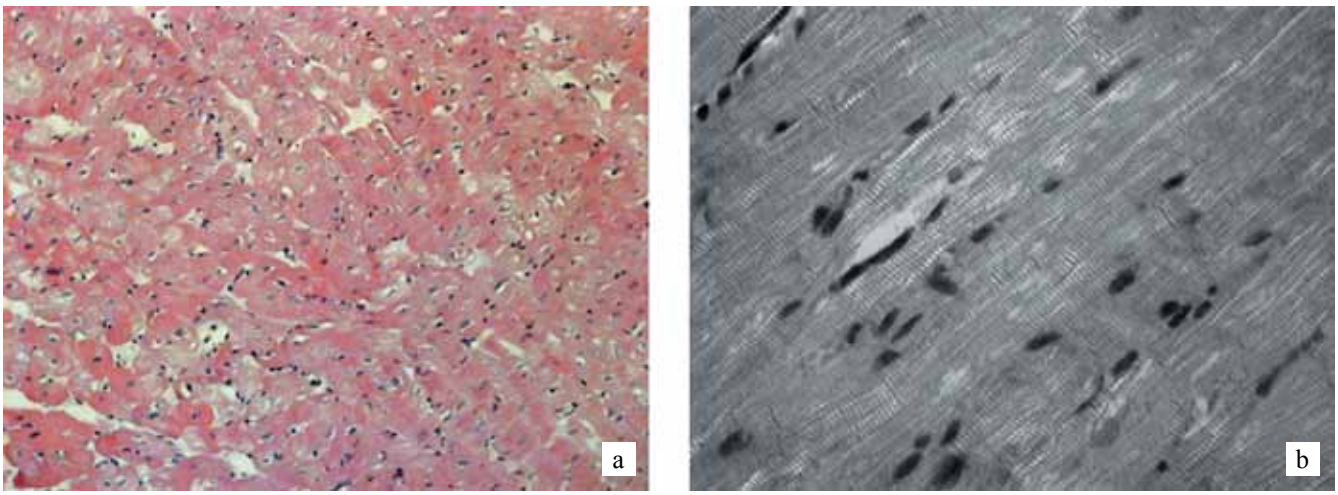

Fig. 3. Left ventricular myocardium after reperfusion, experimental group: a, H&E stain, magnification 400×; b, polarized light microscopy, magnification 630×

CONCLUSION

This study demonstrated significant advantages of normothermic autoperfusion of the CPC over static cold cardioplegia. However, pathomorphological changes caused by reduced blood flow in pulmonary circulation and in heart cavities, leading to leukocyte sequestration and pulmonary edema, require modification of the circulation circuit with inclusion of an effective cardiac output receiver and a pathway of blood return to the right heart.

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The authors declare no conflict of interest.

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