Is profound hypothermia required for storage of cardiac allografts?

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Background: Improved methods of cardiac allograft protection are required to expand the pool of potentially available organs and to enhance the recovery of grafts subjected to prolonged ischemia. We have previously demonstrated that limited coronary perfusion provided by donor blood harvested at the time of organ procurement can improve both metabolic and functional recovery after transplantation. In this study we evaluated the hypothesis that limited coronary perfusion may enable prolonged cardiac storage while avoiding the potentially detrimental effects of profound hypothermia.

Methods: Fourteen orthotopic cardiac transplants were performed in female Yorkshire pigs by using donor blood perfusion during 5 hours of either tepid (25°C) or cold (4°C) storage. Assessments of myocardial metabolism and function were performed at baseline and after 45 minutes of normothermic (37°C) reperfusion.

Results: Hearts protected with tepid perfusion displayed improved recovery of myocardial function (89% ± 18% vs 63% ± 25%, P = .05). Diastolic compliance was adversely affected in both groups after transplantation. Aerobic myocardial metabolism was better preserved in the tepid group.

Conclusions: Profound hypothermia results in depressed myocardial metabolic and functional recovery after transplantation. Limited coronary perfusion with shed donor blood can permit cardiac allograft storage at tepid temperatures, resulting in improved myocardial performance.
was responsible for almost 50% of the early mortality after isolated heart transplantation. Improved methods of allograft preservation are required to prevent primary graft dysfunction so that marginal donor organs can continue to be used.

We have previously developed a technique to harvest shed donor blood from the chest cavity at the time of organ procurement for subsequent allograft perfusion during storage. In a porcine model of orthotopic cardiac transplantation, we found that a simple perfusion apparatus making use of un oxyg enated donor blood provided superior myocardial protection compared with our current clinical technique of static hypothermic storage. However, we noted persistent inhibition of normal aerobic myocardial metabolism, which led to depressed left ventricular functional recovery after transplantation. In an attempt to reverse the inhibition of aerobic metabolism, we supplemented our blood perfusate with insulin. Insulin-treated hearts displayed greater oxygen extraction than control blood-perfused hearts but only after a normothermic infusion of blood cardioplegic solution. The increase in myocardial oxygen extraction resulted in improved functional recovery.

In previous studies with blood perfusion, the average coronary flow rate during storage was less than 15 mL/min at a myocardial temperature of 4°C. Buckberg and colleagues classic description of myocardial metabolism during cardioplegic arrest demonstrated that oxygen consumption in the normothermic arrested heart was reduced by 80% compared with that in the empty beating state. The addition of mild hypothermia (22°C) reduced oxygen consumption by an additional 15%. On the basis of these observations, we hypothesized that the limited coronary flow provided by our perfusion apparatus may be sufficient to support aerobic myocardial metabolism at room temperature (25°C-27°C). Our previous clinical experience in coronary bypass operations suggested that hypothermic cardioplegic arrest produced a delay in myocardial metabolic and functional recovery that was partially prevented by the use of normothermic blood cardioplegia.

The present study was designed to evaluate myocardial metabolic and functional recovery after orthotopic cardiac transplantation in a porcine model by using donor blood perfusion and storage at profound hypothermia (4°C) versus at mild hypothermia (25°C-27°C).

Methods
The experimental protocol was approved by our institutional animal care committee and was in accordance with 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 1996. Twenty-eight female Yorkshire pigs (50-60 kg) were used to perform 14 orthotopic cardiac transplants with either profound hypothermia (cold, 4°C; n = 7) or mild hypothermia ( tepid, 25°C; n = 7). The size mismatch between donor and recipient was less than 5% of body weight in all experiments.

Donor Operation
The animals were anesthetized with intramuscular ketamine (30 mg/kg) and isoflurane and intubated and ventilated with 100% oxygen to maintain normocarbia. After sternotomy, the heart and great vessels were exposed. Systemic anticoagulation was achieved with the intravenous injection of 10,000 units of heparin.

A purse-string suture was placed in the ascending aorta to permit placement of a cardioplegia cannula. Arterial and coronary sinus blood samples were obtained just before aortic crossclamping, and then 1 L of a hyperkalemic crystalloid solution (Na+, 127 mmol/L; K+, 20 mmol/L; Mg2+, 6 mmol/L; Cl−, 7 mmol/L; SO4 2−, 6 mmol/L tris-hydroxymethyl aminomethane, 4 mmol/L and dextrose, 135 mmol/L) was infused into the aortic root to achieve cardioplegic arrest. In the cold group cardioplegic solution was infused at 4°C, and then the donor heart was extracted, placed in a bag containing 300 mL of hypothermic cardioplegic solution, and stored on ice. In the tepid group cardioplegic solution was infused at room temperature (25°C), and after extraction, the donor heart was stored in a plastic bag containing 300 mL of room temperature cardioplegic solution. The cardioplegia cannula and aortic crossclamp were left in place in all hearts to permit donor blood perfusion during storage.

In both groups donor blood was harvested from the chest after organ extraction. After filtration for particulate matter and the addition of 10,000 units of heparin, the blood was stored in standard transfusion bags (Travenol; Baxter Healthcare Corp, Deerfield, Ill). Blood perfusion was initiated within 10 minutes of cardioplegic arrest and was delivered at room temperature (20°C) at a vertical height of 100 cm (to correspond to a perfusion pressure of 60 mm Hg) by using a standard intravenous transfusion apparatus (Fenwal, Baxter Healthcare Corp). Blood perfusion was maintained in all hearts throughout the 5-hour storage period before commencing the recipient operation. A myocardial probe was inserted into the apex of the left ventricle in all hearts to monitor temperature during storage. In the cold group myocardial temperatures were maintained between 4°C and 7°C with topical iced saline solution, and in the tepid group the apical temperature was allowed to equilibrate with room temperature. Coronary flow rate was determined as the total volume of perfusate delivered corrected for storage time in minutes.

Recipient Operation
Preoperative sedation and anesthesia were performed as in the donor protocol. In addition, a marginal ear vein was used for intravenous access and kept open with a 50 mL/h 5% dextrose infusion. Continuous electrocardiographic monitoring was used, and a carotid artery line was inserted to measure arterial pressure.

After sternotomy, the heart and great vessels were exposed. Umbilical tapes were placed around the superior and inferior vena cavae. Systemic anticoagulation was achieved by injecting heparin into the pump prime (10,000 U) in addition to an intravenous dose of 10,000 U. Ascending aortic and bicaval cannulation were used to place the recipient on cardiopulmonary bypass. Flow rates were adjusted to maintain systemic perfusion pressures above 50 mm Hg. No vasoactive medications were administered during cardiopulmonary bypass. Systemic perfusion was maintained at 37°C in both groups.

After the aorta was crossclamped, the recipient heart was extracted, maintaining a cuff of right and left atrium. The left hemiazygos vein was ligated at its insertion into the coronary sinus. The anastomotic margins were then inspected and trimmed in preparation for orthotopic transplantation by a standard atrial-to-atrial technique.
In all groups donor blood perfusion of the allograft was stopped, and an initial 350-mL blood cardioplegic dose was infused at a flow rate of 100 mL/min. Cardioplegic protection consisted of a 2:1 mixture of blood/crystalloid and was delivered at 4°C or 27°C, depending on the randomization group. Cardioplegic solution was infused after the completion of each atrial anastomosis and the pulmonary arterial anastomosis. Arterial and coronary sinus blood samples were obtained at each cardioplegic infusion, at the time of crossclamp removal, and every 15 minutes during reperfusion. If ventricular fibrillation occurred during reperfusion, 3 attempts were made to defibrillate the heart. If unsuccessful, 100 mg of lidocaine was delivered intravenously, and defibrillation was attempted once again.

At the completion of 45 minutes of reperfusion on cardiopulmonary bypass, hemodynamic measurements were obtained. After completion of all measurements, the recipient was weaned off bypass if possible. In all hearts calcium chloride (1 g) was given, and if additional inotropic support was required, an isoproterenol (INN: isoprenaline) drip was established (0.4 mg/min). If a mean arterial pressure greater than 60 mm Hg could not be maintained for 30 minutes despite inotropic support, weaning was deemed to be unsuccessful. Ventricular pacing was used if necessary to maintain the heart rate at greater than 80 beats/min. Thirty minutes after discontinuation of cardiopulmonary bypass, decannulation was performed, and the animal was killed by means of intravenous potassium chloride injection.

**Assessment of Left Ventricular Function**

A fluid-filled latex balloon connected to a Millar micromanometer catheter (Millar Instruments, Inc, Houston, Tex) was inserted through the apex of the left ventricle to permit on-line measurements of heart rate and left ventricular systolic and diastolic pressures. Measurements of left ventricular end-systolic pressure, left ventricular end-diastolic pressure (LVEDP), and developed pressure (DP) were performed at 5-mL increments as the balloon volume was increased from 0 to 50 mL.

In addition to the assessment of the pressure-volume relationships, the average recovery of DP was calculated as the ratio of the postreperfusion DP to the baseline DP at the same balloon volume.

The average percentage recovery of developed pressure (%DP) was determined by means of the trapezoidal rule, as described previously by Jayawant and colleagues:

\[
\%DP = \left(100\right)\int_{V_a}^{V_b} \frac{DP\text{(Postreperfusion)}}{DP\text{(baseline)}} \cdot \frac{dV}{(V_b - V_a)}
\]

where \(V_a\) is the smallest matching postreperfusion balloon volume and \(V_b\) is the largest matching postreperfusion balloon volume. This integration of the pressure-volume curve is a surrogate measurement of myocardial work and was performed both before and after transplantation.

Similarly, the change in diastolic compliance after transplantation was calculated by determining the ratio of the integrated areas of the postreperfusion and baseline LVEDP-balloon volume relationship:

\[
\text{Mean LVEDP ratio} = \left[\int_{V_a}^{V_b} \frac{P_R}{P_B} \cdot \frac{dV}{(V_b - V_a)}\right]
\]

where \(P_B\) is the LVEDP at baseline and \(P_R\) is the LVEDP after reperfusion. The mean LVEDP ratio reflects the average change in LVEDP for a given change in balloon volume corrected for the pre-transplant compliance. Therefore, a ratio of 1 indicates that the change in LVEDP for a given change in balloon volume was identical before and after transplantation with no change in diastolic compliance. A ratio greater than 1 is indicative of decreased diastolic compliance.

**Biochemical Measurements**

Arterial and coronary sinus blood samples were assayed for the \(P_{O_2}\), \(P_{CO_2}\), \(pH\), hemoglobin concentration (Hb), and oxygen saturation (Sao\(_2\)). Oxygen content (\(O_2\text{Con}\)) was calculated from the following formula:

\[
O_2\text{Con} = 1.39 \cdot \text{Hb} \cdot \text{Sao}_2 + 0.003 \cdot P_{O_2}
\]

Blood samples for lactate determination were mixed with a measured volume of 6% perchloric acid. Lactate concentration was measured in the protein-free supernatant with a commercially available assay (Rapid Lactate Stat Pack; Calbiochem-Behring, La Jolla, Calif).
Statistical Analysis

Statistical analysis was performed with the SAS statistical software program (SAS Institute, Inc, Cary, NC). Categorical data were analyzed by the 2-tailed Fisher exact test. The left ventricular DP–balloon volume relation was analyzed with 2-way analysis of variance (ANOVA), evaluating the main effects of group and balloon volume. Similarly, 2-way ANOVA was used to evaluate the LVEDP–balloon volume relation. The average recovery of DP and the mean LVEDP ratio were analyzed with paired *t* tests.

Continuous metabolic data are expressed as the mean ± standard deviation and were analyzed with 2-way ANOVA, evaluating the main effects of group and time, as well as the interactive effect between group and time (group · time). When appropriate, a post hoc Duncan multiple-range test was performed to specify differences between groups.

Results

All hearts in both groups were successfully weaned from cardiopulmonary bypass. Inotropic support was required to wean from cardiopulmonary bypass more frequently in the cold group (4/7 vs 2/7, *P* = .59). Similarly, more hearts in the cold group required lidocaine therapy than in the tepid group (7/7 vs 3/7, *P* = .02). There was no difference between groups in heart rate, either before or after transplantation.

Recovery of Left Ventricular Function

Figure 1 shows the DP–balloon volume relationship before and after transplantation in both groups. There was no significant interactive effect between group and balloon volume either before (group · volume: F = 1.57, *P* = .213) or after (F = 0.04, *P* = .847) transplantation. The integrated DP–volume relation was not different between groups before transplantation (cold: 4110 ± 952 mm Hg/mL vs tepid: 4090 ± 538 mm Hg/mL, *P* = .96); however, this value trended higher in the tepid group after transplantation (3805 ± 906 mm Hg/mL vs 2791 ± 999 mm Hg/mL, *P* = .07) Figure 2 shows the average percentage recovery of DP in both groups after transplantation. When corrected for pretransplantation values, hearts in the tepid group displayed significantly better recovery of myocardial function than hearts in the cold group (89% ± 18% vs 63% ± 25%, *P* = .046).

Figure 3 illustrates the LVEDP–balloon volume relationship before and after transplantation in both groups. There was no significant interactive effect between group and balloon volume before transplantation (group · volume: F = 1.6, *P* = .208); however, there was a significant interactive effect after transplantation and reperfusion (F = 9.29, *P* = .003). The diastolic pressure-volume relationship was significantly worse in the cold group. Figure 4 displays the mean LVEDP ratio in both groups. Hearts in both groups displayed diastolic dysfunction after transplantation (ratio >1), but there were no significant differences between groups (*P* = .985).

Recovery of Myocardial Metabolism

Figure 5 demonstrates myocardial lactate release–extraction during the experimental protocol. There was no interactive effect between group and time (group · time effect: F = 1.04, *P* = .414); however, lactate release increased significantly after storage and then returned to baseline values after 45 minutes of reperfusion (time effect: F = 2.56, *P* = .013). There were no differences in lactate release between groups at any time point (group effect: F = 0.05, *P* = .830).

Figure 6 demonstrates myocardial acid release (pH difference between arterial and coronary sinus blood) during the experimental protocol. There was no significant interactive effect between group and time (group · time effect: F = 1.40, *P* = .204); however, acid release increased in both groups after the initial cardioplegic infusion, returning to baseline levels after reperfusion (time effect: F = 7.57, *P* <
There was an overall trend toward greater acid release in the cold group (group effect: F = 3.37, P = .069). Figure 7 demonstrates myocardial oxygen extraction during the experimental protocol. There was no significant interactive effect between group and time (group · time effect: F = 0.78, P = .618); however, myocardial oxygen extraction was severely inhibited in both groups during cardioplegic arrest (time effect: F = 15.59, P < .001). Hearts that received tepid perfusion demonstrated improved myocardial oxygen extraction compared with hearts that were perfused at cold temperatures (group effect: F = 16.70, P < .001).

Discussion
Primary graft dysfunction remains the predominant cause of perioperative death after cardiac transplantation. Improved methods of myocardial preservation during allograft procurement and storage are therefore required to reduce the risk of early death. In addition, recent evidence suggests that perioperative ischemic-reperfusion injury may influence the late development of graft vascular disease. Graft vascular disease represents the leading cause of late death after heart transplantation. Thus, novel techniques of organ preservation may lead to improvement in both short- and long-term survival after transplantation.

Several investigators have reported the beneficial effects of allograft perfusion during storage. Unfortunately, the perfusion systems described by these authors have not become widely adopted because of either cost, lack of portability, or complexity. We have previously described a simple technique of organ perfusion that makes use of donor blood harvested from the mediastinum at the time of organ procurement. Although perfusion with donor blood provided superior myocardial protection compared with our clinical technique of static hypothermic storage, we noted persistent inhibition of normal aerobic myocardial metabolism, which led to depressed left ventricular functional recovery after transplantation. In previous cell-culture studies we demonstrated that insulin was capable of stimulating aerobic metabolism in human ventricular cardiomyocytes subjected to ischemia and reperfusion. Therefore, in an attempt to reverse the metabolic inhibition observed in our porcine studies, we supplemented our blood perfusate with insulin and found that transplanted hearts displayed greater oxygen extraction than control blood-perfused hearts. However, this metabolic difference occurred only after a normothermic infusion of blood cardioplegic solution near the end of organ implantation. The increase in myocardial oxygen extraction resulted in a further improvement of functional recovery.

We hypothesized that maintaining higher temperatures during organ storage and implantation may permit higher myocardial oxygen extraction and lead to improved functional recovery after reperfusion. Because our primary focus was to develop a simplified, clinically useful perfusion apparatus, we chose to compare hearts stored at profoundly hypothermic temperatures (4°C, which is similar to our current clinical practice) with hearts that were stored at temperatures of 25°C to 27°C. We were able to achieve this level by simply allowing the apical temperature to equili-
brate with room temperature. This avoided complicating our apparatus with cumbersome heater-cooler machinery. In previous studies we found that cooling the donor blood perfusate to 4°C resulted in rouleau formation and an unacceptable viscosity.3,4 Therefore, in both groups the blood perfusate was delivered at room temperatures. However, measurements of the left ventricle, right ventricle, and interventricular septum confirmed profoundly hypothermic (4°C) temperatures in the cold group compared with temperatures of 27°C in the tepid group.

Previous studies examining the optimal storage temperatures have yielded conflicting results.16-19 Hendry and colleagues17 demonstrated, in an ultrastructural canine study, that current techniques of hypothermic storage result in unacceptably low myocardial temperatures that cause reversible myocardial injury. In a subsequent functional study Hendry and colleagues17 showed that myocardial preservation at 4°C versus 12°C resulted in similar preservation of intracellular energy stores and functional recovery. A study by Suehiro and colleagues18 demonstrated that warm blood perfusion (25°C) in a canine model of 60-minute global ischemia provided superior protection to hypothermic (4°C) preservation in University of Wisconsin solution. In contrast, studies by Masters and colleagues19 and Ueno and colleagues12 both demonstrated that storage temperatures of 4°C resulted in the optimal recovery of myocardial function. However, the study by Masters and colleagues19 did not include myocardial perfusion during storage, which may have adversely affected the group subjected to 15°C storage. In the study by Ueno and colleagues,12 which used continuous perfusion for 24 hours in a rabbit model, the authors compared storage at 0°C, 4°C, and 10°C. They found that the intermediate temperature provided optimal protection but did not evaluate continuous perfusion at higher temperatures similar to that used in our present protocol. Furthermore, anatomic differences between rabbit, canine, and human myocardium make it difficult to extrapolate these studies to the clinical realm. In contrast, the porcine heart has remarkably similar coronary anatomy to that of human subjects and as such represents a more clinically relevant model.

In this study we found that tepid perfusion permitted an earlier recovery of aerobic metabolism compared with standard hypothermic temperatures. Unfortunately, we were unable to demonstrate a difference in either lactate release or acid release between groups, although there was a trend toward less acidosis in the tepid group (P = .07). At crossclamp release, hearts in the cold group displayed twice as much lactate release as those in the tepid group (1.6 ± 0.3 vs 0.8 ± 0.4 mmol/L, P = .04). Although this difference did not persist during reperfusion, we have previously shown that early lactate release within the first 5 minutes of reperfusion can predict the development of posts ischemic ventricular dysfunction.20 Therefore, strategies aimed at stimulating aerobic metabolism during this critical phase of reperfusion may have significant effects on myocardial function. The major metabolic difference between groups remained the increased oxygen extraction at all time points in the tepid group. The improved aerobic capacity in the tepid group resulted in significantly greater functional recovery (Figure 2).

In summary, continuous coronary perfusion during allograft storage enabled successful transplantation while avoiding the detrimental effects of profound hypothermia. Refinements to both perfusate composition and delivery may further enhance myocardial tolerance to prolonged ischemia and hopefully improve both the short- and long-term outcomes of clinical heart transplantation.

References
5. Buckberg GD, Braizer JR, Nelson RL, Goldstein SM, McConnell DH, Cooper N. Studies of the effects of hypothermia on regional myocar-