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EFFECT OF PROLONGED CARDIAC GRAFT PRESERVATION ON ADHESION PROTEIN ACTIVATION AND SYNTHETIC ENDOTHELIAL FUNCTION

M.O. Zhulkov[1], N.A. Karmadonova[1], M.A. Surovtseva[1, 2], I.I. Kim[1, 2], O.V. Poveshchenko[1, 2], I.S. Zykov[1], A.R. Tarkova[1], D.A. Sirota[1, 3], A.V. Protopopov[1], A.G. Makaev[1], F.Yu. Kosimov[1], M.N. Murtazaliev[1], A.V. Guseva[1], K.A. Agaeva[1]

[1] Meshalkin National Medical Research Center, Novosibirsk, Russian Federation
[2] Research Institute of Clinical and Experimental Lymphology, Novosibirsk, Russian Federation
[3] Novosibirsk State Medical University, Novosibirsk, Russian Federation

Objective: to conduct a comparative study of the efficacy of Custodiol® cardioplegia (Custodiol HTK, Dr. Franz Köhler Chemie GmbH, Bensheim, Germany) and normothermic autoperfusion of heart graft as a part of an *ex vivo* cardiopulmonary complex (CPC). **Methods.** Landrace pigs weighing 50 ± 5 kg and aged 4–5 months (n = 10) were used as the model for a series of acute experiments. In the experimental group (n = 5), the CPC was conditioned by autoperfusion for 6 hours. In the control group, the heart's pumping function was restored after a 6-hour cold preservation with Custodiol®. The effectiveness of cardiac graft preservation methods was evaluated by measuring myocardial ischemic markers, endothelial synthetic function, and endothelial cell activation markers (E- and P-selectins, endothelial growth factor). **Results.** Following cardiac graft reperfusion, the control group exhibited a statistically significant increase in the concentration of myocardial ischemia markers; also, there was a significant decrease in the synthesis of endothelium-derived relaxing factor in the Custodiol® solution preservation group (378.5 [226.4; 539.7] *vs*. 542.1 [377.6; 853.2] μM/mL in the autoperfusion group, $p < 0.05$). The degree of coronary endothelial reperfusion injury/activation was several times higher in the control group than in the normothermic autoperfusion conditioning group. Moreover, cardiac output after a 6-hour graft conditioning was 0.63 [0.37; 0.80] and 0.37 [0.23; 0.37] L/min in the experimental and control groups, respectively ($p < 0.05$). **Conclusion.** Normothermic autoperfusion showed a significant advantage in preserving the morphofunctional status of the donor heart compared with cold preservation with Custodiol® during 6 hours of *ex vivo* graft conditioning.

Keywords: autoperfusion, heart preservation, normothermic perfusion, reperfusion injury, heart transplantation, cold preservation.

INTRODUCTION

Primary graft dysfunction is the leading cause of death and morbidity in cardiac transplant recipients [1]. Factors such as ischemia time, composition of the preservation solution, and preservation method may contribute to initial endothelial dysfunction and potentially influence long-term endothelial changes, including graft vasculopathy. This has led to an increasing use of myocardial and endothelial markers in both experimental and clinical studies to assess the quality of graft function preservation [2].

One of the inevitable events during the transplant reperfusion phase is the interaction between circulating neutrophils and the coronary endothelium. Endothelial injury represents the primary consequence of reperfusion, initiating a cascade that includes calcium overload in cardiomyocytes (the "calcium paradox"), tissue edema, and generation of reactive oxygen species by neutrophils. This damage begins within 2.5 to 5 minutes after the onset of reperfusion. It involves the initial slowing down or "rolling" of neutrophils along the endothelium, followed by firm adhesion and diapedesis of neutrophils into the myocardium. Once in the tissue, neutrophils interact with cardiomyocytes, leading to cellular necrosis [3].

Leukocyte adhesion to the vascular wall marks the early stage of both the immune and inflammatory responses to reperfusion. In post-ischemic myocardial tissue, neutrophil infiltration significantly impairs cardiac function [4]. Ischemia followed by reperfusion disrupts basal and agonist-stimulated nitric oxide (NO) synthesis [5], a factor known to modulate leukocyte adhesion to the endothelium. Reduced NO availability has been shown to increase leukocyte-endothelial interactions. The onset of reperfusion also triggers a sharp decline in endothelium-derived relaxing factor, alongside a spike in free radical production and P-selectin expression [3, 7].

Anti-adhesion therapy represents a promising new approach to mitigating ischemia-reperfusion injury (IRI).

Corresponding author: Alexander Makaev. Address: 15, Rechkunovskaya str., Novosibirsk, 630055, Russian Federation. Phone: (905) 198-33-31. E-mail: makaev_a@meshalkin.ru

One such strategy involves the use of monoclonal antibodies targeting specific adhesion molecules [8]. These adhesion-blocking antibodies help to reduce the extent of myocardial injury following reperfusion [9]. Although the therapeutic use of such anti-adhesion strategies shows potential for enhancing myocardial and coronary function recovery after cardiac surgery or transplantation, their use remains largely experimental at this stage.

Therefore, the continued investigation and clinical implementation of effective, cost-efficient methods for long-term conditioning of donor hearts is essential. Such advancements not only have the potential to expand the geographic scope of donor organ availability, thereby increasing transplant opportunities, but also to significantly improve long-term outcomes by reducing the incidence of graft vasculopathy.

MATERIALS AND METHODS

Landrace pigs (females), weighing 550 ± 5 kg and aged 4–5 months (n = 10), were used as the animal model for this series of experiments. Animal care, experimental procedures, monitoring, and euthanasia were conducted in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986). The study was approved by the local bioethics committee (Protocol No. 2, dated September 1, 2022).

In the experimental group (n = 5), heart conditioning was performed using a 6-hour normothermic autoperfusion of the cardiopulmonary complex (CPC) *ex vivo*, followed by 1 hour of cold cardioplegia with Custodiol® HTK solution at 4 °C, and subsequent reperfusion via a circulatory assist device. The control group (n = 5) consisted of hearts preserved for 6 hours using standard cold cardioplegia with Custodiol® HTK solution, following conventional protocols (Fig. 1).

Preoperative preparation and anesthesia

On the day of the experiment, all animals were premedicated with Zoletil® 100, administered on an empty stomach. The dosage was individually adjusted based on the animal's weight and body size. Once anesthesia was induced, the surgical field and the neck area for vascular catheterization were prepared. The animal was then transferred to the operating table and positioned supine for tracheal intubation and placement of central arterial and venous catheters.

The procedures were performed under endotracheal anesthesia using sevoflurane and muscle relaxation with rocuronium bromide. Mechanical ventilation was delivered via a Fabius® Plus anesthesia-breathing workstation (Dräger, Germany) with inspiratory positive pressure of 20–30 cm H_2O, expiratory pressure of 5–8 cm H_2O, tidal volume of 8 ml/kg, and a respiratory rate of 12–14 breaths per minute.

Physiological parameters were continuously monitored using the IntelliVue MP70 patient monitor (Philips, Netherlands). During the procedure, we recorded invasive blood pressure within the heart chambers and major vessels, electrocardiographic data for arrhythmia detection, and core temperature of the organ complex.

Hematological parameters were assessed using an ABL 800 FLEX automatic blood analyzer (Radiometer, Denmark), in accordance with the manufacturer's instructions. Central hemodynamic monitoring was conducted via right heart catheterization using a Swan–Ganz catheter, complemented by a portable multifunctional ultrasound system (Philips CX50, Philips Ultrasound, USA) with ECG synchronization.

Coronary vascular resistance (CVR) was calculated using the following formula:

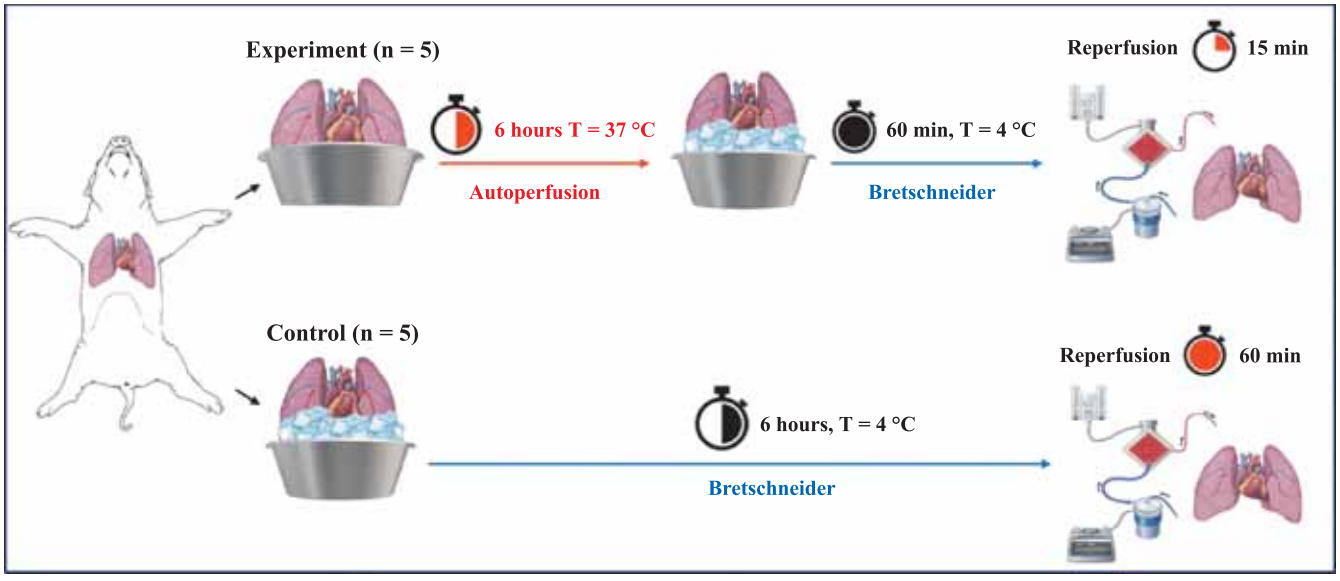

Fig. 1. Study design

$$CVR = \frac{mAP - mRAP}{CBF \times 100\ g},$$

where, mAP – mean aortic pressure, mRAP – mean right atrial pressure, and CBF – coronary blood flow.

Surgical technique for the experiment

A functional CPC was procured through midline sternotomy. Isolation of the CPC was started with removal of the pericardium and mobilization of the superior vena cava (SVC), then the brachiocephalic trunk (BCT), left subclavian artery (LSA), inferior vena cava (IVC) were isolated. The trachea was carefully separated from the esophagus using an electrocoagulator, achieving hemostasis. After heparin (3 mg/kg body weight) had been administered, the LSA was ligated as distally as possible, and an introducer was placed through the arterial stump to measure the aortic pressure and to guide diagnostic catheters. Then, the BCT was ligated and crossed, and an 18 Fr arterial cannula was inserted into the arterial stump and connected to the arterial reservoir. After clamping the descending thoracic aorta at the isthmus level, the arterial trunk was opened, and arterial blood was drawn into the reservoir. After blood level and arterial pressure were stabilized, 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 (38 °C), the arterial trunk was clamped, and observation was continued for 6 hours (Fig. 2).

Throughout the autoperfusion period, a continuous infusion of 5% calcium chloride solution (3–5 mL/hour) and 10% glucose (5–10 mL/hour) was administered to maintain electrolyte and glucose levels within the physiological reference range. After 6 hours of normothermic autoperfusion of the CPC, cardioplegia was induced by injecting 2 liters of Custodiol® solution (Custodiol® HTK, Germany) into the aortic root. The CPC was subsequently stored in Custodiol® solution at 4 °C for 1 hour.

After cold storage, the heart was reperfused for 15–20 minutes using a cardiopulmonary bypass (CPB) machine primed with the animal's autologous blood. Electrical defibrillation was performed as needed. Once normothermia and spontaneous cardiac activity were restored, the CPC was filled with blood, isolated, and assessed via ultrasound imaging.

Tissue samples for histological examination were collected from the apex of the left ventricle and the middle lobes of the left and right lungs. Samples were fixed in 10% neutral buffered formalin, dehydrated through a graded series of ethanol solutions (increasing alcohol

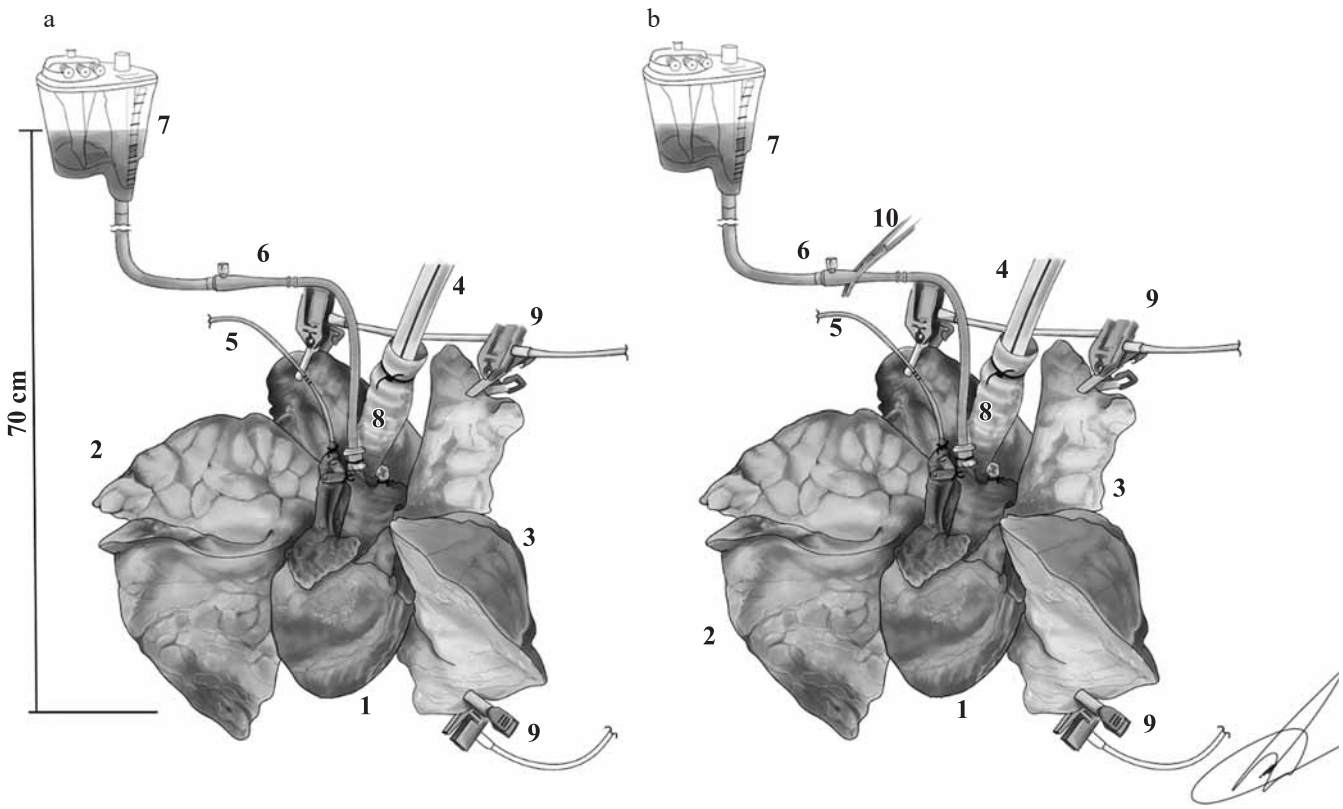

Fig. 2. Diagram of the isolated cardiopulmonary complex: a, stage of blood exfusion into the reservoir and preparation for transfer of the complex into a container; b, stage of final hemodynamic isolation of the cardiopulmonary complex; 1 – heart, 2 – right lung, 3 – left lung, 4 – intubation tube, 5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

by volume, ABV), and embedded in paraffin using a dispenser with integrated heating and cooling plates. Histological sections, 4–5 μm thick, were cut from paraffin blocks using a Microm HM 550 microtome (Thermo Scientific, Waltham, USA).

Prior to staining, the sections were deparaffinized in two changes of pure xylene for 10–15 minutes, then rehydrated through a graded series of ethanol (decreasing ABV, absolute to 70%) and finally rinsed in distilled water. Standard histological stains were applied, including hematoxylin and eosin, Van Gieson's stain with orcein for elastic fibers, and the periodic acid–Schiff (PAS) reaction.

Polarized light microscopy of the myocardium was performed using an Axio Scope.A1 microscope (Zeiss, Germany), equipped with an analyzer and polarizer, AxioCam HRm and HRc cameras (Zeiss, Germany), and ZEN Blue imaging software (Zeiss, Germany).

To prepare the extracts, left ventricular myocardial tissue was weighed, minced, and suspended in 1 mL of PBS, then stored at –70 °C. Samples were homogenized using a KZ-III-FP low-temperature tissue homogenizer (Servicebio Technology Co., Wuhan, China) at –40 °C with 3 mm ×2 and 4 mm ×1 steel balls, following the manufacturer's instructions. levels were centrifuged at 16,100×g for 5 minutes to remove tissue debris. Vascular endothelial growth factor (VEGF) and NO concentrations in tissue extracts were normalized to the total protein content of each sample. VEGF levels were quantified using a commercial ELISA kit (Vector-BEST, Novosibirsk, Russia), and NO levels were determined by measuring nitrite levels, a stable end product, using the Griess reagent (Sigma-Aldrich, Darmstadt, Germany), per the manufacturer's protocol. Briefly, 50 μL of tissue extract was mixed with 50 μL of Griess reagent in a 96-well plate, and absorbance was measured at 492 nm using a Stat FAX-2100 microplate reader (Awareness Technology Inc., USA). Nitrite levels were calculated from a standard calibration curve.

Serum troponin I was measured using a chemiluminescent immunoassay with ARCHITECT STAT Troponin-I reagents on the Architect i2000SR analyzer (Abbott, USA). To assess serum levels of troponin T, heart-type fatty acid-binding protein (H-FABP), E-selectin (SelE), and P-selectin (SelP), blood samples were centrifuged at 1,000×g for 20 minutes. Serum was aliquoted and stored at –80 °C until analysis. These biomarkers were quantified using sandwich ELISA kits (Cloud-Clone Corp., China) specific to swine antigens.

Statistical analysis was performed using Statistica 10.0 software (StatSoft Inc., USA). Descriptive statistics were applied to summarize the data. The significance of differences between groups was evaluated using the nonparametric Mann–Whitney U test for independent groups and the Wilcoxon signed-rank test for dependent groups. A p-value of less than 0.05 was considered statistically significant, in accordance with standard criteria for biomedical research.

RESULTS

In all experiments, graft reperfusion was performed using a CPB machine, maintaining consistent perfusion parameters (300–350 ml/min). However, by the 15th minute, a significant increase in aortic pressure and vascular resistance was observed in all hearts from the control group (Table 1).

At the same time, in all experiments within the control group, restoration of heart rhythm required multiple electrical defibrillation attempts (up to 10 discharges), followed by electrical cardiac stimulation. The reperfusion time required to wean the CPC from CPB, while maintaining an aortic root pressure of no less than 60 mmHg independently, was 87 [67; 102] minutes in the control group, compared to 19 [17.5; 22.5] minutes in the experimental group ($p < 0.05$).

The degree of ischemia and the effectiveness of the conditioning technique were assessed by measuring the levels of lactate, troponin I, troponin T, and H-FABP in

Table 1

Main hemodynamic parameters

Parameter \ Group	Control (n = 5)		Experimental (n = 5)		
	Before preservation	After reperfusion	T1	T6	After reperfusion
CO (L/min)	0.83 [0.74; 1.86]	0.37* [0.23; 0.37]	0.84 [0.78; 0.94]	0.57 [0.26; 0.88]	0.63# [0.37; 0.8]
HR (bpm)	96 [86; 105]	100 (ЭКС)	87 [78; 96]	98 [83; 116]	100 (ЭКС)
iABP (mmHg)	110 [75; 130]	162* [158; 210]	115 [65; 134]	112 [57; 128]	108 [84; 137]
CVR (mmHg·min/mL/100 g)	5.4 [4.2; 7.6]	13.9* [9.6; 15.8]	6.3 [5.3; 8.7]	7.1 [6.1; 10.3]	8.8# [5.3; 10.7]

Note. Data are presented as Me [Q1; Q3]. CO, cardiac output; HR, heart rate; iABP, invasive arterial blood pressure (aortic root); CVR, coronary vascular resistance; T1, 1st hour of autoperfusion; T6, 6th hour of autoperfusion; *, $p < 0.05$ compared with baseline (before preservation); #, $p < 0.05$ compared with control group after reperfusion.

Table 2

Myocardial ischemic markers

Indicator \ Group	Control (n = 5)		Experimental (n = 5)		
	Before preservation	After reperfusion	T1	T6	After reperfusion
Lactate (mmol/L)	3.3 [2.2; 4.5]	11.8* [10.1; 13.5]	5.8 [5.1; 6.7]	5.3 [4.7; 5.9]	7.1# [6.3; 8.4]
Troponin I (nmol/L)	175.84 [57.7; 309.9]	317,803.98* [44,509.9; 500,000.0]	144.8 [87.5; 187.7]	–	126,069*# [42,437.5; 141,583.1]
Troponin T (nmol/L)	0	988* [648; 1815.5]	0	442* [86.3; 881]	104.5*# [55.3; 344.3]
H-FABP (pg/mL)	0.2 [0.02; 1.1]	2.1* [0.1; 2.1]	0	0	0

Note. Data are presented as Me [Q1; Q3]; H-FABP, heart-type fatty acid-binding protein; T1, 1st hour of autoperfusion; T6, 6th hour of autoperfusion; *, $p < 0.05$ *vs.* baseline (before preservation); #, $p < 0.05$ *vs.* control group after reperfusion.

Table 3

Results of the study of myocardial extracts from the left ventricle of the heart

Indicator \ Group	Control (n = 5)		Experimental (n = 5)		
	Before preservation	After reperfusion	T1	Before preservation	After reperfusion
NO (μM/mL)	524.3 [335.1; 733.2]	378.5* [226.4; 539.7]	626.8 [566.5; 1288.5]	593.1 [442.8; 1003.8]	542.1# [377.6; 853.2]
VEGF (pg/mL)	701.8 [397.3; 1034.2]	978.1 [732.8; 1265.7]	742.3 [464.2; 1152.1]	789.3 [465.2; 1115.1]	777.8 [407.6; 1140.8]
SelE (ng/mL)	0.3 [0.05; 2.3]	4.4* [0.3; 8.1]	0	0.2 [0.1; 0.4]	0.2# [0.05; 0.2]
SelP (ng/mL)	1.8 [0.9; 2.6]	5.6* [2.8; 9.1]	1.3 [0.8; 1.8]	1.6 [1.1; 2.1]	2.4# [1.2; 3.2]

Note. Data are presented as Me [Q1; Q3]; NO, endothelium-derived relaxing factor; VEGF, vascular endothelial growth factor; SelE, selectin E; SelP, selectin P; T1, 1st hour of autoperfusion; T6, 6th hour of autoperfusion; *, $p < 0.05$ *vs.* baseline (before preservation); #, $p < 0.05$ *vs.* control group after reperfusion.

the blood flowing from the coronary sinus (Table 2). The control group showed statistically significant increases in lactate, troponin I, and troponin T levels following the reperfusion phase and restoration of cardiac function, compared to the autoperfusion group (Table 2).

The preservation of synthetic endothelial function was assessed by measuring the levels of endothelium-derived vasorelaxing factor (NO), endothelial growth factor (VEGF), and adhesion molecules E- and P-selectins (Table 3).

The study revealed that after 6 hours of preservation with Custodiol® solution, NO levels were significantly lower compared to the normothermic autoperfusion group (378.5 μM/mL *vs.* 542.1 μM/mL, respectively, $p < 0.05$). Additionally, the concentrations of adhesion molecules (E- and P-selectins) were significantly higher in the Custodiol® group compared to the autoperfusion group (4.4 ng/mL *vs.* 0.2 ng/mL for E-selectin and 5.6 ng/mL *vs.* 2.4 ng/mL for P-selectin, respectively, $p < 0.05$).

DISCUSSION

Myocardial reperfusion injury is primarily an iatrogenic phenomenon. A clear cause-and-effect relationship has been established between the degree of endothelial injury and post-ischemic contractile dysfunction in heart transplants [10]. Despite cold cardioplegia being the standard for donor organ preservation, graft function can deteriorate after four hours, particularly in organs from older donors [11]. This organ preservation method remains the leading risk factor for primary allograft dysfunction and mortality [12].

Despite the numerous benefits of *ex vivo* machine warm perfusion, this technology has not been widely adopted in most transplant centers over recent decades. The primary barrier is the high cost of such systems, which hinders their broader implementation in clinical practice [13]. However, evidence suggests that normothermic autoperfusion, as a method of prolonged *ex vivo* normothermic conditioning, is superior to static cold preservation [14]. Unlike machine perfusion techniques, autoperfusion of the donor heart provides optimal conditions for oxygen and energy substrate delivery to the graft, while preserving coronary blood flow's vasomotor autoregulation without subjecting the endothelial layer to excessive shear stress [15].

Recent studies have shown that reperfusion injury involves various components of the inflammatory response, with leukocyte-endothelial interactions playing a

central role [16]. The initial interaction between leukocytes and the endothelium triggers the subsequent pathophysiological stages of reperfusion injury – adhesion and migration of neutrophils across the endothelial barrier. Once in close proximity to cardiomyocytes, neutrophils release numerous cytotoxic factors that can lead to myocyte necrosis. The process of leukocyte adhesion to the endothelium begins with rolling along the endothelial surface, a process mediated by the release of adhesion molecules [17]. In the present study, it was demonstrated that 6-hour preservation of cardiac grafts with Custodiol® solution resulted in a significant increase in P-selectin levels compared to normothermic conditioning under autoperfusion conditions. The study of P-selectin is particularly significant because its expression is believed to play a critical role in leukocyte rolling and adhesion to the graft's endothelium [18].

Another reason for the interest in studying the expression of adhesion molecules and endothelial function is the high incidence of graft vasculopathy and the lack of effective treatment for this complication. Previous studies have shown that the intensity of arterial intimal thickening correlates with expression of P-selectin and vascular cell adhesion molecule-1 on endothelial cells in a rat model of chronic heart allograft rejection [19]. Administration of antibodies against P-selectin during reperfusion has been shown to reduce infarct size, decrease leukocyte adhesion to the coronary endothelium, and promote endothelial preservation [20]. Of particular interest are studies that have demonstrated P-selectin expression activation when isolated hearts were subjected to continuous perfusion with a blood-based perfusate [10].

These findings suggest that P-selectin release may not only indicate ischemia and reperfusion (where reperfusion acts as a rapid trigger for increased P-selectin expression) but could also be a general consequence of continuous perfusion through an extracorporeal circulation circuit. Interestingly, prior studies have shown that perfusion of rat hearts with crystalloid solution without an extracorporeal circulation circuit did not result in increased P-selectin levels [19]. In our study, we also observed increased P-selectin expression in the autoperfusion group after cardiac recovery using an extracorporeal circulation circuit. However, despite a twofold increase in P-selectin expression post-reperfusion compared to initial values, these changes were not statistically significant ($p > 0.05$). Similar results were observed for E-selectin expression, which was significantly increased in the control group, suggesting a higher degree of endothelial reperfusion injury in the control group compared to the autoperfusion group.

Another biomarker for myocardial ischemic injury is H-FABP, which plays a role in cellular fatty acid metabolism by reversibly binding and transporting long-chain polyunsaturated fatty acids from cell membranes to mitochondria. Plasma H-FABP levels begin to rise within 1 hour after myocardial ischemia, peak at 4–6 hours, and return to baseline within 24 hours [7]. In the present study, a statistically significant increase in H-FABP levels was observed in the control group compared to the autoperfusion group. There was no increase in H-FABP in the autoperfusion group, even after 6 hours of *ex vivo* cardiac conditioning, 60 minutes of cold ischemia, and reperfusion. This suggests the high efficiency of autoperfusion as a method for prolonged protection of the donor heart. Similar results were observed for lactate, troponin I, and troponin T levels in the blood flowing from the coronary sinus, highlighting the insufficient efficiency of Custodiol® solution for prolonged (6 hours) cardiac graft preservation. However, further research is needed to explore the predictive value of biomarkers of myocardial injury and endothelial dysfunction in determining the functional outcomes of transplantation.

CONCLUSION

Prolonged normothermic autoperfusion of a cardiac graft, compared to static cold preservation with Custodiol®, can better maintain the physiological conditions of the coronary endothelium and promote the synthesis of regulatory agents by endothelial cells. This, in turn, reduces the severity of IRI. The findings suggest that this method of long-term conditioning for cardiac transplants has significant potential in preventing vasculopathy.

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The authors declare no conflict of interest.

REFERENCES

1. *Hosenpud JD, Bennett LE, Keck BM, Fiol B, Boucek MM, Novick RJ*. The Registry of the International Society for Heart and Lung Transplantation: sixteenth official report – 1999. *J Heart Lung Transplant*. 1999 Jul; 18 (7): 611–626.
2. *Stoica SC, Goddard M, Large SR*. The endothelium in clinical cardiac transplantation. *Ann Thorac Surg*. 2002 Mar; 73 (3): 1002–1008.
3. *Tsao PS, Aoki N, Lefer DJ, Johnson G 3rd, Lefer AM*. Time course of endothelial dysfunction and myocardial injury during myocardial ischemia and reperfusion in the cat. *Circulation*. 1990 Oct; 82 (4): 1402–1412.
4. *Kupatt C, Habazettl H, Zahler S, Weber C, Becker BF, Messmer K, Gerlach E*. ACE-inhibition prevents postischemic coronary leukocyte adhesion and leukocyte-dependent reperfusion injury. *Cardiovasc Res*. 1997 Dec; 36 (3): 386–395.
5. *Ma XL, Weyrich AS, Lefer DJ, Lefer AM.* Diminished basal nitric oxide release after myocardial ischemia and

reperfusion promotes neutrophil adherence to coronary endothelium. *Circ Res*. 1993 Feb; 72 (2): 403–412.
6. *Kubes P, Suzuki M, Granger DN*. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proc Natl Acad Sci USA*. 1991 Jun 1; 88 (11): 4651–4655.
7. *Patel KD, Zimmerman GA, Prescott SM, McEver RP, McIntyre TM.* Oxygen radicals induce human endothelial cells to express GMP-140 and bind neutrophils. *J Cell Biol*. 1991 Feb; 112 (4): 749–759.
8. *Boyle EM Jr, Pohlman TH, Cornejo CJ, Verrier ED.* Endothelial cell injury in cardiovascular surgery: ischemia-reperfusion. *Ann Thorac Surg*. 1996 Dec; 62 (6): 1868–1875.
9. *Forbess JM, Hiramatsu T, Nomura F, Miura T, Farrington GK, Sokolowski K et al.* Anti-CD11b monoclonal antibody improves myocardial function after six hours of hypothermic storage. *Ann Thorac Surg*. 1995 Nov; 60 (5): 1238–1244.
10. *Lefer AM, Tsao PS, Lefer DJ, Ma XL.* Role of endothelial dysfunction in the pathogenesis of reperfusion injury after myocardial ischemia. *FASEB J*. 1991 Apr; 5 (7): 2029–2034.
11. *Costanzo MR, Dipchand A, Starling R, Anderson A, Chan M, Desai S et al.* The International Society of Heart and Lung Transplantation Guidelines for the care of heart transplant recipients. *J Heart Lung Transplant*. 2010 Aug; 29 (8): 914–956.
12. *Banner NR, Thomas HL, Curnow E, Hussey JC, Rogers CA, Bonser RS et al.* The importance of cold and warm cardiac ischemia for survival after heart transplantation. *Transplantation*. 2008 Aug 27; 86 (4): 542–547.
13. *Pettit SJ, Petrie MC*. Transplantation of Hearts Donated After Circulatory-Determined Death. *Circ Heart Fail*. 2019 Apr; 12 (4): e005991.
14. *Tarkova AR, Zykov IS, Zhulkov MO, Protopopov AV, Smirnov YaM, Makaev AG et al.* Normothermic *ex vivo* heart and lung autoperfusion: assessment of functional status and metabolism. *Russian Journal of Transplantology and Artificial Organs.* 2023; 25 (4): 150–159.
15. *Zhulkov MO, Tarkova AR, Zykov IS, Makaev AG, Protopopov AV, Murtazaliyev MN et al.* Long-term normothermic autoperfusion of the cardiopulmonary complex *ex vivo* as a method of effective graft conditioning: an experimental study. *Circulatory pathology and cardiac surgery*. 2023; 27 (4): 33–42.
16. *Zhou M, Yu Y, Luo X, Wang J, Lan X, Liu P et al.* Myocardial Ischemia-Reperfusion Injury: Therapeutics from a Mitochondria-Centric Perspective. *Cardiology*. 2021; 146 (6): 781–792.
17. *Lasky LA*. Selectin-carbohydrate interactions and the initiation of the inflammatory response. *Annu Rev Biochem*. 1995; 64: 113–139.
18. Cell adhesion molecules: selectins and integrins – PubMed [Electronic resource]. URL: https://pubmed.ncbi.nlm.nih.gov/10647744/ (accessed: 18.01.2024).
19. *Koskinen PK, Lemström KB*. Adhesion molecule P-selectin and vascular cell adhesion molecule-1 in enhanced heart allograft arteriosclerosis in the rat. *Circulation*. 1997 Jan 7; 95 (1): 191–196.
20. *Weyrich AS, Ma XY, Lefer DJ, Albertine KH, Lefer AM. In vivo* neutralization of P-selectin protects feline heart and endothelium in myocardial ischemia and reperfusion injury. *J Clin Invest*. 1993 Jun; 91 (6): 2620–2629.

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