Published in final edited form as:

Ann Transplant. ; 19: 409–416. doi:10.12659/AOT.890797.

Preservation of Donor Hearts Using Hypothermic Oxygenated Perfusion

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Abstract

Background—Hypothermic machine perfusion of donor hearts enables continuous aerobic metabolism and washout of toxic metabolic byproducts. We evaluated the effect of machine perfusion on cardiac myocyte integrity in hearts preserved for 4 h in a novel device that provides pulsatile oxygenated hypothermic perfusion (Paragonix Sherpa Perfusion™ Cardiac Transport System).

Material/Methods—Pig hearts were harvested and stored in Celsior® solution for 4 h using either conventional cold storage on ice (4-h CS, n=6) or the Sherpa device (4-h pulsatile perfusion (PP), n=6). After cold preservation, hearts were evaluated using a non-working heart Langendorff system. Controls (n=3) were reperfused immediately after organ harvest. Biopsies were taken from the apex of the left ventricle before storage, after storage, and after reperfusion to measure

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^AStudy Design

^BData Collection

^CStatistical Analysis

^DData Interpretation

^EManuscript Preparation

^FLiterature Search

^GFunds Collection

Conflict of interest

Sebastian G. Michel, Glenn M. La Muraglia II, Maria Lucia L. Madariaga, James S. Titus, Martin K. Selig, Evan A. Farkash and James S. Allan: nothing to disclose. Joren C. Madsen received grant support from Paragonix Technologies, Inc. to perform this study as part of a Phase I SBIR grant (1R43HL115852-01) awarded to Paragonix Technologies, Inc. (Braintree, MA). Lisa M. Anderson is an employee at Paragonix Technologies, Inc.

ATP content and endothelin-1 in the tissue. Ultrastructural analysis using electron microscopy was performed.

Results—Four-hour CS, 4-h PP, and control group did not show any significant differences in systolic or diastolic function (+dP/dt, -dP/dt, EDP). Four-hour PP hearts showed significantly more weight gain than 4-h CS after preservation, which shows that machine perfusion led to myocardial edema. Four-hour CS led to higher endothelin-1 levels after preservation, suggesting more endothelial dysfunction compared to 4-h PP. Electron microscopy revealed endothelial cell rupture and damaged muscle fibers in the 4-h CS group after reperfusion, but the cell structures were preserved in the 4-h PP group.

Conclusions—Hypothermic pulsatile perfusion of donor hearts leads to a better-preserved cell structure compared to the conventional cold storage method. This may lead to less risk of primary graft failure after orthotopic heart transplantation.

MeSH Keywords

Heart Transplantation; Organ Preservation; Pulsatile Flow

Background

According to the International Society for Heart and Lung Transplantation Registry, primary graft failure is the leading cause of early death after heart transplantation [1]. Its multifactorial pathogenesis involves the neurohormonal consequences of brain death, ischemia-reperfusion injury, and recipient factors such as pulmonary hypertension [2]. The registry data show that ischemic preservation times of longer than 200 min increase mortality after heart transplantation [3]. However, ischemic times have become longer, most likely due to an increasing number of assist-device patients who subsequently undergo heart transplantation [4]. Providing oxygen and washing out toxic metabolites via machine perfusion during the preservation time could reduce the ischemic damage to the donor heart and improve post-transplant ventricular function. This would reduce the incidence of primary graft dysfunction and, in the long run, cardiac allograft vasculopathy (CAV) [5]. Longer safe preservation intervals would not only extend the geographic boundaries for organ retrieval – the additional time that a heart can be stored without damage would also enable tissue typing and exact human leukocyte antigen (HLA) matching of donor and recipient. Although currently not routine in clinical practice, HLA-matching has been shown to improve survival in a retrospective study [6]. Multiple studies in animal models using machine perfusion have demonstrated the advantages of ongoing aerobic metabolism during the transport period and its positive effects on cell integrity and cell functions [7–10]. Enhanced preservation of ATP stores and clearance of toxic metabolites like lactate were associated with better ventricular performance after reperfusion [11–14]. In this study, we aimed to compare the conventional static immersion storage method with hypothermic machine perfusion and test their effects on the preservation of myocardial metabolism and cell structures. We used the Paragonix Sherpa Perfusion™ Cardiac Transport System (Paragonix Technologies Inc., Braintree, MA, Figure 1) in a pig model because the pig heart is similar to human hearts in anatomy and physiology. We chose a clinically acceptable cardiac preservation time of 4 h for this study.

Material and Methods

Animals

We used 15 Yorkshire swine (weight 45–50 kg) as heart and blood donors and divided them into 3 groups: n=6 for 4 h of CS, n=6 for 4 h of PP, and n=3 for control (immediate reperfusion). The protocol was approved by the institutional review board and all procedures were in compliance with “The Principles of Laboratory Animal Care” formulated by the National Society for Medical Research and the “Guide for the Care and Use of Laboratory Animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 2011.

Experimental procedure

Surgery—Anesthesia was induced with 4.4 mg/kg Telazol, 2.2 mg/kg xylazine and 0.04 mg/kg atropine intramuscularly and maintained with isoflurane after endotracheal intubation. Median sternotomy was performed and heparin (300 units/kg) was given intravenously. An aortic root cannula was inserted and 1.5 L of blood harvested into 2 transfusion bags. After that, the ascending aorta was cross-clamped and 1 L of Celsior® (Genzyme, Cambridge, MA, USA) cardioplegia was administered through the cannula. The heart was vented through the left atrial appendage, and superior and inferior vena cava. Donor cardiectomy was performed, the heart was weighed, and biopsies were taken from the left ventricular (LV) apex. Biopsies were snap-frozen in liquid nitrogen and stored at –80°C until use for assays or stored in glutaraldehyde and processed for transmission electron microscopy.

Myocardial preservation—Hearts were either statically stored for 4 h in Celsior® on ice (4-h cold storage (CS): n=6) or underwent continuous perfusion in a pulsatile fashion with Celsior® for 4 h (4-h PP: n=6) with the Paragonix Sherpa Perfusion™ Cardiac Transport System (Paragonix Technologies, Braintree, MA, USA, Figure 1). A cannula was inserted in the aortic root and the heart connected to the Sherpa organ carrier, which had been primed with 3.8 L of Celsior® solution without blood. The donor heart was completely immersed in Celsior® solution, which was placed into the Sherpa shell, which provided a second, rigid barrier to protect the donor heart. This assembly was then inserted into the Sherpa shipper, which created a homogenous cooling environment. The Sherpa shipper is capable of maintaining temperature between 4°C and 8°C for 12 h. To operate the device in research mode, the Sherpa Perfusion™ CTS was instrumented with multiple pressure and temperature sensors, which enabled the continuous monitoring of pressures, flows, and temperature during operation. Organ perfusion using the Sherpa Perfusion™ CTS, is achieved by the cyclical pressurization of 2 chambers: a lid and an organ container that are connected via a port through which solution enters the aortic root. Pulsatile perfusion (PP) pressures between 2 and 6 mmHg achieved coronary flows between 35 to 65 mL/min and a pO₂ of 200–350 mmHg in the solution entering the aortic root.

Hearts of the control group (n=3) did not undergo preservation but were reperfused on the Langendorff system immediately after organ harvest to see what effect (particularly edema) reperfusion with this system has by itself on the hearts.

Hemodynamic assessment of the left ventricle with a Langendorff perfusion model

Hearts were weighed before reperfusion and biopsies were again taken from the LV apex. The heart was then connected to a non-working heart Langendorff system in which the coronary arteries are perfused with a roller pump and the heart is beating against the pressure of a latex balloon in the left ventricle without any cardiac output. Priming volume was approximately 2 L and consisted of 1200 mL of autologous donor blood and 800 mL of Krebs-Henseleit buffer. Affinity NT® hollow fiber membrane oxygenators with a heat exchanger (Medtronic Inc., Minneapolis, MN, USA) were used to keep pO₂ between 500 and 600 mmHg, pCO₂ at 25 to 35 mmHg and the temperature at 37°C with a temperature probe in the right ventricle. The applied oxygen/CO₂ mixture was approximately 98%/2%. Electrolytes and pH were corrected to physiological values. The coronary perfusion pressure in the aortic root was set at 80 mmHg. A latex balloon in the LV was filled with 10, 20, 30, 40, 50, and 60 mL of saline, and the systolic (+dP/dt, rate of pressure change) and diastolic (−dP/dt, negative rate of pressure change and EDP [end diastolic pressure]) function of the LV were assessed. Developed LV pressures were measured every 15 min for 1 h using a 3F Millar Micro-Tip catheter (Millar Instruments Inc., Houston, TX, USA) placed inside the balloon. Heart rate was controlled using pacing wires sutured onto the right ventricle (90–100 beats per min). After 1 h of reperfusion, hearts were weighed again to assess the development of myocardial edema, and biopsies were taken from the LV apex.

Assays—Frozen myocardial biopsies were used to determine levels of adenosine triphosphate (ATP, a marker of energy storage) and endothelin-1 (ET-1, a marker of endothelial dysfunction) in the 4-h CS and 4-h PP groups before preservation, after preservation, and after reperfusion using commercially available assay kits. ATP concentration was determined in swine heart lysates by using the Abcam® ATP Colorimetric Assay Kit (cat# ab83355, Abcam®, Cambridge, MA, USA). ET-1 concentration was measured by using the R&D Systems Endothelin-1 Quantikine ELISA Kit (cat#DET100, R&D Systems, Inc., Minneapolis, MN, USA).

Histology—Samples from the LV apex were obtained after organ harvest, after preservation, and after reperfusion. The tissue was formalin-fixed, paraffin-embedded, and stained with hematoxylin and eosin for light microscopy. A pathologist graded edema (1+ dilated lymphatics, 2+ perivascular edema, 3+ parenchymal edema) and myocyte injury (present/absent).

Electron microscopy—Electron microscopy was performed on all hearts as described previously [15]. In brief, biopsy samples from the LV apex were obtained after organ harvest (baseline), after preservation, and after reperfusion, and then were immediately fixed for 2.5 h with 2.5% glutaraldehyde, 2.0% paraformaldehyde, and 0.025% calcium chloride, in a 0.1 M sodium cacodylate buffer at pH 7.4. Tissues were further processed in a Leica Lynx automatic tissue processor. Briefly, tissues were post-fixed with osmium tetroxide, dehydrated in a series of ethanol solutions, en bloc stained during the 70% ethanol dehydration step for 1 h, infiltrated with propylene oxide epoxy mixtures, embedded in pure epoxy, and polymerized overnight at 60°C. One-micron – thick sections were cut using glass

knives and a Sorvall MT-1 (Dupont) ultra microtome, and floated on water droplets on glass slides. The slides were dried in a humidity chamber on a warm hotplate. Toluidine blue stain (0.5% toluidine blue in aqueous 0.5% sodium borate) was pipetted over the sections and placed onto the hotplate until a slight gold rim could be seen around the stain droplet. The sections were rinsed in a stream of distilled water, dried, cover-slipped, and examined by light microscopy.

Representative areas were chosen and thin sections cut using a diamond knife and an LKB 2088 ultra microtome and placed on copper grids. Thin sections were stained with lead citrate and examined with an FEI Morgagni transmission electron microscope. Images were captured with an AMT (Advanced Microscopy Techniques) 2K digital CCD camera.

Statistical analysis

Data are shown as mean $\pm$ standard deviation. Statistical analysis was performed with a 1-way ANOVA test (Prism 5, Graph Pad, Inc., San Diego, CA, USA). Differences were considered to be significant at $p < 0.05$.

Results

Characteristics of pulsatile perfusion

The device was able to provide for relatively high flows (44.2 ± 8.71 mL/min), while keeping the pressure across the organ very low (3.43 ± 1.3 mmHg). An average pO_2 of 310 mmHg was achieved by the end of the preservation interval. Hypothermic oxygenated perfusion did not affect either pH or glutathione levels in the Celsior® solution, as evidenced by the lack of changes in pH and concentration of reduced glutathione, (e.g., pH went from 7.3 ± 0.2 to 7.20 ± 0.06). Four-hour CS led to a slight, statistically not significant, drop in the pH of the Celsior solution from 7.3 ± 0.2 to 7.14 ± 0.03 ($p > 0.05$).

Myocardial edema caused by hypothermic machine perfusion

PP led to significantly more weight gain after 4 h in comparison to CS (mean $10.1\% \pm 1.28$ vs. $1.16\% \pm 1.00$ compared to baseline, $p < 0.001$), which shows that machine perfusion led to myocardial edema. After reperfusion on the Langendorff system, however, both 4-h PP and 4-h CS hearts had undergone a similar weight gain (mean $26.7\% \pm 7.27$ vs. $29.9\% \pm 13.9$) compared to baseline (Figure 2A, 2B).

LV function after reperfusion

The 4-h CS, 4-h PP, and control group did not show any significant differences in systolic or diastolic function ($+dP/dt$, $-dP/dt$, Figure 3A). The control group, which was reperfused immediately after organ recovery, had the lowest EDP values (Figure 3B). This difference, however, was not statistically significant. Of note, 4-h CS hearts showed more arrhythmia (both supraventricular and ventricular extra-systolic beats) compared to 4-h PP and controls.

Endothelin-1 and ATP levels in the heart tissue

CS resulted in increased ET-1 levels (a marker for endothelial dysfunction) compared to PP following the preservation interval of 4 h (Figure 4A). No significant difference was found in ATP levels before and after 4 h of preservation with CS or PP (Figure 4B).

Histology

Four out of 6 hearts in the CS group but only 2 out of 6 hearts in the PP group showed signs of myocyte injury after reperfusion (Figure 5, not statistically significant). PP, however, led to more edema after preservation – 5 out of 6 hearts in the PP group but only 3 out of 6 hearts in the CS group showed edema grade 1+ or 2+ after preservation (Figure 6, not statistically significant).

Electron microscopy findings

Representative images after reperfusion are shown (Figure 7). Electron microscopy revealed endothelial cell rupture (Figure 7, red arrow) and damaged muscle fibers in the 4-h CS group after reperfusion. In contrast, the same cell structures showed no abnormality in the 4-h PP group.

Discussion

According to the most recent report of the International Society for Heart and Lung Transplantation, primary graft failure is responsible for 34% of deaths in the first month after heart transplantation [1]. A significant difference in 1-year mortality is observed when ischemic times extend to periods longer than 4 h [15]. Although machine perfusion during the preservation time has been shown to reduce ischemic damage to the donor heart in various animal models, it is not the current clinical standard [16]. The aim of this study was to establish whether pulsatile perfusion with the single-use Paragonix Sherpa Perfusion™ Cardiac Transport System would provide a safe preservation strategy for the currently acceptable transport time of 4 h compared to the conventional cold storage method.

Compared to CS, where the temperature control is not accurate, the Paragonix Sherpa Perfusion™ CTS can regulate the temperature precisely at around 4–8°C, which is the most desirable for hypothermia. With conventional CS, temperatures often drop to close to 0°C, which can result in microvasculature injury [17].

It has been shown that application of cardioplegic solution in a pulsatile fashion improves subendocardial distribution [18,19], which is particularly beneficial in hypertrophied hearts [20]. The lack of pulsatility in the human circulation has been studied in patients undergoing cardiac surgery on cardiopulmonary bypass [21] as well as in congenital heart disease that requires a Fontan operation (cavopulmonary shunt with bypassing the morphological right ventricle) [22]. Having an unphysiological continuous rather than a pulsatile flow resulted in a worse end-organ microcirculation and in a worse endothelial-dependent vasorelaxation response of the pulmonary arteries.

Myocardial edema is the greatest concern of all *ex vivo* perfusion devices. In this study, the perfusion pressure was kept extremely low (3.43 ± 1.25 mmHg) to avoid edema formation.

These low pressures were still able to generate enough flow of the oxygenated Celsior solution (44.18 ± 8.71 mL/min) for effective perfusion and preservation. The edema observed under these conditions, although significant compared to the conventional cold storage method, did not lead to diastolic impairment of the ventricle (Figure 3A, 3B) and resolved during the reperfusion period. Therefore, we do not expect it to be of clinical relevance following organ transplantation. Nonetheless, the use of a more oncotic perfusion solution such as University of Wisconsin solution would most likely reduce or eliminate edema formation [23]. In this study, we used Celsior® because it has been proven effective and is approved for heart transplantation.

In our non-working heart model, we did not observe differences in hemodynamic parameters between CS and PP hearts. Ventricular contractility (+dP/dt) and diastolic function (−dP/dt, EDP) were similar in both groups (Figure 3A, 3B). In contrast, previous studies showed improved functional recovery of perfused canine hearts using a Langendorff system [17,24]. In these studies, however, prolonged preservation times of 8 and 24 h were tested. Our study focused on the currently clinically relevant preservation interval of 4 h that applies to most human heart transplants. Given the high success rate of human heart transplantation of hearts stored for 4 h, gross functional impairment of the donor heart preserved using CS was not expected and, indeed, no differences between the groups were observed in the non-working heart model.

However, our study revealed biochemical and microscopic injury in 4-h CS hearts, which was not observed in the PP hearts. This was associated with higher levels of ET-1 in the 4-h CS group, which may be due to endothelial dysfunction. Indeed, endothelial injury was also observed by electron microscopy (Figure 7). Pulsatile perfusion, on the other hand, was associated with the preservation of endothelial cell integrity, which could have significant benefits on clinical outcomes both in the short-term (less primary graft failure) and long-term (less CAV). There was no significant ATP depletion after 4 h in either group, which is consistent with the literature [25].

The higher occurrence of arrhythmia in the 4-h CS group compared to 4-h PP group may be due to the susceptibility of the conduction system to ischemia and is consistent with the perfused donor hearts suffering less ischemic damage.

The use of non-heart-beating donors for heart transplantation is still controversial [26–29]. Besides being an additional source of organs, donors after cardiac death have the advantage of avoiding the deleterious effects that brain death has on allografts. Brain death induces a cytokine storm and a pro-inflammatory milieu leading to myocardial tissue injury [30] and increasing antigenicity of the graft, with higher risk of rejection. This has been well described in kidney transplantation comparing living and cadaveric donors [31]. If this extra source of donor organs became available for heart transplantation as it is already for lung transplantation, it is very likely that some kind of *ex vivo* perfusion device would be required to resuscitate the heart and confirm its suitability before transplantation. Normothermic perfusion as the most physiological technique may be better in such a scenario. This technique has been shown to be effective in a pilot study of 12 lung transplant patients and is currently being studied in a prospective randomized trial [32]. Normothermic machine

perfusion can also be used for viability testing of explanted organs [33]. Besides the much lower cost, there is, however, a big advantage of hypothermic over normothermic perfusion techniques during the transport of a donor organ –if anything technical goes wrong, it is much safer to have the organ at 4°C compared to 37°C because at the higher temperature the oxygen demand for metabolism is much higher [34]. The time for potential cardiac repair without damaging the heart is very limited at normothermia. The technical equipment and expertise necessary for the operation of a hypothermic device are also much less demanding, which limits pitfalls in the already complex heart transplant procedure.

Conclusions

Hypothermic pulsatile perfusion of donor hearts during the storage interval is a simple technique that leads to a better-preserved cell structure compared to the conventional cold storage method. This may lead to less risk of primary graft failure after orthotopic heart transplantation and may enable longer safe ischemic times. This, however, needs to be evaluated in a preclinical orthotopic heart transplant model.

Acknowledgments

Source of support: This work was supported by a Phase I SBIR grant (1R43HL115852-01) awarded to Paragonix Technologies Inc.

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Figure 1.
Paragonix Sherpa Perfusion™ Cardiac Transport System.

Figure 2.

Weight gain of hearts caused by edema in% compared to baseline (**A**) and absolute numbers in grams (**B**). Machine perfusion led to more weight gain during the 4-h storage period compared to conventional cold storage. All groups show a similar weight after reperfusion on the Langendorff system.

Figure 3.

(A) +dp/dt and -dp/dt. No significant difference in contractility and diastolic function was seen between groups (dp/dt, rate of pressure change; LVEDV, left ventricular end-diastolic volume). (B) EDP. No significant difference in diastolic function in all groups (EDP, end-diastolic pressure; LVEDV, left ventricular end-diastolic volume).

Figure 4.

(A) Endothelin-1 levels in the donor hearts. Higher levels of ET-1 after preservation in the 4-h CS group were found, reflecting endothelial dysfunction. (B) ATP content in the tissue. No significant depletion of ATP stores after a 4-h storage period in either group was seen.

Figure 5. Histology of hearts after reperfusion on the Langendorff system. Representative H&E stains show signs of myocyte injury in the 4-h CS group (A) and no injury in the 4-h PP group (B).

Figure 6. Histology of hearts after preservation. Representative H&E stains show less edema in the 4-h CS group (**A**) than in the 4-h PP group (**B**).

Figure 7.

Electron microscopy of hearts after reperfusion on the Langendorff system. Representative images of endothelial cells and muscle fibers are shown **left**: 4-h CS with ruptured endothelium (**top**, red arrow), damaged muscle fibers, and edema (**bottom**); **right**: 4-h PP with intact endothelium and preserved muscle fibers).