Effects of Antegrade and Retrograde Machine Perfusion Preservation on Cardiac Function After Transplantation in Canines

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ABSTRACT

Introduction. Most studies investigating machine perfusion preservation for heart transplantation perfuse through the aortic root (antegrade), but the coronary sinus (retrograde) is a potential option. We hypothesized that retrograde machine perfusion provides better functional protection than static storage, while avoiding the potential irregular perfusion seen when aortic insufficiency occurs with antegrade perfusion.

Materials and Methods. Eighteen canine donor hearts were arrested, procured, and stored in modified Celsior solution for 4 hours by using either static storage at 0°C to 4°C (n = 6) or machine perfusion preservation at 5°C via the aortic root (antegrade, n = 6) or coronary sinus (retrograde, n = 6). Lactate and myocardial oxygen consumption were measured in perfused hearts. Hearts were reimplanted and reperfused for 6 hours with hourly function calculated by using the preload recruitable stroke work (PRSW) relation. Myocardial water content was determined at the end of the experiment.

Results. Storage lactate levels and myocardial oxygen consumption were comparable in both perfused groups. The PRSW was increased immediately after bypass in the antegrade group (120.6 ± 19.1 mm Hg) compared with the retrograde (75.0 ± 11.3 mm Hg) and static (78.1 ± 10.5 mm Hg) storage groups ($P < .05$). At the end of reperfusion, PRSW was higher in the retrograde group (69.8 ± 7.4 mm Hg) compared with the antegrade (40.1 ± 6.8 mm Hg) and static (39.9 ± 10.9 mm Hg) storage groups ($P < .05$). Myocardial water content was similar among groups.

Conclusions. Both antegrade and retrograde perfusion demonstrated excellent functional preservation, at least equivalent to static storage. Initial function was superior in the antegrade group, but the retrograde hearts displayed better function late after reperfusion. Neither perfused group developed significant edema. Machine perfusion preservation is a promising technique for improving results of cardiac transplantation.

MACHINE perfusion for the preservation of donor organs has already been established clinically as superior for early graft function and long-term graft survival in kidney transplantation compared with static cold storage [1,2]. This method has also been evaluated for >50 years for cardiac transplantation [3] but has not yet been applied to clinical practice. The current standard remains hypothermic static storage, which limits safe ischemic times to <6 hours. However, review of registry data suggests that although results are acceptable at ischemic times up to 6 hours, there is an increase in the relative risk of recipient mortality, both 1-year and long-term, when the ischemic times are extended beyond 3 to 4 hours [4]. The potential benefits of machine perfusion preservation in cardiac transplantation include improved immediate and long-term graft function, tolerance of longer ischemic times, better donor–recipient matching, resuscitation of injured or circulatory death hearts, and improved results

The perfusion device prototype, LifeCradle, was provided by Organ Transport Systems Inc, Frisco, Texas. The only support received for this study was for this prototype perfusion device.

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from extended criteria donors. Studies in small- and large-animal models in our laboratory and by others for both short- and long-term preservation have shown improved functional results, higher ATP stores, and improved lactate-to-alanine ratios in the cardiac tissue with cold machine perfusion preservation compared with static storage [5–7].

Most studies examining machine perfusion preservation for hearts use antegrade perfusion through the coronary arteries via the aortic root. Although this technique has been shown in previous studies to be superior to standard cold storage [6,7], there is the potential for aortic valve incompetence, resulting in inconsistent perfusion and nonnutrient flow [8]. Under hypothermic conditions, at standard ischemic intervals, the heart would essentially undergo static cold storage; however, for longer ischemic times, normothermic perfusion, and procurement of extended criteria hearts, nonnutrient flow could have significant implications for graft function. We previously demonstrated that the use of an initially high loading flow rate, followed by normal flow rates of perfusion solution, may result in better apposition of the aortic valve leaflets and potentially avoid this problem [8]. However, it is difficult to assess and verify adequate leaflet closure during machine perfusion. In addition, unlike the controlled, stable laboratory setting, during transport of donor hearts, intermittent distraction of the aortic valve leaflets could occur, resulting in inadequate perfusion.

A potential alternative to antegrade perfusion is retrograde perfusion through the coronary venous system via a catheter inserted in the coronary sinus. Retrograde cardioplegia is already frequently used during cardiac surgery for myocardial protection [9–11]. Using this technique, the issue of aortic valve incompetence, especially in relation to potential movement during transport, would be avoided. An additional benefit to this technique is that the heart could continue to be perfused with oxygenated blood cardioplegia during implantation, essentially eliminating the warm ischemic interval [12–14]. One concern with retrograde perfusion is decreased perfusion to certain regions of the heart, especially the right ventricle [15,16]. We previously reported some decrease in the perfusion of the right ventricle; however, the implications of this finding on reperfusion graft function have not been completely investigated [17].

The purpose of the present study was to compare hypothermic retrograde machine perfusion with both hypothermic antegrade machine perfusion and conventional hypothermic static storage over a clinically relevant ischemic interval in a large animal model. We hypothesized that retrograde machine perfusion would provide better functional protection at standard storage intervals than static storage, while avoiding the potential irregular perfusion seen when aortic insufficiency occurs with antegrade perfusion.

MATERIALS AND METHODS

Experimental Protocol

The protocol used in 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).

Thirty-six adult mongrel dogs between 24 and 39 kg were used, divided into 18 donor–recipient pairs. The first 12 pairs were part of a previously published study comparing hypothermic static preservation versus hypothermic antegrade perfusion preservation using a perfusion device (LifeCradle, Organ Transport Systems Inc, Frisco, Tex, United States) [7]. The functional data from these experiments were reanalyzed by using updated software and improved filtering techniques for use in this study. For further comparison, 6 additional pairs were then subjected to hypothermic retrograde perfusion preservation using the same device with an adaptable coronary sinus catheter (Medtronic, Inc, Minneapolis, Minn, United States) and using the same perfusion solution. The functional data from these animals were analyzed by using the same updated techniques.

All hearts were stored for 4 hours, then reimplanted into recipient animals and reperfused for 6 hours. Celsior organ preservation solution (Genzyme Corporation, Cambridge, Mass, United States) supplemented with 1 g/L (5.5 mmol/L) of glucose was used in all the storage techniques.

Anesthetic Protocol

The animals were premedicated with atropine (0.07 mg/kg intramuscularly) and Telazol (tiletamine/zolazepam) (4.4 mg/kg intramuscularly) and then intubated and ventilated with 100% oxygen at a rate of 10/min, a tidal volume of 10 mL/kg, and positive end-expiratory pressure of 5 cm H_2O. Isoflurane at 1% to 4% was used to maintain anesthesia. Electrocardiograms and arterial pressure were continuously monitored. Arterial blood gas measurements were obtained and used to adjust the ventilator settings to maintain a pH of 7.35 to 7.45, partial pressure of carbon dioxide of 35 to 45 mm Hg, and oxygen saturation >95%.

Donor Protocol

After induction of anesthesia, a sternotomy was performed and the heart exposed. Each animal was given intravenous heparin (300 U/kg), and a cardioplegia catheter was inserted into the ascending aorta. The hearts were instrumented with a left ventricular (LV) pressure catheter and sonomicrometry crystals, and baseline cardiac function was measured. After application of an aortic cross-clamp, the heart was arrested with 1 L of cold modified Celsior solution, and the donor cardiectomy was completed.

Donor hearts randomized to static preservation were stored in an ice chest, in a container of 1 L of modified Celsior. Donor hearts randomized to antegrade perfusion preservation were attached to the perfusion device by a connector in the ascending aorta. This provided continuous antegrade flow of oxygenated, modified Celsior solution at a flow rate of 10 mL/100 g myocardium/minute at a temperature of $5 \pm 2^{\circ}C$. For the antegrade perfusion group, a small polyethylene catheter was secured in the coronary sinus to allow for serial measurements of oxygen tension, lactate, and pH. Donor hearts in the retrograde perfusion preservation group were attached to the perfusion device with a catheter in the coronary sinus. In this group, the polyethylene catheter used for serial measurements was placed in the aortic root to capture coronary effluent.

Recipient Protocol

Each recipient was placed on cardiopulmonary bypass (CPB) after induction of anesthesia and sternotomy. Excision of the heart was

coordinated with the end of the donor storage interval, and the donor heart was then implanted by using a standard bicaval orthotopic transplant technique. Before the aortic clamp was removed, the animals were given 1 g of methylprednisolone. Once reperfusion was established, the hearts were defibrillated with 5 to 20 J if needed, and an infusion of dobutamine was started at 5 μg/kg per minute. After 1 hour of reperfusion, the animals were weaned from CPB. The LV pressure catheter was reinserted, and the previously placed sonomicrometry crystals were reconnected to the preamplifier. Load-independent cardiac functional data were collected at hourly intervals, and at the end of 6 hours, the animal was killed. LV samples were taken for water content analysis.

Measurement of Ventricular Performance

As described in a previous large-animal transplant model [18], a load-independent method was used to quantify LV function. To measure volume, 4 sonomicrometry crystals (Sonometrics Corporation, London, Ontario, Canada) were secured to the subendocardium in the minor and major axis of the left ventricle, and they remained in situ during both storage and reperfusion. For LV pressure monitoring, a micromanometer-tipped catheter (Millar Instruments, Houston, Tex, United States) was inserted into the ventricle through the apex. It was removed for the harvest and storage interval, and then reinserted after reimplantation. Pressure and dimension (volume) data were collected at a rate of 250 Hz, digitized, and stored on a computer. These were later analyzed by using commercially available software (SonoLab and CardioSOFT, Sonometrics Corporation). New advanced techniques were used within this software program to filter the loop data, which had not been implemented in previously reported studies [7]. This method allowed for removal of more background interference in the data and thus utilization of more of the collected loops in the subsequent calculations. Pressure–volume loops were obtained before donor harvest (baseline) and at hourly intervals, for a total of 6 hours of reperfusion. The loops were obtained over a range of filling conditions by draining blood into the cardiotomy reservoir, creating a series of emptying curves. The integral (area) of each pressure–volume loop in the emptying curves was calculated to give the stroke volume and then plotted against the corresponding end-diastolic volumes. The slope of the resulting regression is termed the preload recruitable stroke work (PRSW) and is considered a load-independent index of contractility [18].

Measurement of Myocardial Water Content

LV tissue samples were taken, weighed, and placed in an oven for drying. Daily weights were measured until a consecutive, constant weight was achieved. The myocardial water content was calculated by using the formula: (wet weight − dry weight)/wet weight.

Statistical Analysis

Results are reported as mean values ± SEMs. Commercially available statistical software (SigmaPlot, Systat Software, Inc, San Jose, Calif, United States) was used for calculations. The groups were compared by using a 2-sided *t* test or analysis of variance, as appropriate. A repeated-measures analysis of variance was applied for outcome variables that were measured at multiple time points over the reperfusion interval. A P value $<.05$ was considered significant.

RESULTS

Total ischemic time was just over 5 hours and similar in all groups (static group, 318.0 ± 4.6 minutes; antegrade group, 320.2 ± 4.1 minutes; retrograde group, 318.3 ± 5.3 minutes). All of the hearts were initially able to be weaned from CPB; however, 1 animal in the static group returned to CPB by hour 4 due to cardiac dysfunction, and 1 in the antegrade group returned by hour 4 due to bleeding that could not be controlled surgically (Table 1). Hearts in both the antegrade and retrograde group extracted oxygen throughout the storage interval, with no statistical difference between the 2 perfusion groups (Fig 1). Baseline LV function was similar in all groups. PRSW was increased in all groups at 1-hour postreperfusion, with a significantly higher rise in the antegrade group compared with both the static ($P = .002$) and retrograde ($P < .001$) groups. PRSW decreased over time in both the antegrade and static groups but remained stable in the retrograde group. In hours 5 and 6, PRSW was higher in the retrograde group compared with both the antegrade and static groups (5 hours, $P < .04$; 6 hours, $P < .03$) (Fig 2). Myocardial edema, measured by using the LV water content, was slightly higher in the retrograde group compared with the antegrade and static groups (79.7 ± 0.7% vs 78.3 ± 0.4% and 78.3 ± 0.2%, respectively), but this difference was not statistically significant ($P = .095$).

DISCUSSION

Machine perfusion preservation seems to be a promising strategy to improve results of heart transplantation. A warm, beating heart strategy with a blood-based perfusate and a device by Transmedics, Inc. (Andover, Mass, United States) is currently undergoing clinical trials in the United States and Europe [19]. We chose hypothermic, acellular perfusion in our studies for some of the reasons previously stated. These large-animal functional studies were performed with a planned 4-hour ischemic interval because any clinical trial with this device would be compared with standard cold storage over currently accepted ischemic times. The conventional approach to machine perfusion preservation of any organ is antegrade perfusion through the arterial system. The heart requires a competent aortic valve to ensure adequate delivery of the preservation solution to the myocardium unless the coronary arteries are cannulated directly. However, most cardiac surgeons would be reluctant to instrument these relatively delicate structures. An alternate approach to ensure nutrient flow to the myocardium is retrograde perfusion through the coronary venous system.

One of the potential benefits of machine perfusion preservation is the continued ability of the heart to undergo

Table 1. Total Ischemic Time, Return to CPB, and Myocardial Water Content After Reperfusion

Group	Total Ischemic Time (min)*	Hearts Requiring Return to CPB	LV Water Content (%)*
Static	318.0 ± 4.6	1/6	78.3 ± 0.2
Antegrade	320.2 ± 4.1	1/6	78.3 ± 0.4
Retrograde	318.3 ± 5.3	0/6	79.7 ± 0.7

Abbreviations: CPB, cardiopulmonary bypass; LV, left ventricular.
*Mean values ± SEM.

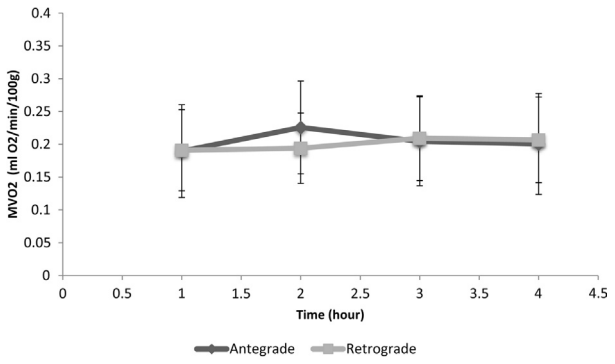

Fig 1. Myocardial oxygen consumption (MVO_2) during preservation interval. Data are expressed as mean values ± SEM. P = NS.

aerobic metabolism and thus have lower lactate levels and higher ATP stores. In previous studies within this laboratory using antegrade perfusion, ongoing oxygen consumption (myocardial oxygen consumption) has been shown during the storage interval [6,7]. Similarly, both the antegrade and retrograde perfused hearts used oxygen continuously during preservation in the present study, with no significant difference between the 2 groups. Thus, despite concerns regarding decreased flow to the right ventricle with retrograde perfusion, both groups seem to undergo oxidative metabolism and with equivalent oxygen consumption.

To the best of our knowledge, our study is the first direct comparison of conventional hypothermic static storage, antegrade machine perfusion preservation, and retrograde machine perfusion preservation for cardiac transplantation. As expected over this conventional ischemic interval, functional recovery of the transplanted hearts was satisfactory in all groups (although 1 organ in the static group required a return to CPB for graft dysfunction and 1 heart in the antegrade group required a return to CPB for technical reasons).

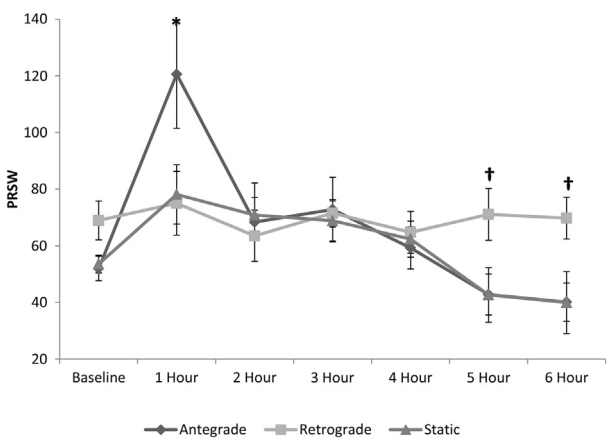

Fig 2. Preload recruitable stroke work (PRSW, mmHg) during reperfusion. Data are expressed as mean values ± SEM. *P <.05 compared with the baseline, static, and retrograde groups. †P < .05 compared with the antegrade and static groups.

When cardiac function was evaluated more closely by using a load-independent measure of performance (PRSW), some interesting findings were noted. All groups experienced an increase in PRSW compared with baseline immediately after separating from CPB following the 1-hour reperfusion period. The initial increase in PRSW was pronounced in the antegrade group compared with the other 2 groups. By the end of the reperfusion period, PRSW was actually higher in the retrograde group compared with the other 2 groups. Reasons for this difference are not entirely clear but may involve reductions in nutrient flow due to some degree of aortic insufficiency, as we previously reported.

PRSW is inotropy dependent, and the infusion of dobutamine in transplanted hearts could at least partially explain this increase in PRSW early after CPB separation. Improved preservation of myocardial energy stores is a likely explanation of the difference in PRSW between the antegrade group and the static group at 1 hour, although the reason for the difference compared with the retrograde group is less clear. One possibility may be related to the degree of myocardial edema that can develop in perfused hearts. We previously reported that antegrade perfused hearts can develop significant myocardial edema when perfused with Celsior over extended storage intervals [20]. Edema seems to develop much sooner in retrograde perfused hearts, likely due to the increased flow rates required to achieve adequate tissue nutrient flow, and unlike in antegrade perfused hearts, this effect seems to be relatively independent of the preservation solution [17]. The present study suggests that development of myocardial edema in perfused hearts seems to be primarily a mechanical rather than an endothelial effect because it resolves over time as the heart resumes contractility; therefore, at the end of the reperfusion period, differences in water content have largely disappeared.

The present study has several limitations. First, the animals were not randomized to specific groups, and retrograde perfusion experiments were not performed concurrently with antegrade perfusion and static storage studies. Nonetheless, the same experimental protocol, preservation solution, and analytical techniques were used for all groups. Also, we did not use a brain death model for these studies. Brain death leads to myocardial dysfunction, and a brain death model might have yielded a greater degree of functional impairment, although differences between groups would probably still have been preserved [21]. Finally, we did not measure right ventricular (RV) function directly. RV protection is a concern with retrograde cardioplegia but should not be worse than RV function in the static group. None of the animals experienced clinically relevant RV failure, but direct measures of RV function were not obtained. This factor is important to consider when more severe conditions of ischemia are evaluated and a greater degree of myocardial dysfunction is expected.

In conclusion, machine-perfused hearts can maintain graft oxidative metabolism over a 4-hour perfusion interval. Compared with static storage, antegrade perfusion offers

improved early graft function, although cardiac function late after reperfusion seems superior with the retrograde technique. Further experiments investigating myocardial preservation and function after transplantation in models of more stringent ischemic conditions are warranted.

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