Magnetic Resonance Spectroscopy on Cardiac Tissue in a Canine Heart Transplant Model

M. Peltz[1], D. H. Rosenbaum[1], M. E. Merritt[2], and M. E. Jessen[1]

[1]Cardiovascular and Thoracic Surgery, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, United States, [2]Advanced Imaging Research Center, University of Texas Southwestern Medical Center at Dallas, Dallas, TX, United States

Introduction

Perfusion preservation techniques in cardiac transplantation are intended to promote myocardial metabolism during storage and maintain the energetic state of the myocardium. Other investigators have used this strategy to extend the viability of donor organs [1]. However, clinically, hearts rarely experience prolonged ischemia because most organs are allocated locally [2]. Effects of perfusion storage on myocardial preservation during these relatively short storage intervals have been infrequently studied. We previously demonstrated in a small animal *ex-vivo* model that the lactate:alanine ratio by MRS correlated with myocardial high-energy phosphate stores, oxidative metabolism during storage, and improved myocardial function after reperfusion even after relatively modest ischemia [3]. We hypothesized that similar advantages to perfusion storage could be demonstrated in a large animal model of heart transplantation. We therefore compared myocardial metabolism using MRS in a canine model of heart transplantation after conventional storage or perfusion storage.

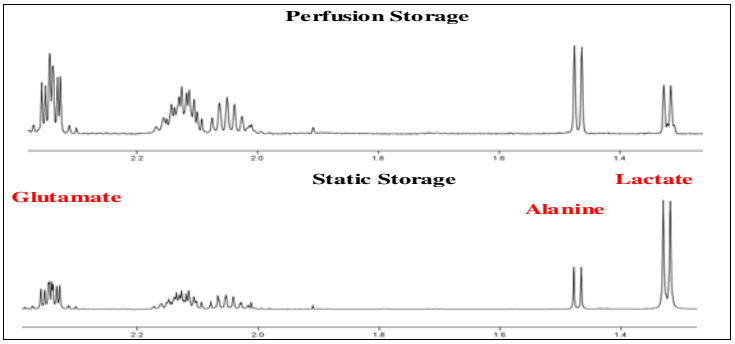
Methods

Experimental Design: Hearts from mongrel dogs (6 per group) were harvested after arrest with Celsior organ preservation solution. Animals were then randomized to either conventional static storage or perfusion preservation in the Lifecradle® (Organ Transport Systems, Inc) at 4°C in Celsior supplemented with 1g/L U-^{13}C labeled glucose. All hearts were stored for 4 hours, implanted into recipient animals, and reperfused for six hours.
Metabolic Studies: Just prior to reimplantation, a left atrial appendage sample was removed, freeze clamped with liquid nitrogen cooled tongs and stored at -80°C for subsequent MRS. Cardiac tissue was extracted with perchloric acid and lyophilized. Extracts were reconstituted in D_2O and high-resolution ^{1}H MR spectra were obtained on a 14.1T Varian Inova spectrometer.
Reperfusion: Cardiac function was measured by preload-recruitable stroke work (PRSW) using sonomicrometry crystals placed in the left ventricular short and long axis and an intraventricular micromanometer-tipped catheter. After 6 hours reperfusion, serum was collected for measurement of CKMB release. Left ventricular tissue was collected and preserved in 4% paraformaldehyde for subsequent histology. TUNEL assays using the Promega Dead End TUNEL kit were performed on tissue sections from each heart to determine apoptotic cell death. Apoptosis was reported as the number of TUNEL positive nuclei per high-power field.
Statistics: Data for all groups are reported as the mean ± SEM. Groups were compared by two-sided t-tests. A p-value of less than 0.05 was considered significant.

Results

After six hours of reperfusion, function in both groups was similar as measured by PRSW. Apoptotic myocardial cell death was reduced in the perfusion preservation group. CKMB release was greater in the static storage group but this difference was not significant. Perfusion preservation did not result in myocardial edema. See Table. Contrary to our previous results, virtually no metabolism of exogenous ^{13}C glucose by glycolysis or oxidative metabolism was detected in either group. Despite this apparent lack of exogenous substrate metabolism, spectral differences were noted between groups. As in our prior model, the lactate:alanine ratio was reversed in static and perfusion preserved hearts. See Figure.

Group	Static Storage	Perfusion Storage
Water Content (%)	78.3±.4	78.7±.2
Lactate:Alanine Ratio	3.0±.5	0.3±.4*
Reperfusion Function (PRSW)	39.6±11	39.5±7
CKMB Release (ng/mL)	6.9±.5	2.9±.2
Apoptosis (TUNEL+cells/HPF)	1.1±.3	.11±.07*



*- p<.05 vs Static Storage

Conclusions

Contrary to our previous small animal experiments, myocardium in the current model appears to principally rely on metabolism of endogenous substrates under the preservation conditions studied. ^{1}H MRS revealed significant differences in myocardial metabolism between static and perfusion storage. These data suggest that perfusion storage reduces lactate accumulation, and improves myocyte survival even after a routine preservation interval. MRS appears to be a promising technique for evaluating cardiac metabolism during hypothermic storage. Further studies will be required to assess whether metabolic parameters can be identified that can predict function prior to reimplantation.

References

1. Mozes MF, Finch WT, Reckard CR, Merkel FK, Cohen C. Comparison of cold storage and machine perfusion in the preservation of cadaver kidneys: A prospective, randomized study. *Transplant Proc* 1985;17:1474-77.
2. Taylor DO, Edwards LB, Mohacsi PJ, Boucek MM, Trulock EP, Keck BM, Hertz MI. The Registry of the International Society for Heart and Lung Transplantation: Twentieth Official Adult Heart Transplant Report – 2003. *J Heart Lung Transplant* 2003;22:616-624.
3. Peltz M, He TT, Adams GA, Koshy S, Burgess SC, Chao Y, Meyer DM, Jessen ME. Perfusion Preservation Maintains Myocardial ATP Levels and Reduces Apoptosis in an ex vivo Rat Heart Transplantation Model. *Surgery* 2005;138(4):795-805.