Transplant International

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Continuous perfusion of donor hearts with oxygenated blood cardioplegia improves graft function

Fan Zhang,[1*] Ansheng Mo,[2,3*] Zhaoke Wen,[3] Yifan Zhou,[3] Shengjing Liang[3] and Hui Lin[2,3]

1 Department of Anesthesiology, Renmin Hospital of Wuhan University, Wuhan, China
2 Department of Cardiothoracic Surgery, Renmin Hospital of Wuhan University, Wuhan, China
3 Department of Cardiothoracic Surgery, Renmin Hospital of Guangxi Zhuang Autonomous Region, Nanning, China

Keywords
heart transplantation, heart–lung machine, organ preservation.

Correspondence
Hui Lin MD, Department of Cardiothoracic Surgery, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan; and Renmin Hospital of Guangxi Zhuang Autonomous Region, 6 Taoyuan Street, Nanning, China. Tel.: 86-13878133622; fax: +86-07712802018; e-mail: linhui33622@yahoo.com.cn

*These authors contributed equally to this work.

Received: 26 November 2009
Revision requested: 13 January 2010
Accepted: 26 April 2010
Published online: 25 May 2010

doi:10.1111/j.1432-2277.2010.01112.x

Summary

Donor hearts cannot be preserved beyond 6 h using cold storage (CS). Improving preservation methods may permit prolonged storage of donor heart. We compared graft function in large animal model after prolonged preservation (8 h) using continuous perfusion (CP) and CS method. Twenty-four miniature pigs were used as donors and recipients. Donor hearts were either stored in University of Wisconsin solution (UW solution) for 8 h at 0–4 °C (CS group, $n = 6$) or were continuously perfused with oxygenated blood cardioplegia at 26 °C for 8 h (CP group, $n = 6$). After preservation, hearts were transplanted into recipients and reperfused for 3 h. Left ventricular (LV) function, cardiac output (CO), malondialdehyde (MDA) and adenosine triphosphate (ATP) levels, and water content were measured. Although water content of CP hearts was higher than that of CS, LV contractility and diastolic function of CP hearts were superior to those of CS. In addition, CP hearts performed better than CS hearts on CO in working heart state. ATP was better preserved and MDA levels were lower in CP hearts compared with those of CS ($P < 0.0001$). Donor hearts can be preserved longer using continuous perfusion with oxygenated blood cardioplegia and this method prevents time-dependent ischemic injury.

Introduction

Cardiac transplantation remains limited as there is a global shortage of donor heart availability. Another key factor is that current preservation method of static cold storage (CS) of donor hearts is limited by functional viability from 4 to 6 h outside the body. Many distant transplantation centers are penalized because of this short window for procurement of donor hearts. Two major drawbacks with CS are hypothermia-induced injury (at 0–4 °C) and myocardial ischemia and hypoxia. It has been reported that continuous perfusion (CP) of harvested hearts with oxygen and metabolic substrates provides better support in preservation, by maintaining myocardial integrity during organ transport [1–5]. However, in most previous studies, subjects were small animals, which do not have a very similar anatomy and physiology to the human. Moreover, these studies used *ex vivo* nonworking Langendorff heart models for functional evaluation, instead of orthotopic transplanted hearts [1–8]. To assess whether CP preservation is superior to CS preservation, swine models were chosen for our study because of the similarities between human and swine in terms of anatomic and physiologic characteristics [9].

Methods

After the approval of Animal Care Committee of our institution and in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health, 24 male Guangxi Bama miniature pigs (weighing 25–30 kg) were selected for the study. Twelve animals were heart donors ($n = 6$ for CS

group and $n = 6$ for CP group) and orthotopic cardiac transplants were performed in the remaining 12 swine.

Donor preparation

All of the animals were anesthetized with intramuscular ketamine (30 mg/kg), intubated, and ventilated with 100% oxygen, at a frequency of 15–20 breaths per min with a tidal volume of 10 ml/kg. Anesthesia was maintained with 1–2% isoflurane. Marginal ear veins were cannulated for intravenous infusion of 5% glucose, at a rate of 50 ml/h. Donor and recipient pairs were determined by cross-matching of blood. After sternotomy, the heart and great vessels were exposed. Heparin 300 U/kg was infused intravenously.

Cold storage group

After aorta was cross-clamped, the heart was arrested with 4 °C St Thomas solution followed by infusion of UW solution (ViaSpan®; Bristol-Myers Squibb, Princeton, New Jersey, USA, together with 40 U of regular insulin, 16 mg of dexamethasone, and 200 000 U of penicillin G). Donor hearts were then harvested in standard classical method and immediately immersed in sterile plastic bags filled with 500 ml of UW solution, and stored on ice for 8 h.

Continuous perfusion group

After being arrested by St Thomas solution, the donor hearts were extracted, and placed in sterile bags containing 500 ml of hypothermic cardioplegia. A perfusion cannula was placed into the aortic root and connected to a perfusion circuit. Donor blood was harvested, filtrated through leukocyte-depleting filters, and transfused into the perfusion circuit. The perfusion device is a small cardiopulmonary bypass (CPB). The system consists of a roller pump, small heat exchanger, gas mixer, membrane oxygenator of infant and blood reservoir, a self-designed organ preservation chamber, filtration system and tubing system. The blood in the perfusion circuit was oxygenated with 95% oxygen and 5% carbon dioxide with a hollow-fiber membrane oxygenator (TERUMO®, BABY-RX; Terumo Corporation, Tokyo, Japan). Blood perfusion solution was supplemented with fructose-1,6-bisphosphate, adenosine, insulin, antibiotics and Solu-Medrol. Blood perfusion was initiated within 10 min after cardioplegic arrest and was maintained at room temperature (26 °C). Pump flow was adjusted to achieve a perfusion rate of 40 ml/min, corresponding to a perfusion pressure of approximately 35–45 mmHg. At 4 h of preservation, 200 ml of circulating perfusion solution was drained off the circuit and replaced by a similar volume of fresh blood cardioplegia to continue perfusion preservation. During 8-h perfusion period, the hearts were suspended in the organ chamber of the perfusion device and the left and right ventricles were vented. Perfusion solution, which returned via coronary sinus, was vented and harvested to the blood reservoir.

Recipient preparation

Anesthesia was similar to the donor protocol. ECG monitoring was carried out and carotid arteries and veins were isolated and cannulated for hemodynamic monitoring and support. Heparin was infused after isolation of the great vessels of the thorax. Ascending aortic and bicaval cannulation were used to connect the recipients for CPB. After the aorta was cross-clamped, the recipient heart was extracted. The anastomotic margins were then inspected and trimmed for preparation of standard orthotopic transplantation. Ten minutes prior to declamping of the aorta, 250 mg of Solu-Medrol was infused into the CPB. After declamping of the aorta, the heart was allowed to fibrillate for 1–2 min. If sinus rhythm did not resume, three attempts of direct-current shock at increments of 5 J were applied to defibrillate the heart. If unsuccessful, 100 mg of lidocaine was delivered intravenously and defibrillation was attempted again.

The hearts were sustained in an empty and beating state under CPB for 2 h, and the bypass was weaned off. Inotropic support, with dopamine and/or isoproterenol infusions, was administered, to maintain the mean arterial pressure above 60 mmHg. One hour after weaning off the bypass, the hearts were arrested with cardioplegia solution. Left ventricular biopsies were obtained and flash frozen in liquid nitrogen for subsequent analysis. The ventricles were cut into five horizontal sections and left overnight in 10% formaldehyde.

Measurement of cardiac function

After 2 h of reperfusion, LV function was measured using a 9F Millar catheter (Arrow, Arrow International Inc., Reading, PA, USA) placed through the apex of the left ventricle. All pressure related data were continuously recorded directly on a computer-based data acquisition system (Technology & Market Co., Chengdu, China), including LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, +dP/dt: the maximum rate of developed pressure, and −dP/dt: maximum negative rate of developed pressure. After weaning off CPB for 30 min, the monitoring of hemodynamics was performed by a Swan-Ganz catheter (Arrow, Arrow International Inc., Reading, PA, USA). The ability of the donor hearts to return to sinus rhythm, with or without defibrillation and the numbers of those successfully weaned from CPB were recorded.

Measurement of blood, cardiac metabolism and water content

Arterial blood gases, hemoglobin, electrolytes and percent oxygen extraction were determined. Blood samples were obtained before aortic cross-clamping (baseline), at 4-h intervals during perfusate preservation ($T_{4\text{-h}}$, $T_{8\text{-h}}$) in the CP group, and at 2 h of reperfusion in both groups. Percent oxygen extraction was calculated as: $100 \times (PaO_2 - PvO_2)/PaO_2$ (where PaO_2 and PvO_2 are arterial and venous PO_2, respectively). After ventricular biopsies were obtained, the heart specimens were weighed and labeled as wet weight. Each specimen was then dried in an 80 °C oven for 48 h to obtain its dry weight. Myocardial water content (MWC) was calculated as follows:

$$MWC = [(\text{Wet weight} - \text{Dry weight})/(\text{Wet weight})] \times 100\%$$

Biochemical measurements

After 11 h (8 h of preservation + 3 h of reperfusion), left ventricular biopsies were obtained to measure ATP and MDA levels. ATP concentrations using the bioluminescent technique (Luciferase-luciferin, Beyotime Institute, Jiangsu, China) were measured, and the levels of MDA, marker of oxidative damage, were measured using the biochemical assay of TBA (Jiancheng Institute, Nanjing, China).

Statistical analysis

SPSS 13.0 software package was used for statistical analysis (Spss Inc., Chicago, IL, USA). Categorical data were analyzed using the chi-square test and Fisher's exact test. Continuous data were expressed as the mean ± SD and were analyzed by a two-tailed Student's *t*-test and one-way analysis of variance. Significance was set at $P < 0.05$.

Results

Cardiac function

All hearts in CS group required direct-current defibrillation and one heart required four attempts and still could not resume pump function. Only one heart in CP group needed defibrillation, and the other five hearts returned to normal sinus rhythm without direct-current shock. All hearts of CP group were successfully weaned off bypass, but for CS group, only four hearts were weaned off successfully (Table 1). There were significant differences between two groups for corresponding parameters of LV function (LVSP: 77.8 ± 3.3 vs. 92.5 ± 2.7 mmHg; LVEDP: −1.7 ± 1.4 vs. −8.0 ± 0.6 mmHg; +dP/dt: 806 ± 17 vs. 1616 ± 94 mmHg/s; −dP/dt: −520 ± 23 vs. −632 ± 33 mmHg/s, CS group vs. the CP group, $P < 0.0001$, Fig. 1a and b). There was a significant difference in the

Table 1. Myocardial recovery data.

	CS group (n = 6)	CP group (n = 6)	χ^2
Hearts receiving defibrillation	6	1	0.015
Hearts returned to normal sinus rhythm	5	6	1.000
Hearts successfully weaned from bypass	4	6	0.121

Data are expressed as the number of hearts.
CP, continuous perfusion; CS, cold storage.

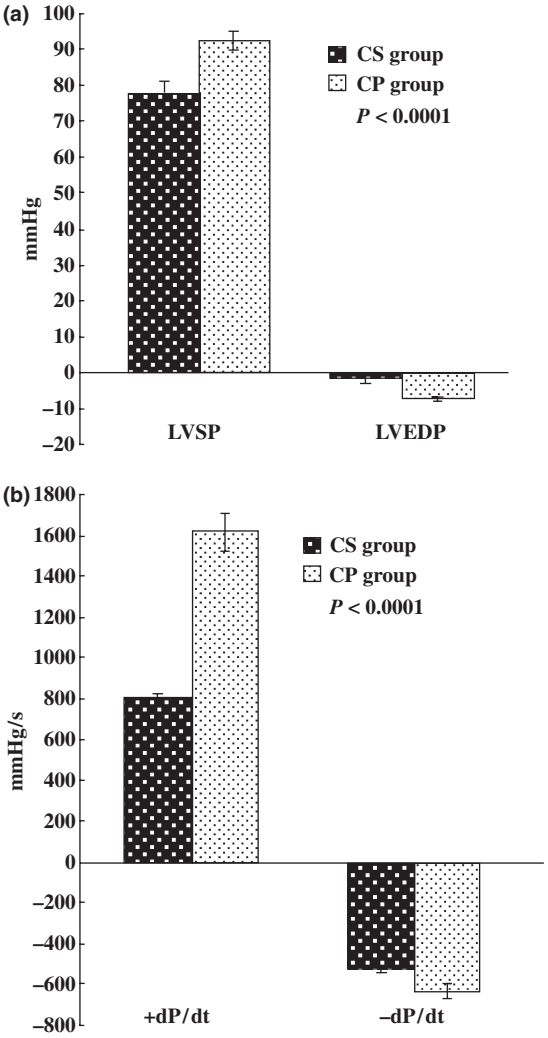

Figure 1 (a) LVSP and LVEDP of cold storage (CS) and continuous perfusion (CP) groups after 2 h of reperfusion. (b) +dP/dt and −dP/dt for CS and CP groups after 2 h of reperfusion. The difference between the two groups was significant for each parameter of LV function ($P < 0.0001$). LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure, +dP/dt: the maximum rate of developed pressure, −dP/dt: maximum negative rate of developed pressure.

CO between the groups (2.34 ± 0.53, vs. 1.79 ± 0.27, $P < 0.05$).

Blood characteristics

Blood components were adequately maintained throughout the 8-h preservation period and 2-h reperfusion period. The use of a leukocyte-depleting filter significantly reduced the white blood cell and platelet count in CP group at T_0, $T_{4\text{-h}}$, and $T_{8\text{-h}}$ time points. During the reperfusion period, intergroup comparison did not show any significant differences (Table 2).

Cardiac metabolism and water content

Figure 2 illustrates myocardial percent oxygen extraction at baseline, $T_{4\text{-h}}$ and $T_{8\text{-h}}$, which were the three different time points during preservation period in CP group. A significant decline in percent oxygen extraction was observed during the perfusion preservation period ($T_{4\text{-h}}$ and $T_{8\text{-h}}$) vs. baseline (59 ± 3% and 61 ± 4% respectively vs. 77 ± 3%, $P < 0.0001$). There was no statistical difference in percentage oxygen extraction between $T_{4\text{-h}}$ and $T_{8\text{-h}}$ during preservation. There was a significant difference in MWC between the groups after 11 h (8-h preservation + 3-h reperfusion). The degree of myocardium edema in CP hearts was higher than that in CS hearts (81.5 ± 1.3% vs. 79.6 ± 0.9%, $P = 0.003$).

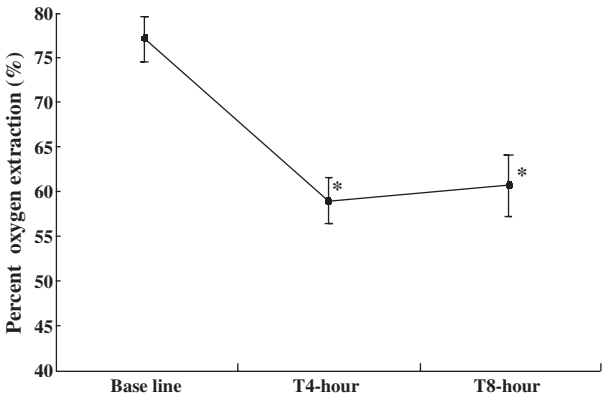

Figure 2 Myocardial percent oxygen extraction at baseline, $T_{4\text{-h}}$ and $T_{8\text{-h}}$, which were the three time points during the preservation period of continuous perfusion (CP) heart. There was a significant decline of oxygen extraction during the perfusion preservation period versus baseline ($P < 0.0001$).

Biochemical measurements

Adenosine triphosphate and MDA levels in myocardium in both groups are shown in Fig. 3. After 11 h, significant

Table 2. Arterial blood gases, blood count, and electrolytes.

		Preservation period			Reperfusion	
		CP group $n = 6$				
	Baseline	T_0	$T_{4\text{-h}}$	$T_{8\text{-h}}$	CS group $n = 5$	CP group $n = 6$
WBC($\times 10^9$/l)	16 ± 4	0.5 ± 0.3	0.5 ± 0.4	0.8 ± 0.3	6.3 ± 1.7	7.0 ± 1.7
Hb (g/l)	109 ± 13	93 ± 11	86 ± 5	81 ± 7	95 ± 1	97 ± 12
Hct (%)	30 ± 3	21 ± 2	19 ± 1	18 ± 1	26 ± 4	26 ± 3
PLT	336 ± 103	15 ± 6	36 ± 9	34 ± 17	115 ± 32	122 ± 30
pH (unit)	7.40 ± 0.04	7.38 ± 0.11	7.36 ± 0.06	7.37 ± 0.06	7.41 ± 0.03	7.39 ± 0.02
PaO_2 (mmHg)	312 ± 52	154 ± 25	157 ± 17	159 ± 26	585 ± 59	602 ± 31
PvO_2 (mmHg)	71 ± 5	–	64 ± 8	63 ± 10	68 ± 3	65 ± 6
$PaCO_2$ (mmHg)	37 ± 5	41 ± 5	32 ± 4	42 ± 5	37 ± 5	39 ± 4
SO_2 (%)	100	99.7 ± 0.6	100	100	100	100
Na^+ (mM)	144 ± 7	148 ± 7	150 ± 6	152 ± 5	145 ± 3	145 ± 4
K^+ (mM)	4.1 ± 0.7	13.3 ± 2.9	14.4 ± 1.0	16.2 ± 1.1	5.4 ± 0.9	5.1 ± 0.7
Ca^{2+} (mM)	1.7 ± 0.4	1.2 ± 0.3	1.09 ± 0.2	1.01 ± 0.2	2.2 ± 0.2	1.9 ± 0.3
HCO_3^- (mM)	23 ± 4	22 ± 3	21 ± 4	22 ± 3	22 ± 2	23 ± 4
BUN (mM)	3 ± 1	2.3 ± 0.4	2.1 ± 0.3	2.2 ± 0.5	3.5 ± 0.5	3.5 ± 0.5
Creatinine (μM)	66 ± 16	44 ± 7	42 ± 6	41 ± 7	57 ± 9	60 ± 11
Glucose (mM)	9.5 ± 2.3	12.8 ± 1.9	10.1 ± 1.6	8.3 ± 1.9	9.3 ± 1.0	9.5 ± 2.2

Data are expressed as mean ± SD.

CS, cold storage; CP, continuous perfusion; WBC, White blood cell; Hb, hemoglobin; Hct, hematocrit; PLT, platelet; PaO_2, arterial oxygen tension; PvO_2, venous oxygen tension; $PaCO_2$, carbon dioxide tension; SO_2, oxygen saturation; BUN, blood urea nitrogen; T_0, time at initiation of perfusion; $T_{4\text{-h}}$,$T_{8\text{-h}}$, 4 h and 8 h after initiation of continuous perfusion of oxygenated blood cardioplegia.

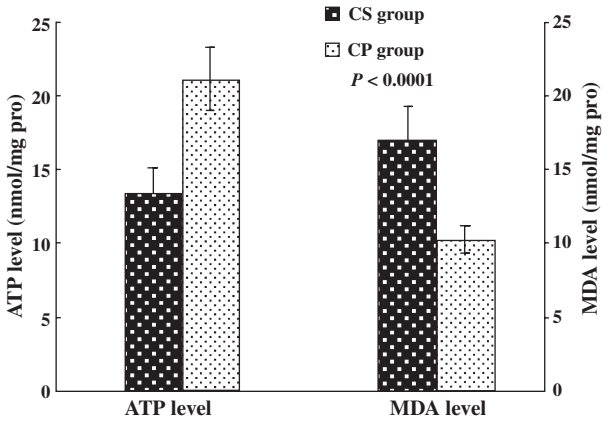

Figure 3 Myocardial levels of adenosine triphosphate (ATP) and malondialdehyde (MDA). After 11 h (8 h of preservation + 3 h of reperfusion), significant differences in ATP and MDA levels were observed between the two groups ($P < 0.0001$).

differences were observed between the two groups (ATP: 13.33 ± 1.78 vs. 21.17 ± 2.09 nmol/mg pro; MDA: 17.16 ± 2.34 vs. 10.34 ± 0.94 nmol/mg pro, CS vs. CP, $P < 0.0001$, Fig. 3).

Discussion

The purpose of this study was to investigate whether continuous perfusion with oxygenated blood of *ex vivo* swine hearts is more effective for preserving myocardial function after prolonged storage of 8 h outside the body than conventional static CS. In our study, most of CP hearts automatically reverted to normal sinus rhythm, whereas all hearts in CS group required DC shock and one of the hearts could not be successfully reverted. Each CP heart was successfully weaned off the bypass, whereas only four hearts of CS group could be successfully weaned off. Parameters of myocardial function during systolic and diastolic phases were better preserved in CP hearts compared with those of CS hearts. Continuous perfusion hearts performed significantly better with regard to viability markers, with better ATP preservation and lower levels of MDA (oxidative injury) compared with those in CS hearts. Our results suggested that CP preservation was more beneficial than conventional CS preservation.

In this study, we found that donor hearts sustained with the CS method, suffered energy depletion, oxidative injury and myocardial dysfunction, while these insults appeared to be minimal using CP during preservation. Although CS is universally accepted, it is an imperfect method for heart preservation. It is thought that myocardial protection using cold cardioplegia is less effective because of a prolonged disturbance of cardiac metabolism and ion homeostasis. In particular, ATP-dependent reactions are impaired, resulting in negative effects on membrane stability, energy production, enzyme function, aerobic glucose utilization, ATP generation and utilization, cyclic adenosine monophosphate production, and osmotic homeostasis [10–12]. Thus, after heart transplant, there is severe manifestation of ischemia/reperfusion injury. Primary graft dysfunction affects approximately 3% of clinical heart transplants performed worldwide, and accounts for 26% of deaths in the first 30 days after surgery [12,13].

Continuous perfusion preservation has three basic advantages over the simple immersion technique. First, a constant oxygen supply is delivered to the myocardium, thus preventing the occurrence of ischemia, anaerobic respiration and reperfusion injury. Second, the perfusate contains nutritional supplementation and metabolic substrates, which are available to myocardial cells, and it acts as a free radical scavenger and a potent buffer. Third, continuous perfusion preservation enables the clearance of metabolic waste of cardiac cells through the active coronary circulation [8,14–16]. It has been suggested that CP improves microvascular protection by promoting a more even, optimal pressure and cooling temperature [17]. In addition, the previous studies have reported adverse effects of leukocytes and platelets in ischemia/reperfusion injury of the heart [18,19]. Activation and accumulation of neutrophils and platelets lead to capillary plugging, impaired perfusion and spiking of oxygen free radicals, which may cause myocardial stunning, cardiac contractile dysfunction, endothelial injury, and further allograft vasculopathy [20]. To minimize these adverse effects, we used a leukocyte-depleting filter during the preservation period. Platelet and white cell counts were significantly suppressed in our study (Table 2).

Aupperle *et al.* [21] reported using lower perfusion pressure (40–50 mmHg) that resulted in a better preservation of the ultrastructure in explanted hearts connected to the Langendorff system. Jones *et al.* [4] experimented on perfusion with a PEG-Hb solution at 20 °C and 30 mmHg for 24 h in the rabbit model. A coronary perfusion pressure that is too low (15 mmHg) may result in inhomogeneous tissue perfusion and oxygen delivery [16]. It has been observed that the empty beating, normothermic heart requires 75–90% less oxygen than does the normal working heart. A further reduction in temperature to 22 °C results in a 70% decrease in oxygen demand, which equates to 0.3 ml/min per 100 g of myocardium [22]. In view of the above findings, we opted to use a 26 °C temperature perfusate in our experiment, which equates to a 90% decrease in oxygen demand of myocardium compared with the normothermic empty beating heart. Based on the results of our pilot studies, we selected a perfusion flow rate of 40 ml/min, which

corresponded to a perfusion pressure of approximately 35–45 mmHg. The percent of oxygen extraction was not different between two intervals ($T_{4\text{-h}}$ and $T_{8\text{-h}}$) during preservation period in CP hearts, representing a balance of oxygen supply and demand, and a stable homeostasis of the internal environment, indicating adequate myocardial perfusion. In addition, at 4 h of the preservation, blood exchange transfusions were used to provide substrate, to ensure an adequate supply of normal red blood cells, to improve oxygen delivery to the heart, and to reduce hemolysis. A volume of 200 ml of circulating perfusion solution was drained off the circuit and replaced by a similar volume of fresh blood cardioplegia to continue perfusion preservation.

Although several studies have reported that CP hearts may develop myocardial edema, which may negatively affect post-transplant diastolic recovery [16,23,24], the impact of myocardial weight gain during preservation is unclear [25,26]. The degree of myocardial edema in CP hearts was higher than that of CS hearts in our study, while CP hearts performed significantly better than CS hearts for all measured parameters of systolic and diastolic function, LVSP, LVEDP, and ±dP/dt as well as the CO in the working heart state. In the previous studies [16,23,24], asanguinous perfusates were used, which are different from blood perfusates, and the perfusion pressure was not suitable for tissue perfusion [16]. Moreover, isolated heart models were used instead of orthotopic heart transplants to evaluate myocardial recovery. The nonworking Langendorff model is less effective for analyzing diastolic function [27]. Edema in the setting of CP is a complex phenomenon affected by hydrostatic pressure created by CP and colloid oncotic pressure of the perfusate. Edema may also result from delivery of the perfusate to the capillaries using a nonphysiologic pattern of flow. Unlike conditions existing during conventional CP, normal physiologic pressure and flow in the coronary vasculature are not constant but show normal variations over the course of the cardiac cycle [28]. Nevertheless, the composition of the ideal perfusate and optimal preservation conditions remain incompletely defined. More studies need to be performed to determine this issue.

Adenosine triphosphate levels of CS hearts were approximately only 62% to that of CP hearts, despite 2 h of reperfusion with whole blood containing all necessary substrates for energy repletion. This finding suggests that there was energy depletion during cold storage. To avoid invasive injury to the grafts before implantation, we preferred not to carry out myocardial biopsies at baseline and at the end of the preservation period, which is a limitation of the study. However, the significant difference of ATP levels between two groups indicates that there is better energy preservation with CP.

In conclusion, our study further confirms that continuous perfusion with oxygenated blood cardioplegia can extend the current preservation period of donor hearts and also prevents time-dependent ischemic injury, thereby allowing distant availability of harvested hearts. Before proceeding to clinical trials, further studies are required to improve the perfusion system and minimize potential side effects. Unlike CS, CP provides the opportunity for dynamic assessment of the organ before committing to transplantation. Organ utilization is maximized while recipient risk is minimized with the incorporation of CP.

Authorship

FZ: contributed to the research/study, collected data, analyzed data and wrote the paper. AM: contributed to the research/study and collected data. ZW, YZ, and SL: contributed to the research/study. HL: designed and contributed to the research/study.

Acknowledgements

The authors thank Bristol-Myers Squibb Company for providing the UW preservation solution and Terumo Corporation for the hollow-fiber membrane oxygenator.

References

1. Stowe DF, Camara AK, Heisner JS, Aldakkak M, Harder DR. Low-flow perfusion of guinea pig isolated hearts with 26 degrees C air-saturated Lifor solution for 20 hours preserves function and metabolism. *J Heart Lung Transplant* 2008; **27**: 1008.
2. Peltz M, He TT, Adams GA, *et al.* Perfusion preservation maintains myocardial ATP levels and reduces apoptosis in an ex vivo rat heart transplantation model. *Surgery* 2005; **138**: 795.
3. Cressoni ES, Avanci LE, Braile DM, *et al.* Effects of myocardial protection in hypertrophic rabbit hearts: structural and ultra structural analysis. *Rev Bras Cir Cardiovasc* 2007; **22**: 24.
4. Jones BU, Serna DL, Smulowitz P. Extended ex vivo myocardial preservation in the beating state using a novel polyethylene glycolated bovine hemoglobin perfusate based solution. *ASAIO J* 2003; **49**: 388.
5. Li G, Sullivan JA, Hall RI. Functional recovery in rabbit heart after preservation with a blood cardioplegic solution and perfusion. *J Heart Lung Transplant* 1993; **12**: 263.
6. Sellke FW, Richter HW, Dunphy G, Azodi M, Ely DL. Twenty-four-hour heart preservation using continuous cold perfusion and copper (II) complexes. *J Surg Res* 1998; **80**: 171.

7. Ferrera R, Marcsek P, Larese A, *et al.* Comparison of continuous microperfusion and cold storage for pig heart preservation. *J Heart Lung Transplant* 1993; **12**: 463.
8. Ozeki T, Kwon MH, Gu J, *et al.* Heart preservation using continuous ex vivo perfusion improves viability and functional recovery. *Circ J* 2007; **71**: 153.
9. Bretschneider HJ. Myocardial protection. *Thorac Cardiovasc Surg* 1980; **28**: 295.
10. Calafiore AM, Teodori G, Mezzetti A, *et al.* Intermittent antegrade warm blood cardioplegia. *Ann Thorac Surg* 1995; **59**: 398.
11. Rivard AL, Gallegos RP, Bianco RW, Liao K. The basic science aspect of donor heart preservation: a review. *J Extra Corpor Technol* 2004; **36**: 269.
12. Stringham JC, Southard JH, Hegge J, *et al.* Limitations of heart preservation by cold storage. *Transplantation* 1992; **53**: 287.
13. Taylor DO, Edwards LB, Boucek MM, *et al.* Registry of the International Society for Heart and Lung Transplantation: twenty-second official adult heart transplant report. *J Thorac Cardiovasc Surg* 2005; **24**: 945.
14. Smulowitz PB, Serna DL, Beckham GE, Milliken JC. Ex vivo cardiac allograft preservation by continuous perfusion techniques. *ASAIO J* 2000; **46**: 389.
15. Franke UF, Korsch S, Wittwer T, *et al.* Intermittent antegrade warm myocardial protection compared to intermittent cold blood cardioplegia in elective coronary surgery – do we have to change? *Eur J Cardiothorac Surg* 2003; **23**: 341.
16. Tsutsumi H, Oshima K, Mohara J, *et al.* Cardiac transplantation following a 24-h preservation using a perfusion apparatus. *J Surg Res* 2001; **96**: 260.
17. Poston RS, Gu J, Prastein D, *et al.* Optimizing donor heart outcome after prolonged storage with endothelial function analysis and continuous perfusion. *Ann Thorac Surg* 2004; **78**: 1362.
18. Hayashi Y, Sawa Y, Nishimura M, *et al.* Clinical evaluation of leukocyte depleted blood cardioplegia for pediatric open heart operation. *Ann Thorac Surg* 2000; **69**: 1914.
19. Koch A, Bingold TM, Oberländer J, *et al.* Capillary endothelia and cardiomyocytes differ in vulnerability to ischemia/reperfusion during clinical heart transplantation. *Eur J Cardio Thorac Surg* 2001; **20**: 996.
20. Sakamoto Y, Wei LH, Buckberg GD, Youg HH. Effects of leukocyte-depleted reoxygenation on endothelial and ventricular function: with observation of a short time period. *Ann Thorac Cardiovasc Surg* 2002; **8**: 343.
21. Aupperle H, Garbade J, Ullmann C, *et al.* Comparing the ultrastructural effects of two different cardiac preparation- and perfusion-techniques in a porcine model of extracorporal long-term preservation. *Eur J Cardiothorac Surg* 2007; **31**: 214.
22. Buckberg GD, Brazier JR, Nelson RL, Goldstein SM, McConnell DH, Cooper N. Studies of the effects of hypothermia on regional myocardial blood flow and metabolism during cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 1977; **73**: 87.
23. Serna DL, Powell LL, Kahwaji C, *et al.* Cardiac function after eight hour storage by using polyethylene glycol hemoglobin versus crystalloid perfusion. *ASAIO J* 2000; **46**: 547.
24. Okada K, Yamashita C, Okada M, Okada M. Efficacy of oxygenated University of Wisconsin solution containing endothelin-A receptor antagonist in twenty-four-hour heart preservation. *J Heart Lung Transplant* 1996; **15**: 475.
25. Oshima K, Morishita Y, Yamagishi T, *et al.* Long-term heart preservation using a new portable hypothermic perfusion apparatus. *J Heart Lung Transplant* 1999; **18**: 852.
26. Cooper DK, Wicomb WN, Rose AG, Barnard CN. Orthotopic allotransplantation and autotransplantation of the baboon heart following 24-h storage by a portable hypothermic perfusion system. *Cryobiology* 1983; **20**: 385.
27. Sutherland FJ, Hearse DJ. The isolated blood and perfusion fluid perfused heart. *Pharmacol Res* 2000; **41**: 613.
28. Collins MJ, Moainie SL, Griffith BP, Poston RS. Preserving and evaluating hearts with ex vivo machine perfusion: an avenue to improve early graft performance and expand the donor pool. *Eur J Cardiothorac Surg* 2008; **34**: 318.