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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