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Benefits of Perfusion Preservation in Canine Hearts Stored for Short Intervals

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Background. **Continuous perfusion of donor hearts for transplantation has been proposed to improve graft function or extend preservation intervals, but the effects on cellular metabolism, myocyte loss, and myocardial edema are not well-defined.**

Methods. **Hearts from mongrel dogs were instrumented with sonomicrometry crystals and left ventricular (LV) catheters. LV function was quantified by the preload-recruitable stroke work (PRSW) relationship. Hearts were arrested with a modified Celsior solution, and stored in cold solution ($n = 6$) or placed in a device providing continuous perfusion of this solution at 10 mL/100 *g*/min ($n = 6$). After 4 h of storage, left atrial samples were frozen, extracted, and analyzed by magnetic resonance spectroscopy (MRS). Hearts were then transplanted into recipient dogs and reperfused for 6 h with function measured hourly. At end-experiment, LV specimens were assayed for water content and apoptosis. Serum CK-MB levels were measured.**

Results. **LV functional recovery was excellent in both groups over 6 h of reperfusion. MRS revealed a dramatic decrease in tissue lactate in hearts protected with continuous perfusion ($P < 0.01$). Apoptotic cell counts were significantly lower in post-reperfusion heart tissue in animals undergoing a continuous perfusion strategy ($P < 0.01$). CK-MB levels and LV water content were similar in both groups.**

Conclusions. **Although both methods of preservation lead to good early graft function after 4 h of protected ischemia, continuous preservation dramatically reduces tissue lactate accumulation without increasing myocardial edema and may reduce tissue damage during storage and reperfusion. It appears promising as a method to improve results of cardiac transplantation.**

Key Words: **cardiovascular surgery; heart transplantation; organ preservation; metabolism; spectroscopy.**

INTRODUCTION

Cardiac transplantation has emerged as a life-saving therapy for patients with end-stage cardiac failure. In clinical practice, donor hearts are protected by infusing an arresting preservation solution and storing the organ under hypothermic conditions prior to implantation. Using this strategy, good results have been described with preservation intervals of up to 6 h. However, closer inspection of registry data suggests that prolonging graft ischemic time beyond 3 to 4 h leads to a significant increase in the relative risk of recipient mortality at 1 year [1]. Thus, many investigators have sought alternative techniques for heart preservation to offer longer preservation intervals or to improve early graft function during reperfusion.

A variety of devices have been devised to provide continuous perfusion to donor organs to achieve these goals. In experimental and clinical studies of renal transplantation, beneficial outcomes have been observed with machine perfusion [2]. Cardiac preservation with devices providing continuous perfusion has been limited to animal studies and, in general, functional recovery following preservation with this technique has been superior to that observed following standard cold storage [3–10].

Continuous preservation strategies have an attractive theoretical basis. With a machine providing a continuous supply of oxygen and substrate, the myocardium may continue to undergo oxidative metabolism

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for better preservation of high-energy phosphates, maintenance of transmembrane ionic gradients, and support of cellular repair in ischemic myocardium. As well, continuous perfusion of a solution across the coronary vasculature may lead to an ongoing washout of metabolites such as lactate or adenosine. In theory, this process could avert the development of intracellular acidosis or attenuate reperfusion injury mediated via the xanthine oxidase pathway. The relative importance of these potential effects is unknown despite encouraging functional results in experimental animal studies.

Perfusion preservation strategies may also offer another benefit. As the preservation solution provided by the device represents the first "reperfusion" seen by the myocardium, there is an opportunity to alter reperfusion conditions, potentially allowing "resuscitation" of injured or "marginal" donor organs from patients with pre-explant ischemia or even from non-heart-beating donors. If successful, these techniques could expand the donor pool. Additionally, this technique may enable surgeons to safely extend the donor ischemic time to allow for improved donor-recipient matching or transplantation on a more elective basis. While some experimental studies suggest that these benefits are possible, initial clinical trials of perfusion preservation are likely to involve conventional storage intervals until the safety and efficacy of these techniques are better established. Our current understanding of metabolic events that occur within the myocardium during perfusion preservation are rudimentary, and a more complete understanding of graft metabolism during storage will be required if this technique is to gain clinical acceptance.

This study was designed to test a continuous perfusion strategy for cardiac preservation in a large animal model over a clinically relevant storage interval. It was conducted using techniques that allow the precise quantification of functional recovery and direct tracking of substrate metabolism during the storage interval. Metabolic studies were designed to assess both the oxidation of exogenously administered substrate (aerobic metabolism) and the accumulation of the byproducts of glycolysis (anaerobic metabolism). Additionally, quantification of myocyte death following ischemia and reperfusion was also undertaken to advance our understanding of the potential benefits of this technology.

MATERIALS AND METHODS

Experimental Protocol

The protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. All animals were treated in accordance with guidelines set forth in the *Guide for the Care and use of Laboratory Animals* (National Institutes of Health Publication No. 86-23, revised 1996).

Twenty-four adult mongrel dogs were used in this experiment. Twelve donor-recipient pairs were randomized to either conventional hypothermic static preservation ($n = 6$) or hypothermic preservation with a perfusion device ($n = 6$) that provided a continuous perfusion of oxygenated fluid through a calibrated pump system that enabled the control of flow rate, oxygenation, and fluid temperature. The flow rate was based on the recommendations of the manufacturer of the device (LifeCradle; Organ Transport Systems Inc., Frisco, TX). Excised donor hearts were stored for 4 h, reimplanted into recipient animals, and reperfused for 6 h. Commercially available Celsior organ preservation solution (SangStat Medical Corp., Fremont, CA) was used for storage of all hearts. Celsior was supplemented with 1 g/L (5.5 mmol/L) of U-^{13}C-labeled glucose to provide a substrate for evaluation of cellular metabolism during the storage period.

Anesthetic Protocol

Each animal was premedicated with 0.07 mg/kg atropine IM and 4.4 mg/kg telazol IM. The animal was intubated and ventilated with 100% oxygen at V_T of 10 mL/kg, rate of 10/min, and PEEP of 5 cm H_2O. Anesthesia was maintained with 1% to 4% isofluorane. Central venous pressure, arterial pressure, and a surface electrocardiogram were continuously monitored. Ventilator settings were adjusted based on arterial blood gas measurements to keep the pCO_2 at 35 to 45 mmHg, pH 7.35 to 7.45, and oxygen saturation >95%.

Donor Protocol

After sternotomy and exposure of the heart, animals were administered 300 units/kg of heparin intravenously and an ascending aortic cardioplegia catheter was inserted. Baseline myocardial function was measured (see below). The aorta was clamped and the heart was arrested with 1 L of cold modified Celsior solution (Table 1). The inferior vena cava and right superior pulmonary vein were incised to decompress the right and left ventricles, and the donor cardiectomy was completed.

Animals randomized to the static preservation group were stored in a container filled with 1 L of modified Celsior and placed in an ice chest. Animals randomized to perfusion preservation were attached to the perfusion device via a connector in the ascending aorta providing continuous antegrade flow of oxygenated, modified Celsior solution at a flow rate of 10 mL/100 g myocardium/min at 5 ± 2°C. In the perfusion preservation group, a small caliber polyethylene catheter was placed in the coronary sinus for serial measurements of oxygen tension during preservation; pH, oxygen tensions, and lactate levels in the preservation solution were measured with a commercial analyzer (Radiometer Copenhagen EML 105; Bronshoj, Denmark).

Recipient Protocol

After induction of anesthesia, the recipient animal was placed on cardiopulmonary bypass and the heart was excised to coincide with

TABLE 1

Characteristics of Celsior Solution

pH	7.3
Osmolarity	320–360 mOsm/L
Potassium	15 mmol/L
Sodium	100 mmol/L
Magnesium	13 mmol/L
Lactobionate	80 mmol/L
Mannitol	60 mmol/L
Histidine	30 mmol/L
Glutamate	20 mmol/L
Glutathione (reduced)	3 mmol/L

the end of the donor heart storage interval. The heart was then implanted into the recipient animal using a standard bicaval orthotopic transplant technique. Animals received 1 g of methylprednisolone prior to unclamping of the aorta. After the onset of reperfusion, hearts were defibrillated with 5-20 J, if necessary. An intravenous infusion of dobutamine was started at 5 μg/kg/min. The animals were weaned from cardiopulmonary bypass after 1 h of reperfusion. The left ventricular catheter was reinserted, and the previously placed sonomicrometry crystals were connected to the preamplifier. Pressure-volume data were collected in triplicate over a range of filling conditions produced by draining blood into the cardiotomy reservoir (emptying curves) at 2, 3, 4, 5, and 6 h postreperfusion. After 6 h reperfusion, arterial blood was collected for determination of creatine kinase MB isoenzyme (CK-MB) levels and the animal was euthanized. Left ventricular samples were collected for water content analysis and measurement of apoptosis.

Measurement of Ventricular Performance

LV function was quantified by a load-independent method that has been previously described in a large animal transplant model [11]. Four sonomicrometry crystals (Sonometrics Corp., London, Ontario, Canada) were attached to the subendocardium in the minor axis and major axis of the left ventricle. These remained in situ during storage and reperfusion. A micromanometer-tipper catheter (Millar Instruments, Houston, TX) was inserted into the ventricular cavity through the apex. The catheter was removed prior to harvest and reinserted after reimplantation. Pressure and dimension data were collected at a rate of 250 Hz and digitized. Data were acquired and stored on a computer and later analyzed using commercially available software (SonoLab and CardioSOFT; Sonometrics Corp.). Pressure-volume loops were derived over the cardiac cycle prior to explantation (baseline) and after 2, 3, 4, 5, and 6 h of reperfusion. At each time point, a series of pressure-volume loops was created over a range of filling conditions. Stroke volume was calculated from the integral (area) of each pressure-volume loop and plotted against the end-diastolic volume of each loop. The slope of this regression is termed preload-recruitable stroke work (PRSW), and is considered a load-independent index of contractility [12].

Assessment of Substrate Metabolism

Samples of left atrial appendage tissue were harvested at the end of preservation, immediately freeze-clamped, and cooled in liquid nitrogen. The tissue was stored in an −80°C freezer and subsequently extracted with perchloric acid. Purified extracts were reconstituted in D_2O and pH was adjusted to 7.0 to 7.4 for MRS. MR Spectra were then acquired with a 14.1 tesla Varian spectrometer operating at 600 MHz over a spectral width of 8000 Hz. ^{1}H MR spectra with and without ^{13}C decoupling were obtained for evaluation of metabolism between the preservation strategies. Metabolism of exogenous glucose, either by glycolysis or oxidative metabolism, was determined by inspection of spectra for incorporation of ^{13}C into metabolic intermediates [13]. Lactate to alanine ratios were compared from ^{1}H spectra as measures of cellular metabolism during storage.

Evaluation of Myocyte Apoptosis

Samples of left ventricular tissue were preserved in 4% paraformaldehyde for subsequent histology. Terminal deoxynucleotidyl transferase-mediated dUTP neck-end labeling (TUNEL) assays using the Promega Dead End Fluorometric TUNEL System (Madison, WI) were performed on tissue sections from each heart to determine apoptotic cell death. Thirty-six random high-power fields were evaluated in each group. Apoptosis was reported as the number of TUNEL positive nuclei per high-power field. Only cells with nuclear characteristics consistent with apoptosis were counted.

Measurement of Myocardial Water Content

Samples of left ventricular tissue were collected, blotted free of blood, weighed, and placed in an oven for desiccation. The tissue was weighed daily until a constant weight was reached during consecutive measurements. The myocardial water content was then calculated as ([wet weight-dry weight]/wet weight).

Statistical Analysis

Results are reported as mean and standard error of the mean (SEM). Groups were compared by a two-sided t-test using commercially available statistical software (SigmaStat, Chicago, IL). When outcome variables were measured at multiple time points over the reperfusion interval, a repeated-measures analysis variance was applied. Differences between groups were determined with the Student-Newman-Keuls post hoc test and a P-value less than 0.05 was considered significant.

RESULTS

Cardiac Function

Left ventricular function was similar at baseline in both groups of animals. Function rose slightly in both groups during the initial 3 h postreperfusion; however, by 4 h post-reperfusion, the function returned closer to baseline (Fig. 1). At no time during the 6 h postreperfusion period did LV function differ significantly from baseline in either storage group.

Cardiac Metabolism

Hearts in the perfusion preservation group extracted dissolved oxygen continuously throughout the 4 h storage interval (Table 2). Analysis of left atrial tissue samples by ^{1}H MRS revealed less than 1% glutamate enrichment in both groups of animals. As the natural abundance of ^{13}C is 1.1%, this is below the sensitivity of MRS for detection of glutamate enrichment. Thus, no oxidation of exogenous labeled glucose from the preservation solution during the storage interval could be detected.

Very little intracellular lactate accumulated during organ preservation in the perfused hearts. This was readily apparent from simple inspection of typical spectra of each group and comparison of the lactate peaks to those from intracellular alanine (Fig. 2). Samples collected at the end of the preservation interval from static stored hearts had a greater than 5-fold higher lactate to alanine ratio than those stored with perfusion preservation (0.77 ± 0.23 *versus* 3.97 ± 0.54; $P < 0.01$). Virtually all lactate measured in both groups was unlabeled.

Myocardial Necrosis, Apoptosis, and Edema

Although the overall number of apoptotic cells was low in both groups, significant differences in apoptotic myocytes were noted between groups. The total number of apoptotic nuclei in the perfusion preservation group (0.11 ± 0.07) was one-tenth that of the static

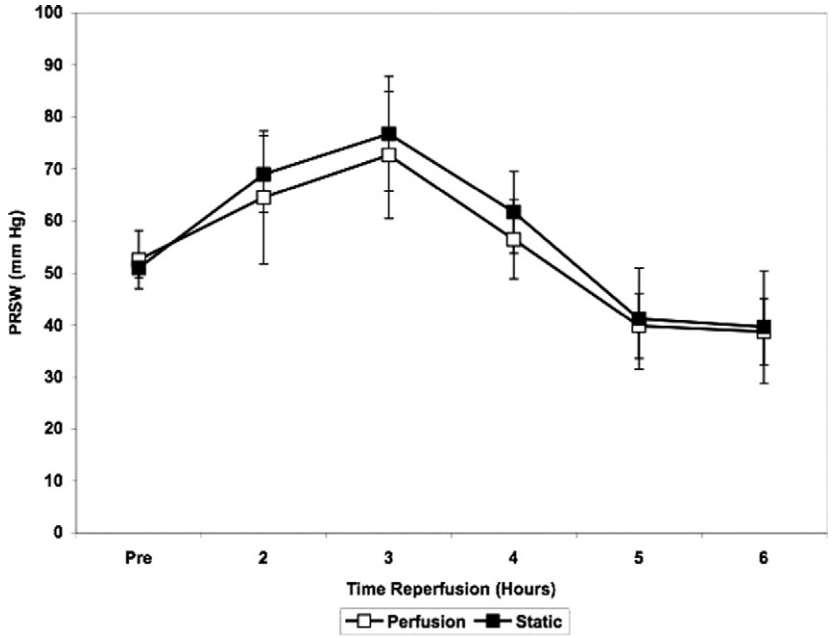

FIG. 1. Left ventricular function post-transplant. Function is quantified according to the preload-recruitable stroke work (PRSW) relationship.

preservation group (1.1 ± 0.3) ($P < 0.01$, see Fig. 3). Myocardial necrosis was estimated from blood levels of CK-MB drawn at 6 h postreperfusion. CK-MB levels were higher in the static preservation hearts compared with animals that received perfusion preserved organs (6.9 ± 2.4 ng/mL *versus* 4.0 ± 1.1 ng/mL). However, this difference was not statistically significant ($P = 0.17$). Myocardial edema (as measured by left ventricular water content) was not significantly different between groups after 6 h postreperfusion (78.3% ± 0.6% *versus* 78.3% ± 0.2%; $P = 1.0$).

DISCUSSION

While perfusion preservation has seen clinical application in transplantation of kidneys, experience with this technique in donor hearts has been limited to animal research. Despite the theoretical benefits of continuous perfusion, surgeons may harbor concerns about the additional expense and complexity of perfusion systems, and may be satisfied with the levels of success achieved with current (static) preservation techniques. However this complacency may be unwarranted, as ISHLT registry data describe 1-y mortality rates in heart transplant recipients in whom donor ischemic times reach 6 h to be double the rates observed when ischemic times are 3 h or less [1]. Primary graft failure remains a major problem and is responsible for 40% of deaths that occur during the first month following cardiac transplantation [1]. Therefore, the results of clinical transplantation could be improved with techniques that offer superior preservation of the donor heart.

If perfusion preservation techniques are to enter the clinical realm, initial use of these devices is likely to remain within conventional storage intervals. As a result, we selected a 4 h preservation interval for this study. The total donor ischemic time in this experiment includes the 4 h of protected storage and an additional interval of donor ischemia during the implantation operation. This latter portion may provide an important ischemic stress to the donor graft. Studies were

TABLE 2

Myocardial Temperature, Oxygen Extraction, and Preservation Fluid Lactate Concentration During Perfusion Preservation

Storage time	Temp (°C)	pO2 Inflow (mmHg)	pO2 Coronary tinus (mmHg)	Δ pO2 (mmHg)	Lactate (mmol/L)
1 hour	5.8 ± 0.7	1127 ± 8	561 ± 98	566 ± 99	0.12 ± 0.05
2 hours	5.9 ± 0.4	1159 ± 5	485 ± 135	674 ± 138	0.15 ± 0.06
3 hours	5.9 ± 0.4	1154 ± 11	543 ± 134	611 ± 140	0.22 ± 0.07
4 hours	6.0 ± 0.4	1121 ± 33	522 ± 144	599 ± 161	0.47 ± 0.26

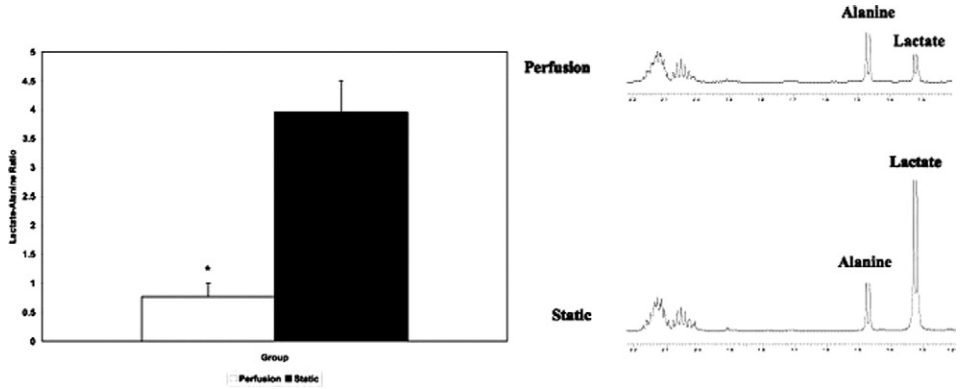

FIG. 2. Representative MR spectra from extracts of left atrial tissue at end-ischemia are shown in the right panel. The ratio of the resonance areas of lactate to alanine in hearts stored with perfusion preservation is much lower than in those stored with traditional static preservation (left panel, $*P < 0.01$).

conducted using an extracellular preservation solution (Celsior) in both groups. U-^{13}C glucose was added to enable quantification of cellular metabolism by MRS. This particular solution was selected based on prior isolated rat heart studies, which identified an increase in metabolism of exogenous glucose and an increase in oxygen consumption when extracellular preservation solutions were selected [7]. The addition of ^{13}C-enriched glucose enables quantification of incorporation of label into citric acid cycle intermediates using techniques that have been previously described [13].

As donor animals were stored for relatively short ischemic interval and all had normal hemodynamics prior to explant, it is perhaps not surprising that functional recovery was good in both groups in this study. Other investigators have found improved functional results with perfusion preservation techniques, but in general, they have selected longer storage intervals where the degree of ischemic stress is increased. It is interesting that ventricular performance as measured by PRSW actually rose to levels above baseline by 3 h postreperfusion. The reason for this is not well defined but may relate to the infusion of dobutamine given to both groups during early reperfusion.

A major focus of this study was to assess the metabolic events that occurred during the preservation interval. Hearts stored in the preservation device were found to extract significant amounts of dissolved oxygen throughout the 4 h storage period. This suggests that oxidative metabolism is maintained during perfusion preservation. Surprisingly, MRS done on left atrial samples at the end of the preservation interval

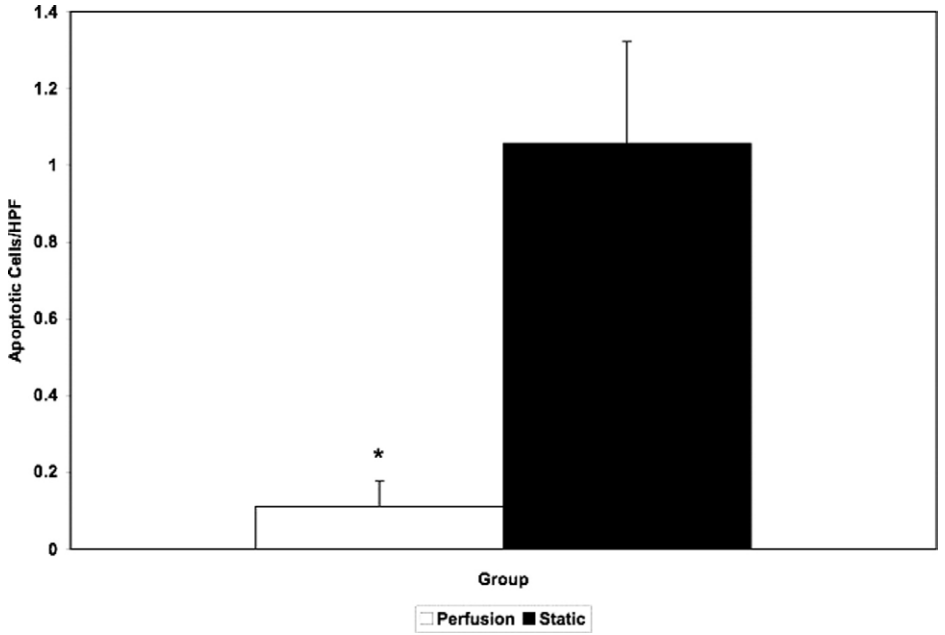

FIG. 3. Apoptotic cells per high power field (HPF) in hearts stored with perfusion preservation *versus* traditional cold storage ($*P < 0.01$).

did not demonstrate any significant incorporation of ^{13}C into citric acid cycle intermediates. This observation is markedly different from that made in rat hearts preserved with an identical solution using the same MRS method [7]. While these results appear to be conflicting, several explanations may apply. Oxygen consumption could occur within the myocardium without evidence of ^{13}C incorporation if unlabeled endogenous energy sources such as glycogen or triglyceride were the substrate used for oxidative metabolism. It is well established that crystalloid-perfused isolated rat hearts will undergo minimal oxidation of endogenous stores when exogenous substrates are in adequate supply. However, in the present study, the flow rate and perfusion pressure are much lower, the myocardial mass is much higher, and the perfusate temperature is much colder than in most small animal studies that have examined substrate metabolism. As well, glucose uptake may be impaired in this model since lower temperatures are used and insulin is not provided. Finally, it should be noted that the MRS analysis was performed in left atrial tissue that may have a much lower metabolic rate than ventricular myocardium.

A novel finding of this study was the dramatic decrease in tissue lactate accumulation in atrial samples collected at the end of the storage interval from hearts in the perfusion preservation group compared with hearts protected with simple cold storage. The presence of oxygen in this system may allow glucose to undergo complete oxidation through the citric acid cycle leading to minimal production of lactate through anaerobic pathways. Alternatively, any lactate formed from anaerobic metabolism may be washed out from the myocardium by the ongoing perfusion. As shown in Table 2, lactate levels in samples of perfusion fluid rose slowly during the 4 h storage interval, suggesting that washout may be partially responsible. Of note, all lactate observed in the MRS spectra was unlabeled, suggesting that glycolysis of glucose in the preservation solution was not a contributor. Regardless of whether decreased lactate accumulation is the result of ongoing aerobic metabolism or washout, lower lactate levels have been suggested to be beneficial. Neely and Grotyohann have demonstrated that accumulation of anaerobic glycolytic products, including lactate, may contribute to irreversible damage to the myocardium [14].

Although myocardial function did not differ between groups, there were some suggestions that myocardial damage may have differed. There was a trend, although not significant, in cardiac enzyme release with higher CK-MB levels observed in static stored hearts. As well, analysis of left ventricular biopsies at the end of reperfusion demonstrated significantly higher counts of TUNEL positive cells in the standard cold storage preservation group. Although the apoptotic cell fraction was low in both groups, these differences may have clinical importance. Data from Anselmi *et al.* suggest that increased cardiomyocyte apoptosis after reperfusion translates into significant long term myocardial dysfunction [15]. In cardiac transplantation, apoptosis may have greater importance as Birks *et al.* have demonstrated that donor heart dysfunction is correlated with increased activation of apoptosis [16]. Apoptotic cell death appears to be involved with graft vasculopathy and allograft survival is associated with the degree of expression of apoptotic regulatory proteins in mitochondria [17].

Any system that provides ongoing perfusion with a crystalloid solution has the potential to create myocardial edema, and several previous experimental studies have suggested increased edema in hearts stored with perfusion techniques [3, 10]. The current study found no difference in myocardial water content after 4 h of preservation and 6 h of reperfusion between the two preservation strategies. Further studies with longer intervals of storage and reperfusion are warranted to confirm these findings.

This study has several limitations. First, this model does not use brain dead donors, which have been shown to display greater myocardial dysfunction and to have an altered metabolic profile [18]. As well, the perfusion preservation group was tested at a single flow rate (10 mL/100 g/min) and other flow rates, perfusion temperatures, or preservation solution compositions may lead to different results. Finally, a species effect may need to be considered and direct comparisons to other models using pigs or other species need to be interpreted with caution. Nevertheless, this study suggests that the strategy of continuous preservation of donor hearts stored for transplantation may have metabolic benefits to the donor heart. Further studies will be required to test this technique under differing perfusion conditions and longer intervals. Perfusion preservation techniques may offer the opportunity to improve results after cardiac transplantation.

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