

Heart Preservation Using Continuous Ex Vivo Perfusion Improves Viability and Functional Recovery

Toshinaga Ozeki, PhD; Michael H Kwon, BS; Junyan Gu, PhD;
Michael J Collins, BS; John M Brassil, BS*; Michael B Miller Jr, BS*;
Rao P Gullapalli, PhD; Jiachen Zhuo, MS; Richard N Pierson III, MD;
Bartley P Griffith, MD; Robert S Poston, MD

Background Cold static storage (CS) is a proven preservation method for heart transplantation, yet early post-operative graft dysfunction remains prevalent, so continuous perfusion (CP) during ex vivo transport may improve viability and function of heart grafts.

Methods and Results Canine hearts underwent CP (n=9) or CS (n=9) for 6 h while intramyocardial pH was continuously monitored. Biopsies were assayed for ATP, caspase-3, malondialdehyde (MDA), and endothelin-1 (ET-1) levels at baseline, after preservation (t_1), and after 1 h of blood reperfusion on a Langendorff model (t_2). Functional recovery was determined at t_2 by +dP/dt, -dP/dt, developed pressure, peak pressure and end-diastolic pressure. CP resulted in higher tissue pH and ATP stores and reduced caspase-3, MDA and ET-1 levels compared with CS at both t_1 and t_2 . Post reperfusion recovery was significantly greater in CP vs CS for all myocardial functional parameters except end-diastolic pressure. Weight gain was significantly increased in CP vs CS at t_1 , but not at t_2 .

Conclusions Low-grade tissue acidosis and energy depletion occur during CS and are associated with oxidative injury and apoptosis during reperfusion. CP attenuates these biochemical and pathologic manifestations of tissue injury, together with improved myocardial recovery, despite mild, transient edema. (Circ J 2007; 71: 153–159)

Key Words: Metabolism; Myocardium; Reperfusion; Transplantation

Because cold static storage (CS) has proven to be simple, inexpensive and reliable, it is the standard of care for preserving donor hearts during the ex vivo transport interval.^{1,2} However, CS is an imperfect method, associated with low-level but persistent anaerobic metabolism that induces discrete changes of myocardial gene expression.^{3,4} These effects contribute to the risk of post-transplantation primary graft dysfunction, a problem that remains pervasive in clinical cardiac transplantation! Although preservation times less than 4–6 h limit these consequences for the “ideal donor”,^{4,5} this time limit poses a significant obstacle for transporting a heart to a geographically remote but otherwise well-matched recipient most in need of timely transplantation. Furthermore, the justifiable perception that ischemia in the setting of less-than-ideal donor heart compounds the risk of poor graft function undermines efforts to use “extended” or “marginal” heart donors.

Prior studies with continuous perfusion (CP) of donor hearts with oxygen and metabolites have demonstrated physiologically important support of aerobic metabolism⁶ needed for maintaining cell integrity and vital cell functions

during the transport period^{6–10} Other potential advantages include myocardial cooling through the native coronary circulation and the ongoing washout of metabolic byproducts. Improving myocardial preservation with CP may reduce the risk of primary graft dysfunction and better microvascular protection may lessen the chance for perioperative endothelial dysfunction, a proven risk factor cardiac allograft vasculopathy (CAV).¹¹

Ex vivo CP of the renal allograft has been used successfully for many years for clinical organ preservation.¹² CP for donor heart preservation is a natural extension of this technology approved by the US Food and Drug Administration. Despite promising evidence of the benefit of CP during the transport of human donor hearts^{8–13} little progress has been made in the clinical development of this technique since initial reports 20 years ago. Concerns about increased myocardial edema and the technical complexity of CP compared to CS have continued to dampen enthusiasm^{6,14,15} The aim of this study was to systematically evaluate a modified, improved approach to CP of heart grafts, with respect to myocardial viability and function, in a preclinical dog model.

Methods

Eighteen mongrel dogs weighing 19–25.5 kg (21.7±1.8 kg) were used as heart and blood donors. Donor hearts were divided into 2 groups (n=9 for CS group, n=9 for CP group). The protocol was approved by the Institutional Animal Care and Use Committee at the University of Maryland Medical Center. All animals received humane

(Received June 7, 2006; revised manuscript received October 11, 2006; accepted October 23, 2006)

Division of Cardiac Surgery, University of Maryland School of Medicine and VA Medical Center at Baltimore, Baltimore, MD, *Organ Recovery Systems, Inc, Des Plaines, IL, USA

Disclosure: John Brassil and Michael Miller disclose that they have a financial interest in Organ Recovery Systems, Chicago, IL, USA.

Mailing address: Robert S Poston, MD, Assistant Professor of Surgery, Division of Cardiac Surgery, N4W94 22 S. Greene St, Baltimore, MD 21201, USA. E-mail: rposton@smail.umaryland.edu



Fig 1. Heart Transporter™, a portable perfusion pump equipped with temperature and perfusion pressure controls, as well as a bubble oxygenator. After baseline analysis in situ, hearts were preserved in cold storage or with continuous perfusion using this apparatus. After preservation, hearts were reperfused with blood at 37°C using an isolated nonworking heart preparation (Langendorff) to simulate heart transplantation.

care in compliance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health publication 85–23, revised 1985), were housed in conformance with National Institutes of Health guidelines, and were fed a routine diet.

Experimental Procedure

Donor Heart Harvest Dogs were anesthetized with 20 mg/kg intramuscular ketamine induction followed by isoflurane titration for maintenance and 100% oxygen via mechanical ventilation. After placement of a femoral arterial line for pressure monitoring, hearts were exposed via median sternotomy. IV heparin (300 IU/kg), followed by timed infusion of 1 L Celsior cardioplegia (Sangstat Corp; Fremont, CA, USA), was administered into the aortic root prior to donor cardiectomy. After exsanguination and harvesting of donor blood into citrate-phosphate-dextrose transfusion bags, the hearts were excised in standard fashion.

Myocardial Preservation Protocol CS hearts were stored for 6 h in an iced (0–4°C) Celsior™ solution (Sangstat Corp). To initiate CP, a perfusion cannula was placed into the aortic root and the hearts were suspended on a Heart Transporter™, a lithium-powered, ultra-lightweight apparatus that includes a bubble oxygenator to maintain PO₂ between 200 and 400 mmHg (Organ Recovery Systems; Des Plaines, IL, USA) (Fig 1). Monitors allow for continuous recording of the infusion temperature of the perfusate, coronary flow, and resistance. CP hearts were perfused with RS-1™ (Organ Recovery Systems), an extracellular solution supplemented with adenine, and fructose-1,6-bisphosphate, adenosine, glutamate, albumin and chlorpromazine with pH titrated to 7.4. CP was performed with continuous 15 mmHg aortic pressure, which could satisfy 80 ml/min of coronary flow during CP at 4–6°C, and with a transient increase in the temperature of the perfusate to 25°C for 30 min at the initiation of CP followed by return to 4–6°C for the remainder of the preservation interval.

Left Ventricular (LV) Function Assessment After the preservation period, autologous, oxygenated whole blood at 37°C was infused at 70 mmHg into the aorta using a

nonworking, standard Langendorff preparation for 60 min in both CS and CP groups. PO₂ (500–600 mmHg) and PCO₂ (25–35 mmHg) were maintained using a membrane oxygenator/heart exchanger (Optima unit, Cat. #050255500, Cobe Cardiovascular; Arvada, CO, USA) ventilated with a 95%/5% oxygenated CO₂ mixture. Electrolyte concentrations were corrected to physiologically normal values in the blood perfusate prior to starting reperfusion and after reassessments performed at 15-min intervals. LV function was measured using an intraventricular 9F Millar catheter placed through the apex during the donor harvest (ie, baseline) and every 15 min during Langendorff reperfusion (ie, recovery) inside a fluid-filled latex balloon inflated to 0, 10 and 20 mL. All pressure data were continuously recorded with a computer-based data acquisition system (Powerlab, ADInstruments, Inc; Colorado Springs, CO, USA). The pressure vs time traces were analyzed via a previously described method of integrating trapezoidal areas under the curve^{16,17} to determine the following LV functional parameters: developed pressure (DP), peak systolic pressure (PSP), maximum rate of DP (+dP/dt), maximum negative rate of DP (–dP/dt), and LV end-diastolic pressure (LVEDP). The development of myocardial edema was assessed by obtaining gross weights of the excised grafts at baseline (*t*₀), at the end of the preservation interval (*t*₁), and at the end of 1 h of Langendorff reperfusion (*t*₂).

Graft Viability and Endothelial Function Assays Myocardial biopsies were obtained from anterior and posterior ventricular walls in all hearts at *t*₀, *t*₁, and *t*₂. Levels of adenosine triphosphate stores (ATP, marker of energy storage), caspase-3 (marker of apoptosis), malondialdehyde (MDA, marker of oxidative damage), and endothelin-1 (ET-1, marker of endothelial damage) were determined by mean values of the myocardial biopsies in both CS and CP groups. These assessments were performed with an ATP bioluminescent somatic cell assay kit (Sigma Chemical, St Louis, MO, USA), caspase-3 activity detection kit (Chemicon; Temecula, CA, USA), Bioxytech MDA-586 assay (OXIS International Inc; Portland, OR, USA), and ET-1 enzyme immunometric assay kit (Assay Designs; Ann Arbor, MI, USA).

pH and Temperature Recording Calibrated pH probes (Khuri™, Terumo Corp, Tokyo, Japan) were inserted on the anterior and posterior surface of the left ventricle with a ground lead placed on the anterior ventricular wall. pH and temperature readings were recorded every 10 s starting from *t*₀ and continuing throughout both *t*₁ and *t*₂.

Tissue Perfusion Imaging To determine tissue perfusion during CP, hearts were analyzed by gadolinium-enhanced magnetic resonance imaging (MRI) during the ex vivo preservation period. All imaging was conducted on a 1.5T Philips Eclipse MRI scanner equipped with echo planar gradients to assess the homogeneity of tissue perfusion using an 8-echo spoiled gradient echo sequence with echo time 2 ms, repetition time 5 ms, Flip Angle 20°, matrix 100×128, field of view 14×16 cm. A total of 3 mid-short-axis slices were acquired with a slice thickness of 6 mm with 5 mm gap, resulting in a temporal resolution of 1.9 ms. Gadolinium-DTPA (0.05 mmol/kg) was injected as a bolus into the aortic perfusion line after acquisition of 4 baseline frames. Signal intensity vs time (SIVT) curves were generated in each of 4 myocardial regions of interest (anterior, posterior, lateral and septal walls) within each of 3 mid-short-axis slices of the heart for a total of 12 curves per heart.

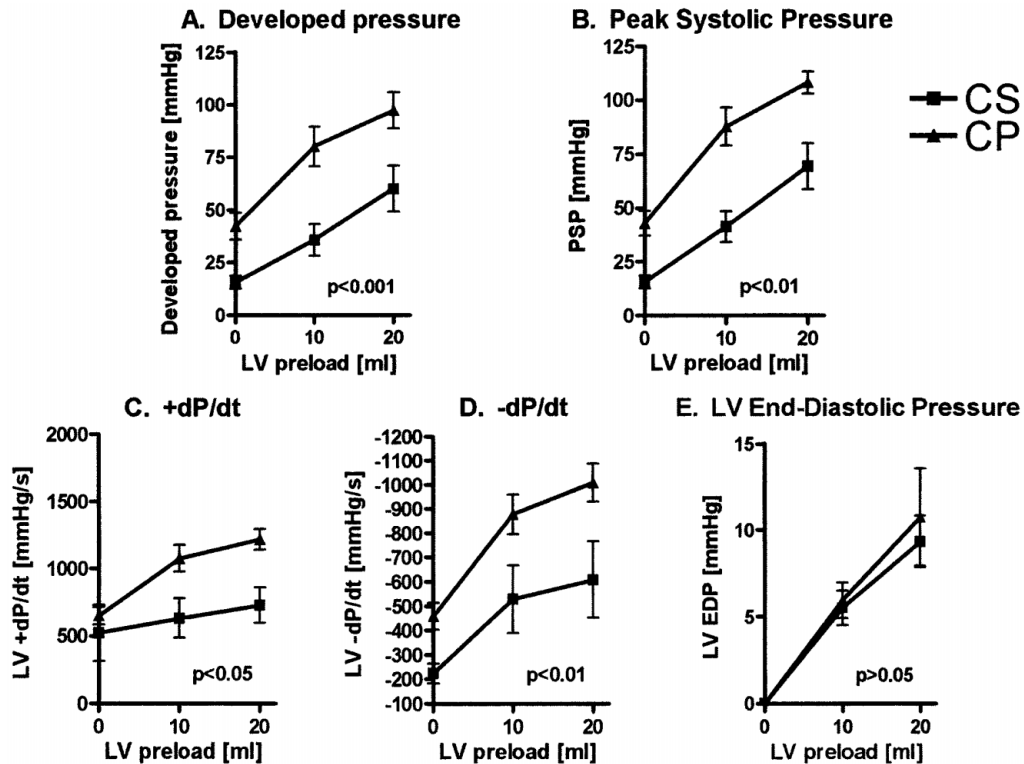


Fig 2. After preservation with either cold storage (CS) or continuous perfusion (CP), hearts underwent blood reperfusion with an isolated nonworking heart preparation (Langendorff). (A–C) During reperfusion, recovery of systolic function at the end of the 1-h reperfusion period, as assessed by developed pressure (DP), peak systolic pressure (PSP), and maximum rate of DP (+dP/dt) was significantly greater in CP vs CS hearts over the full range of left ventricular (LV) preloads (0, 10, 20 ml) on 2-factor repeated measures ANOVA. (D–E) Recovery of diastolic function was significantly improved in CP vs CS hearts as assessed by the maximum negative rate of DP. However, the difference seen in LV end-diastolic pressure was not significant between the 2 groups. -dP/dt, maximum negative rate of DP.

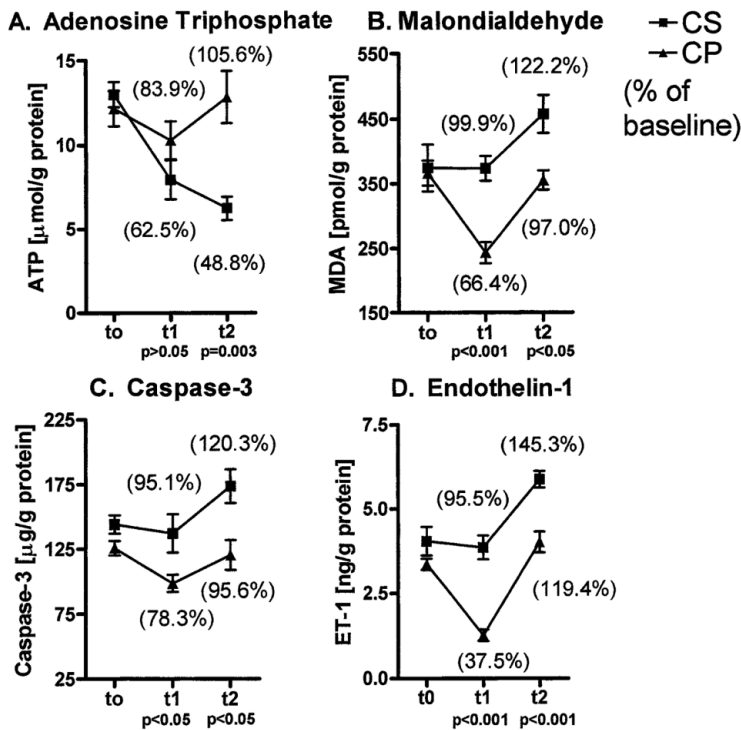


Fig 3. Myocardial viability and endothelial dysfunction was assessed in hearts subjected to continuous perfusion (CP) vs cold storage (CS). Tissue levels of adenosine triphosphate (ATP, marker of energy storage), malondialdehyde (MDA, marker of oxidative injury), caspase-3 (marker of apoptosis), and endothelin-1 (ET-1, marker of endothelial dysfunction) at 3 time points: baseline (t_0), immediately after 6h of preservation (t_1), and immediately after 1h of Langendorff reperfusion (t_2). (A–C) Although there were no differences at baseline, CP hearts showed a trend toward better ATP preservation and significantly lower levels of MDA and caspase-3 at t_1 compared with CS hearts. Differences in ATP and caspase-3 at t_1 further widened at t_2 such that all 3 viability markers were significantly improved in CP vs CS hearts. Only CP hearts were able to fully recover baseline levels of ATP while avoiding rises in MDA and caspase-3 beyond baseline. (D) Although neither method of preservation produced worsening of ET-1 levels at t_1 , CS hearts displayed a sharp rise in ET-1 at t_2 to 45% above baseline. CP provided for significant reductions in ET-1 levels compared to CS at t_1 that persisted significantly into t_2 .

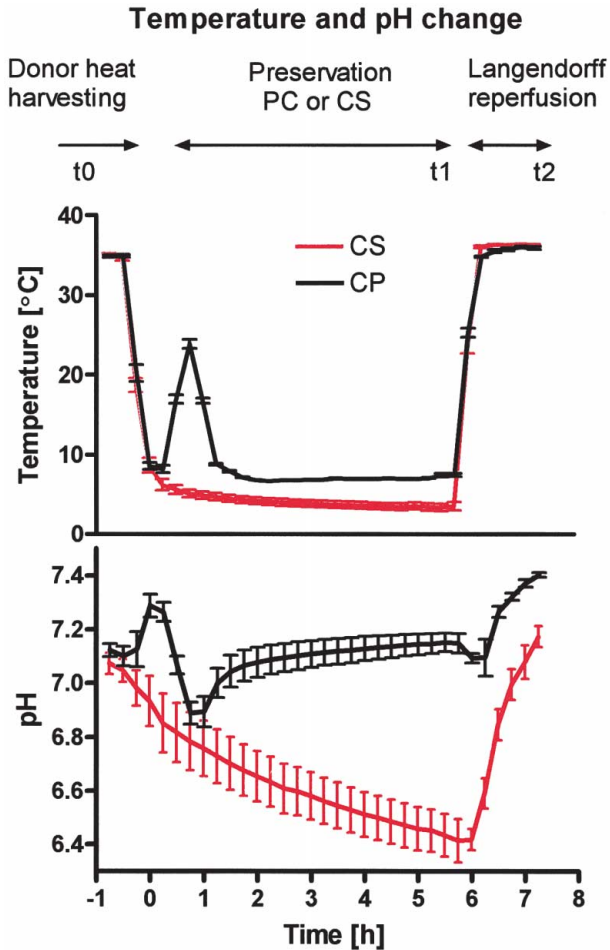


Fig4. Temperature and myocardial pH (mean of anterior and posterior walls of the left ventricle) were recorded continuously over the 6-h preservation interval (hours 0–6) and the 1-h warm-blood reperfusion interval (hours 6–7). Hearts subjected to cold storage (CS) exhibited a steady decrease in pH and developed significant acidosis by the end of the preservation interval and these persisted well into the reperfusion period. Meanwhile, hearts that underwent continuous perfusion (CP) maintained a physiologic pH throughout the majority of the preservation interval and recovered to baseline levels very early during reperfusion.

Statistical Analysis

Results are expressed as the mean \pm standard error of the mean. Statistical analysis was performed with a statistical software package (InStat 3.05 and Prism 4.0, GraphPad, Inc). Student's t-tests were used for testing differences in continuous variables between 2 groups. Two-factor repeated measures ANOVA was used to determine if there was a significant difference in the LV functional parameters described above across the entire series of LV preloads. Differences were considered to be significant at $p < 0.05$.

Results

LV Function

At t_0 , the CP and CS groups were well matched with respect to baseline myocardial function as assessed by functional parameters (DP: 84.5 ± 11.0 vs 83.9 ± 10.3 mmHg; PSP: 90.9 ± 11.0 vs 91.3 ± 13.4 mmHg; $+dP/dt$: $1,141 \pm 286$ vs $1,274 \pm 217$ mmHg/s; $-dP/dt$: $-1,017 \pm 120$ vs $-1,102 \pm 307$ mmHg/s; LVEDP: 7.37 ± 3.13 vs 6.39 ± 2.95 mmHg). At

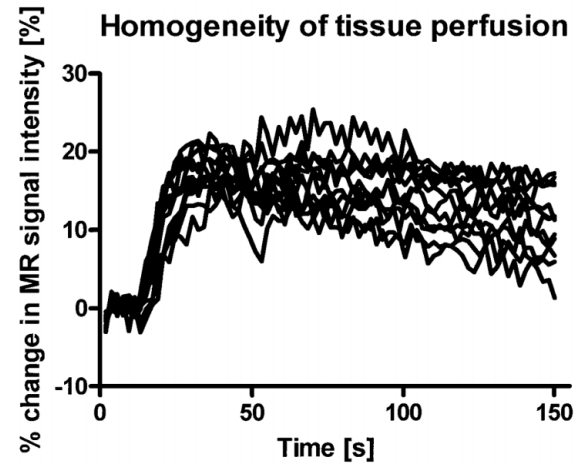


Fig5. Representative traces of signal intensity vs time (SIVT) curves during continuous perfusion (CP). SIVT were generated using standard first-pass imaging methodology for each of 4 areas of interest (posterior, anterior, lateral, and septal walls) in each slice for a total of 12 SIVT curves per heart. Evidence of homogeneous perfusion during CP was found in each heart evaluated.

t_1 , perfused hearts showed more weight gain (16.4 ± 4.2 vs $1.8 \pm 1.6\%$ increase from baseline, CP vs CS group, $p = 0.01$), but at t_2 neither weight gain (43.7 ± 6.1 vs $31.9 \pm 8.5\%$) nor LVEDP (Fig 2E) was significantly different between groups. At t_2 , $-dP/dt$ was significantly greater in CP vs CS (Fig 2D), reflecting improved diastolic relaxation. At t_2 , all parameters of LV systolic function recovery were significantly higher in CP vs CS group (Figs 2A–C), reflecting improved function of the CP group hearts.

Myocardial Viability Tests

Baseline (t_0) ATP, MDA, caspase-3 and ET-1 levels were similar in both groups. At t_1 , these markers each showed strong correlations with $+dP/dt$ at t_2 (R values: ATP 0.41, MDA -0.57 , caspase-3 -0.70 , ET-1 -0.64 ; p-values: ATP 0.049, MDA 0.014, caspase-3 0.001, ET-1 0.004), supporting their value as predictors of myocardial viability. Between t_0 and t_1 , hearts in the CS group experienced a significant reduction in ATP and unchanged levels of MDA, caspase-3, and ET-1, a pattern that continued at t_2 (Figs 3A–D). CP limited these viability changes at t_1 as evidenced by a trend toward higher ATP preservation (10.3 ± 1.1 vs 7.9 ± 1.2 $\mu\text{g/g}$ protein, $p = 0.188$), significantly lower MDA (243.4 ± 16.2 vs 373.7 ± 19.0 pmol/g protein, $p < 0.001$), caspase-3 (98.4 ± 6.7 vs 137.7 ± 14.9 $\mu\text{g/g}$ protein, $p = 0.047$) and ET-1 levels (3.86 ± 0.35 vs 1.26 ± 0.17 ng/g protein, $p < 0.001$) compared with CS hearts. After reperfusion (t_2), CP demonstrated a more complete recovery of ATP (12.8 ± 1.5 vs 6.2 ± 0.7 $\mu\text{g/g}$ protein, $p = 0.003$), suppression of MDA (355.4 ± 15.0 vs 457.3 ± 29.0 pmol/g protein, $p = 0.012$), caspase-3 activity (120.2 ± 11.6 vs 173.3 ± 13.1 $\mu\text{g/g}$ protein, $p = 0.011$) decreased level of ET-1 (4.02 ± 0.30 vs 5.87 ± 0.25 ng/g protein, $p = 0.004$) than CS.

Microvascular Perfusion Between t_0 and t_1 , CS hearts developed a steady drop in tissue pH (from 7.08 ± 0.06 to 6.35 ± 0.02 , $p < 0.001$, paired t-test). In contrast, CP hearts maintained a normal tissue pH during the preservation interval (from 7.11 ± 0.02 to 7.12 ± 0.04), suggesting appropriate tissue perfusion was achieved (Fig 4). As a result, the mean time for tissue pH to return to baseline levels during Langendorff reperfusion was significantly shorter for CP vs

CS (2.5 ± 2.0 vs 42.5 ± 2.9 min, $p < 0.001$). Homogeneity of perfusion was confirmed by gadolinium enhanced MRI examination. The SIVT curves obtained from the 4 myocardial regions of interest within each of the 3 mid-short-axis slices of the heart (ie, total of 12 regions) revealed consistently homogeneous perfusion in CP hearts (Fig 5).

Discussion

The primary finding of this study is that donor hearts undergoing CS suffer energy depletion, oxidative injury and apoptosis, which directly correlate with myocardial and endothelial dysfunction after reperfusion. These injuries appear to be largely avoided by using CP during the ex vivo preservation interval. Perfused hearts showed improvements in a wide range of viability markers coinciding with enhanced myocardial recovery. Although CS is universally accepted as a safe and effective method of heart preservation, primary graft dysfunction continues to affect approximately 3% of clinical heart transplants performed worldwide, and accounts for 26% of deaths in the first 30 days after transplant! Our data suggest that even in hearts from an "ideal donor", injury induced during CS may impact early myocardial performance and play a clinically important role in primary graft dysfunction. If results from this preclinical model are translated into clinical patients, CP could have significant favorable impact on the leading cause of death and morbidity in the early post-transplant period!

The primary aim of this study was to establish whether CP provides a safe means for improving myocardial function in the early period of reperfusion compared with CS. Different preservation solutions were used for this study: Celsior for CS and RS-1 for CP. Celsior is considered to be an extracellular solution with 15 mmol/L of K^+ concentration!⁶ The higher K^+ concentration in the Celsior solution is able to induce more rapid and complete cardiac arrest and prevent deleterious changes in membrane ionic flux during preservation. In contrast, the more physiological level of K^+ in RS-1 (5 mmol/L) appears to be important for homogeneous tissue perfusion by avoiding K^+ induced vasospasm.

Although CP hearts indeed performed significantly better on all measured parameters of systolic function, a consistent concern with CP has been edema, which may negatively affect post-transplant diastolic recovery!^{6,14,15} We confirm a higher degree of weight gain during CP compared with CS. However, the edema advantage of CS found at t_1 was transient and eliminated at t_2 because of the greater weight gain during Langendorff reperfusion of CS compared with CP hearts. Although a relationship to diastolic dysfunction has been suggested by others!⁷ edema at t_1 did not adversely affect myocardial function in our model.

Although primary dysfunction is unusual during clinical transplantation, the relationship between subclinical injury that occurs during CS to myocardial recovery after transplantation has not been fully characterized, and may be underappreciated. Our data show that CS for 6h, an interval at the margins of clinical acceptability, has discrete effects on myocardial metabolism and viability. ATP levels were depleted to two-thirds of baseline levels during CS and failed to recover despite 1h of reperfusion with whole blood containing all necessary substrates for energy repletion. The degree of decline in tissue pH during CS in this

study (6.35 after preservation) was consistent with prior reports!¹⁸ Markers of oxidative injury (MDA), apoptosis (caspase-3), and endothelial dysfunction (ET-1) were induced during CS and all were further exacerbated by reperfusion. In contrast, CP preserved ATP (Fig 3A) and maintained baseline tissue pH (Fig 4) during the preservation interval. As a result, CP hearts showed a rapid restoration of ATP and shorter period of tissue acidosis during reperfusion than the CS group (mean 2.5 vs 42.5 min). The shorter period of acidosis suggests decreased oxygen debt and improved microvascular recovery in CP hearts. The strong correlation of each of these viability markers with systolic function is consistent with previous studies. Suehiro et al previously reported a significant relationship between ATP levels during preservation and the recovery of systolic function!¹⁰ In addition, prior studies show that inhibition of lipid peroxidation!⁹ caspase-3!^{20,21} and ET-1!^{22,23} all decrease ischemia-reperfusion injury.

Continuous cold temperature!²⁴ has been shown to cause vasoconstriction during coronary perfusion. We speculate that transiently increasing the perfusate temperature largely abrogates the adverse effects of low perfusate temperature on rheology and/or endothelial function in flush-preserved hearts, a suggestion that is supported by the gadolinium-enhanced MRI findings (Fig 5). Hearts from the CP group showed a trend towards increased tissue pH above baseline that is consistent with a hyperemic response, providing further evidence of improved microvascular function in CP hearts. These findings also highlight that CP may not provide optimal protection of hearts, and perhaps other organs, without a focused validation of the tissue perfusion that is provided by a specific protocol.

ET-1, a potent vasoconstrictor and established marker of endothelial injury, was found to progressively increase from the baseline level during Langendorff reperfusion (t_2) in CS but not CP hearts (Fig 3D). Our data corroborate the relationship between heightened perioperative levels of ET-1 and early myocardial dysfunction. In addition, ET-1 has been suggested to play a role in late CAV, and reduced overall survival!²⁵⁻²⁸ in clinical cardiac transplantation. ET-1 antagonists improve systolic function in animal models of cardiac transplantation!^{29,30} Perhaps more importantly, clinical studies indicate that ET-1 is one of the strongest alloantigen-independent factors for the subsequent development of CAV!¹¹ By decreasing initial ET-1 expression induced by CS, CP may protect against the development of CAV, the leading cause of graft loss after the first year! The potential to affect this particularly vexing problem provides a compelling rationale for a clinical trial of this CP protocol.

The main limitation of this study is the use of an isolated heart model instead of orthotopic heart transplants to evaluate myocardial recovery. The nonworking Langendorff model is less effective for analyzing diastolic function!³¹ a common cause of graft dysfunction after transplantation!³² and only provides data for the first 2-3h after restoration of blood flow. A major advantage of the Langendorff model is its ability to specifically address our study aim of directly comparing CP with CS, the current gold standard, without the wide range of confounding effects present in a transplantation model!³³ This method is well-established!^{19,20,34-37} and has a track record of predicting the clinical performance of a wide range of clinical protocols in cardiac surgery, including anti-ischemic interventions!³¹ and methods of cardiac preservation!^{38,39} Although the heart rate and preload

dependence of DP, $+dP/dt$ and $-dP/dt$ are well-known potential sources of experimental error, we found a consistent agreement between each of these functional parameters at varying preloads, thus providing confidence in the reliability of the data. The use of young healthy dogs for heart donors does not fully conform with our aim to model the clinical use of CP in hearts from "standard donors" because brain death is a necessary component of clinical organ donation. However, a brain death model is unlikely to have altered our conclusions about the benefits of CP. In fact, the additional inflammatory challenge of brain death would seem to further aggravate the effects of CS on heart function, thereby widening the difference between preservation methods.

In conclusion, compared with the traditional CS method, CP improved myocardial acidosis, energy storage, apoptosis, and oxidative/ischemia-reperfusion injury in a pre-clinical large animal model. In addition to improving outcomes, this technique merits further assessment to extend the allowable transport period and facilitate ex situ pharmacologic interventions in order to potentially expand the donor pool. Despite some concerns regarding myocardial edema, the wide range of benefits of CP established in this preclinical model strongly support the initiation of a clinical trial to assess the safety and efficacy of CP in heart transplantation.

Acknowledgments

This work was funded in part by grants the Office of Naval Research, the Thoracic Surgery Foundation for Research and Education and an intramural grant from the University of Maryland. Supplies necessary for the completion of these studies were donated by Organ Recovery Systems (RS-1™, perfusion materials and technical expertise) and Sangsat (Celsior preservation solution).

References

- Taylor DO, Edwards LB, Boucek MM, Trulock EP, Deng MC, Keck BM, 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**: 945–955.
- Kitamura S, Nakatani T, Yagihara T, Sasako Y, Kobayashi J, Bando K, et al. Cardiac transplantation under new legislation for organ transplantation in Japan: Report of two cases. *Jpn Circ J* 2000; **64**: 333–339.
- Amberger A, Schneeberger S, Hernegger G, Brandacher G, Obrist P, Lackner P, et al. Gene expression profiling of prolonged cold ischemia and reperfusion in murine heart transplants. *Transplantation* 2002; **74**: 1441–1449.
- Buckberg GD, Brazier JR, Nelson RL, Goldstein SM, McConnell DH, Cooper N. Studies of the effects of hypothermia on regional myocardial flow and metabolism during cardiopulmonary bypass. I: The adequately perfused beating, fibrillating and arrested heart. *J Thorac Cardiovasc Surg* 1977; **73**: 87–94.
- Hassanein WH, Zellos L, Tyrrell TA, Healey NA, Crittenden MD, Birjiniuk V, et al. Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function. *J Thorac Cardiovasc Surg* 1998; **116**: 821–830.
- Poston RS, Gu J, Prastein D, Gage F, Hoffman JW, Kwon M, et al. Optimizing donor heart outcome after prolonged storage with endothelial function analysis and continuous perfusion. *Ann Thorac Surg* 2004; **78**: 1362–1370.
- Proctor E, Parker R. Preservation of isolated heart for 72h. *BMJ* 1968; **4**: 296–298.
- Wicomb WN, Cooper DK, Novitzky D, Barnard CN. Cardiac transplantation following storage of the donor heart by a portable hypothermic perfusion system. *Ann Thorac Surg* 1984; **37**: 243–248.
- Tsutsumi H, Oshima K, Mohara J, Takeyoshi I, Aizaki M, Tokumine M, et al. Cardiac transplantation following a 24-h preservation using a perfusion apparatus. *J Surg Res* 2001; **96**: 260–267.
- Suehiro K, Mohri M, Takagaki M, Hisamochi K, Morimoto T, Sano S. The effect of graft perfusion with warm blood cardioplegia for cadaver heart transplantation. *Surg Today* 1999; **29**: 890–896.
- Gaudin PB, Rayburn BK, Hutchins GM, Kasper EK, Baughman KL, Goodman SN, et al. Peritransplant injury to the myocardium associated with the development of accelerated arteriosclerosis in heart transplant recipients. *Am J Surg Pathol* 1994; **18**: 338–346.
- St. Peter SD, Imber CJ, Friend PJ. Liver and kidney preservation by perfusion. *Lancet* 2002; **359**: 604–613.
- Hardesty RL, Griffith BP. Autoperfusion of the heart and lungs for preservation during distant procurement. *J Thorac Cardiovasc Surg* 1987; **93**: 11–18.
- Serna DL, Powell LL, Kahwaji C, Wallace WC, West J, Cogert G, et al. Cardiac function after eight hour storage by using polyethylene glycol hemoglobin versus crystalloid perfusion. *ASAIO J* 2000; **46**: 547–552.
- 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**: 475–484.
- Jahania MS, Sanchez JA, Narayan P, Lasley RD, Mentzer RM Jr. Heart preservation for transplantation: Principles and strategies. *Ann Thorac Surg* 1999; **68**: 1983–1987.
- Hsu DT, Weng ZC, Nicolosi AC, Detwiler PW, Sciacca R, Spotnitz HM. Quantitative effects of myocardial edema on the left ventricular pressure-volume relation: Influence of cardioplegia osmolarity over two h of ischemic arrest. *J Thorac Cardiovasc Surg* 1993; **106**: 651–657.
- Dearani JA, Axford TC, Patel MA, Healey NA, Lavin PT, Khuri SF. Role of myocardial temperature measurement in monitoring the adequacy of myocardial protection during cardiac surgery. *Ann Thorac Surg* 2001; **72**: S2235–S2243; discussion S2243–S2244, S2267–S2270.
- Nishida T, Morita S, Miyamoto K, Masuda M, Tominaga R, Kawachi Y, et al. The effect of lazaroid (U74500A), a novel inhibitor of lipid peroxidation, on 24-hour heart preservation: A study based on a working model using cross-circulated blood-perfused rabbit hearts. *Transplantation* 1996; **61**: 194–199.
- McCully JD, Wakiyama H, Hsieh YJ, Jones M, Levitsky S. Differential contribution of necrosis and apoptosis in myocardial ischemia-reperfusion injury. *Am J Physiol* 2004; **286**: 1923–1935.
- Yang TL, Chen MF, Jiang JL, Xie QY, Li YP, Li YJ. The endothelin receptor antagonist decreases ischemia/reperfusion-induced tumor necrosis factor production in isolated rat hearts. *Int J Cardiol* 2005; **100**: 495–498.
- Naka Y, Sawa Y, Nishimura M, Hirata N, Ueda H, Ohtake S, et al. Participation of caspase-3-like protease in necrotic cell death of myocardium during ischemia-reperfusion injury in rat hearts. *Circ J* 2003; **67**: 248–252.
- Xia Z, Kuo K-H, McNeill JH, Ansley DM. Endothelin A and B receptor antagonist bosentan reduces posts ischemic myocardial injury in the rat: Critical timing of administration. *Can J Physiol Pharmacol* 2005; **83**: 259–266.
- Kevelaitis E, Nyborg NC, Menasche P. Coronary endothelial dysfunction of isolated hearts subjected to prolonged cold storage: Patterns and contributing factors. *J Heart Lung Transplant* 1999; **18**: 239–247.
- Weis M, Wildhirt SM, Schulze C, Rieder G, Wilbert-Lampen U, Wolf WP, et al. Endothelin in coronary dysfunction early after human heart transplantation. *J Heart Lung Transplant* 1999; **18**: 1071–1079.
- Spinale FG. The bioactive peptide endothelin causes multiple biologic responses relevant to myocardial and vascular performance after cardiac surgery. *J Thorac Cardiovasc Surg* 2002; **123**: 1031–1034.
- Ferri C, Properzi G, Tomassoni G, Santucci A, Desideri G, Giuliani AE, et al. Patterns of myocardial endothelin-1 expression and outcome after cardiac transplantation. *Circulation* 2002; **105**: 1768–1771.
- Ravalli S, Szabolcs M, Albala A, Michler RE, Cannon PJ. Increased immunoreactive endothelin-1 in human transplant coronary artery disease. *Circulation* 1996; **94**: 2096–2102.
- Brunner F, Leonhard B, Kukovetz WR, Mayer B. Role of endothelin, nitric oxide and L-arginine release in ischaemia/reperfusion injury of rat heart. *Cardiovasc Res* 1997; **36**: 60–66.
- Fedak PW, Rao V, Verma S, Ramzy D, Tumiali L, Miriuka S, et al. Combined endothelial and myocardial protection by endothelin antagonism enhances transplant allograft preservation. *J Thorac Cardiovasc Surg* 2005; **129**: 407–415.
- Sutherland FJ, Hearse DJ. The isolated blood and perfusion fluid perfused heart. *Pharmacol Res* 2000; **41**: 613–627.
- Kendall SW, Bittner HB, Peterseim DS, Campbell KA, Van Trig P. Right ventricular function in the donor heart. *Eur J Cardiothorac*

- Surg* 1997; **11**: 609–615.
33. Hearse DJ, Sutherland FJ. Experimental models for the study of cardiovascular function and disease. *Pharmacol Res* 2000; **41**: 597–603.
 34. Zhang ZW, Kaneda T, Ku K, Otaki M, Oku H. Ischemic preconditioning and nicorandil pretreatment improve donor heart preservation. *Jpn Circ J* 2001; **65**: 678–682.
 35. Fukuhara S, Matsushita S, Sakakibara Y. Changes in coronary resistance related to the stages of the female life cycle. *Circ J* 2006; **70**: 478–481.
 36. Yoo KJ, Li RK, Weisel RD, Mickle DA, Jia ZQ, Kim EJ, et al. Heart cell transplantation improves heart function in dilated cardiomyopathic hamsters. *Circulation* 2000; **102**(Suppl 3): III-204–III-209.
 37. Yaguchi Y, Satoh H, Wakahara N, Katoh H, Uehara A, Terada H, et al. Protective effects of hydrogen peroxide against ischemia/reperfusion injury in perfused rat hearts. *Circ J* 2003; **67**: 253–258.
 38. Galinanes M, Murashita T, Hearse DJ. Long-term hypothermic storage of the mammalian heart for transplantation: A comparison of three cardioplegic solutions. *J Heart Lung Transplant* 1992; **11**: 624–635.
 39. Menasche P, Pradier F, Grousset C, Peynet J, Mouas C, Bloch G, et al. Improved recovery of heart transplants with a specific kit of preservation solutions. *J Thorac Cardiovasc Surg* 1993; **105**: 353–363.