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

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Background Cold static storage (CS) is a proven preservation method for heart transplantation, yet early postoperative 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 6h 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 (t1), and after 1h of blood reperfusion on a Langendorff model (t2). Functional recovery was determined at t2 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 t1 and t2. 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 t1, but not at t2.

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 expression3,4 These effects contribute to the risk of post-transplantation primary graft dysfunction, a problem that remains pervasive in clinical cardiac transplantation.1 Although preservation times less than 4–6h 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 preservation12 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 hearts8–13 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 enthusiasm6,14,15 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 19–25.5kg (21.7±1.8kg) 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
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 1L 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 6h 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 Plains, 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. #05025500, 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 curve16,17 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 (t0), at the end of the preservation interval (t1), and at the end of 1 h of Langendorff reperfusion (t2).

**Graft Viability and Endothelial Function Assays** Myocardial biopsies were obtained from anterior and posterior ventricular walls in all hearts at t0, t1, and t2. 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), Baxxytech 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 (Karui™, 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 t0 and continuing throughout both t1 and t2.

**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) preload (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 (t₀), immediately after 6 h of preservation (t₁), and immediately after 1 h of Langendorff reperfusion (t₂). (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 t₁ compared with CS hearts. Differences in ATP and caspase-3 at t₁ further widened at t₂ 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 t₁, CS hearts displayed a sharp rise in ET-1 at t₂ to 45% above baseline. CP provided for significant reductions in ET-1 levels compared to CS at t₂ that persisted significantly into t₂.
Results

LV Function

At t₀, 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±286 vs 1,274±217 mmHg/s; −dP/dt: −1,017±120 vs −1,102±307 mmHg/s; LVEDP: 7.37±3.13 vs 6.39±2.95 mmHg). At t₁, perfused hearts showed more weight gain (16.4±4.2 vs 1.8±1.6% increase from baseline, CP vs CS group, p=0.01), but at t₂ neither weight gain (43.7±6.1 vs 31.9±8.5%) nor LVEDP (Fig 2E) was significantly different between groups. At t₁, −dP/dt was significantly greater in CP vs CS (Fig 2D), reflecting improved diastolic relaxation. At t₂, 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 (t₀) ATP, MDA, caspase-3 and ET-1 levels were similar in both groups. At t₁, these markers each showed strong correlations with +dP/dt at t₂ (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 t₀ and t₁, 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 t₂ (Figs 3A–D). CP limited these viability changes at t₁ as evidenced by a trend toward higher ATP preservation (10.3±1.1 vs 7.9±1.2 μg/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 μg/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 (t₂), CP demonstrated a more complete recovery of ATP (12.8±1.5 vs 6.2±0.7 μg/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 μg/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 t₀ and t₁, 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

Statistical Analysis

Results are expressed as the mean±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.
Continuous Ex Vivo Heart Perfusion

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. 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.

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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. We confirm a higher degree of weight gain during Langendorff reperfusion of CS compared with CP. However, the edema advantage of CS found at t1 was transient and eliminated at t2 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, edema at t1 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. 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. In addition, prior studies show that inhibition of lipid peroxidation, caspase-3,21 and ET-122,23 all decrease ischemia–reperfusion injury.

Continuous cold temperature24 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 antagonists improve systolic function in animal models of cardiac transplantation. ET-1 antagonists improve systolic function in animal models of cardiac transplantation. Perhaps more importantly, clinical studies indicate that ET-1 is one of the strongest alloantigen-independent factors for the subsequent development of CAV11 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 function31 a common cause of graft dysfunction after transplantation and only provides data for the first 2–3 h 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. This method is well-established and has a track record of predicting the clinical performance of a wide range of clinical protocols in cardiac surgery, including anti-ischemic interventions31 and methods of cardiac preservation. 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 preclinical 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.

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References


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