Metabolic characteristics of human hearts preserved for 12 hours by static storage, antegrade perfusion, or retrograde coronary sinus perfusion

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Objective: Machine perfusion of donor hearts is a promising strategy to increase the donor pool. Antegrade perfusion is effective but can lead to aortic valve incompetence and nonnutrient flow. Experience with retrograde coronary sinus perfusion of donor hearts has been limited. We tested the hypothesis that retrograde perfusion could support myocardial metabolism over an extended donor ischemic interval.

Methods: Human hearts from donors that were rejected or not offered for transplantation were preserved for 12 hours in University of Wisconsin Machine Perfusion Solution by: (1) static hypothermic storage; (2) hypothermic antegrade machine perfusion; or (3) hypothermic retrograde machine perfusion. Myocardial oxygen consumption (MVO_2), and lactate accumulation were measured. Ventricular tissue was collected for proton and phosphorus 31 magnetic resonance spectroscopy (MRS) to evaluate the metabolic state of the myocardium. Myocardial water content was determined at the end of the experiment.

Results: Stable perfusion parameters were maintained throughout the perfusion period with both perfusion techniques. Lactate/alanine ratios were lower in perfused hearts compared with static hearts ($P < .001$). Lactate accumulation (antegrade 2.0 ± 0.7 mM, retrograde 1.7 ± 0.1 mM) and MVO_2 (antegrade 0.25 ± 0.2 mL, retrograde 0.26 ± 0.3 mL O_2/min/100 g) were similar in machine-perfused groups. High-energy phosphates were better preserved in both perfused groups ($P < .05$). Left ventricular myocardial water content was increased in retrograde perfused hearts (80.2 ± 0.8%) compared with both antegrade perfused hearts (76.6 ± 0.8%, $P = .02$) and static storage hearts (76.7 ± 1%, $P = .02$).

Conclusions: Machine perfusion by either the antegrade or the retrograde technique can support myocardial metabolism over long intervals. Machine perfusion seems promising for long-term preservation of human donor hearts. (J Thorac Cardiovasc Surg 2014;148:2310-5)

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Machine perfusion preservation of donor hearts has potential for improving heart preservation, extending the donor ischemic interval, retrieval of marginal donor organs, and possibly the use of non-heart-beating donors.[1-3] Most studies investigating machine perfusion for heart transplantation either by warm, beating-heart perfusion or cold perfusion use antegrade perfusion of the coronary arteries by delivery of the perfusate into the ascending aorta.[4-6] Research by our laboratory and others have confirmed that machine perfusion preservation is more effective than conventional static storage after standard and long-term ischemic intervals.[5-7] However, we have previously reported that under some conditions, aortic insufficiency and thus nonnutrient flow could occur.[8] In clinical practice, the likelihood of significant aortic valve incompetence would be even greater during the conditions encountered when traveling to procure donor hearts. This is of minor consequence in the cold perfused heart over a standard ischemic interval because these hearts would essentially be undergoing static storage, but may prove devastating during normothermic perfusion or over extended donor ischemic intervals.

Retrograde perfusion through the coronary sinus avoids the possibility of aortic insufficiency and is used routinely for cardioplegia delivery during cardiac surgery[9,10] but its use for machine perfusion of donor hearts is rudimentary

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Abbreviations and Acronyms

ATP = adenosine triphosphate
ANOVA = analysis of variance
MRS = magnetic resonance spectroscopy
MVO_2 = myocardial oxygen consumption
PCr = phosphocreatine
Pi = inorganic phosphate
UWMPS = University of Wisconsin Machine Perfusion Solution

at best.[11] Furthermore, we previously reported that right ventricular perfusion was reduced in canines when using retrograde perfusion, illustrating a potential limitation of this technique.[12]

The ability of either technique to preserve human hearts over extended ischemic intervals has not been well characterized. We therefore decided to investigate the myocardial metabolism of human hearts perfused by either antegrade or retrograde machine perfusion and compare this with conventional static storage. We hypothesized that machine perfusion is more effective than standard cold storage for long-term preservation of human donor hearts for transplantation.

METHODS

This study was conducted in accordance with an organ procurement organization–approved research protocol. Human donor hearts either not offered for transplantation or rejected for transplantation by all centers to which they were offered and for tissue donation were assigned to 1 of 3 preservation techniques: (1) cold storage; (2) antegrade machine perfusion; (3) retrograde machine perfusion. Tissue from additional hearts was procured immediately after cardiectomy to obtain baseline metabolic parameters.

Perfusion Device

Hearts assigned to the perfusion groups were perfused with a prototype perfusion device, the LifeCradle (Organ Transport Systems, Inc, Frisco, Tex). The device delivers temperature-regulated oxygenated perfusate to the heart. Perfusate temperature, flow, and pressure were monitored continuously.

Procurement

Organ retrieval was performed through a median sternotomy. The heart was exposed and the donor was systemically heparinized with 30,000 units of heparin. After adequate time for heparin circulation had elapsed, the aorta was crossclamped and 1 L of ice-cold University of Wisconsin Machine Perfusion Solution (UWMPS) was used to arrest the heart. Hearts were decompressed through the inferior vena cava and the left atrial appendage. The donor cardiectomy was completed.

Preservation Techniques

Hearts were preserved for 12 hours by 1 of the 3 techniques. Conventional hypothermic storage hearts were immersed in 1 L of ice-cold UWMPS. Hearts in the antegrade perfusion group were perfused with UWMPS through the ascending aorta at a flow rate of 10 mL/100 g heart weight/min at 5°C ± 2°C. Donor hearts in the retrograde groups were perfused through a retrograde cardioplegia cannula (Medtronic, Inc, Minneapolis, Minn) sewn into the coronary sinus with UWMPS at a flow rate of 13 to 20 mL/100 g/min, also at 5°C ± 2°C. Perfusion group flow rates were based on previously obtained data on large animals and in some cases for the retrograde group, maximum achievable flow rate of the device.[8,12] Temperature and perfusion pressure were measured continuously in machine-perfused hearts. End perfusion myocardial oxygen consumption (MVO_2) and transmyocardial lactate and perfusate lactate accumulation were determined for most of the perfused hearts using a commercial analyzer (Radiometer America, Inc, Westlake, Ohio). Separate MVO_2 from right and left coronary artery effluent was calculated in retrograde perfused hearts. Myocardial water content was measured from tissue samples at the end of the experiment.

Magnetic Resonance Spectroscopy

The tissue samples were harvested, immediately freeze-clamped, and cooled in liquid nitrogen. The tissue was stored in a freezer at −80°C and subsequently extracted with perchloric acid. Purified extracts were reconstituted in deuterium oxide, and the pH was adjusted to 7.0 to 7.4 for magnetic resonance spectroscopy (MRS). Proton (^{1}H) MR spectra were then acquired with a 14.1-T Varian spectrometer operating at 600 MHz over a spectral width of 8000 Hz. Lactate-to-alanine ratios were compared from ^{1}H spectra as measures of cellular aerobic and anaerobic metabolism during storage.[13,14] Proton-decoupled phosphorus 31 (^{31}P) spectra were obtained on the same spectrometer tuned to the ^{31}P nucleus operating at 243 MHz over a spectral width of 36,000 Hz. Phosphocreatine (PCr)/inorganic phosphate (Pi), γ-adenosine triphosphate (γ-ATP)/Pi, and phosphocreatine/γ-ATP ratios were measured to determine the preservation of high-energy phosphates during the storage interval. Pi, PCr, and ATP standards were applied to selected samples to verify these signals.

Data Analysis

Data are reported as means ± standard error of the mean. Statistical analysis was performed with SigmaPlot statistical software (SyStat Software, Inc, San Jose, Calif) using a 2-sided *t* test or analysis of variance (ANOVA), as appropriate. A $\log_{10}$ function was applied to ^{31}P MRS spectra to normalize the data. ANOVA on ranks using the Kruskal-Wallis test was performed on other variables that demonstrated either unequal variances or a nonnormal distribution. When measurements were collected over multiple time points, data were compared with repeated-measures ANOVA. Adjustments for multiple comparisons were performed using the Fisher least significant difference method or the Dunn method, as appropriate. Relationships between variables were investigated with the Pearson correlation coefficient.

RESULTS

Demographics

Twenty-eight donor hearts were studied with the 12-hour storage protocol: 10 using conventional cold storage, 8 using antegrade perfusion, and 7 using retrograde perfusion. Three additional hearts were used to determine baseline metabolic parameters immediately after procurement. Twenty-one hearts were procured using ground transportation and 7 hearts were procured by air transport. Donors were reasonably matched for age, ejection fraction, cause of death, height, and weight. Donor demographics are shown in Table 1. Seven of the 31 hearts in this study were offered for donation but rejected for transplantation.

Perfusion Characteristics

Both perfusion groups cooled rapidly within 1 hour to the set temperature and maintained a stable temperature over