

Proton Spectroscopy Techniques to Examine Myocardial Metabolic State in Human Hearts Preserved for Transplantation

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Introduction

Heart transplantation is limited by the relatively brief ischemic tolerance of preserved myocardium that restricts optimal organ allocation and utilization of extended donor organs. Perfusion preservation is a promising strategy for extending the ischemic tolerance of myocardium and for recovery of marginal donor hearts.¹ Predicting myocardial function after long term preservation or of suboptimal organs remains a challenge. We previously applied ¹H magnetic resonance spectroscopy (MRS) to a large animal model of perfusion preservation and demonstrated that important metabolic differences could be determined by ¹H high resolution MRS of myocardial extracts compared to static storage. In these studies, the lactate/alanine ratio predicted post-transplant function in stored hearts. This technique was useful in the experimental setting but had limited clinical application because of the tissue manipulation and time required to perform the analysis. We subsequently applied ¹H magic angle spinning MRS (MAS) to tiny left ventricular biopsies and created a mathematical model that was able to identify the preservation strategy.³ Similar applications in the evaluation of human heart donors have not been reported. In the current study, we applied ¹H HRS MRS and ¹H MAS MRS to human hearts that were stored for long intervals either by conventional static storage or perfusion preservation. We hypothesized that these techniques could be used to determine the metabolic state of preserved hearts. This in turn may ultimately predict their suitability for heart transplantation after storage.

Methods

Human hearts unsuitable for transplantation (n=12) were obtained from potential donors throughout our local organ procurement organization. Eight hearts were flushed with University of Wisconsin Machine Perfusion Solution, connected to a perfusion device (Lifecradle®, Organ Transport Systems, Inc) and perfused at 10 mL/100g/min at 5 °C for 12 hours with the same solution. Four hearts underwent conventional hypothermic, static storage in the same organ preservation solution at 0-4°C for 12 hours. Temperature, flow, and pressure were recorded in perfused hearts. After 12 hours of perfusion, hearts were removed and weighed. Left atrial (LA) and left ventricular (LV) tissue samples were collected, frozen in liquid nitrogen and later analyzed by high resolution proton magnetic resonance spectroscopy on a Varian 14.1T Vnmrs spectrometer. ¹H MAS was carried out on small left ventricular cylindrical biopsies (approx 10mg) with a gHx nanoprobe on the same Varian 14.1T Vnmrs spectrometer. The lactate/alanine ratio in proton spectra was used to evaluate the metabolic state of stored hearts as previously described.² MAS spectra of perfused hearts and static storage hearts were compared to identify biomarkers that were indicative of the storage method. Data are reported as mean ± SEM. Differences between groups were determined by t-tests. A p-value < .05 was considered significant.

Table 1 – Lactate/Alanine Ratios

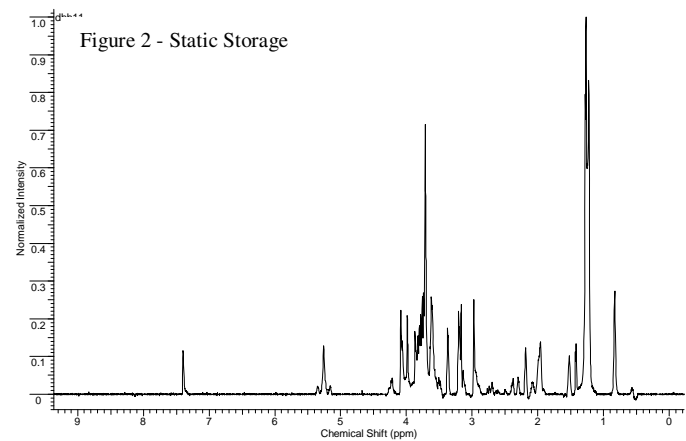
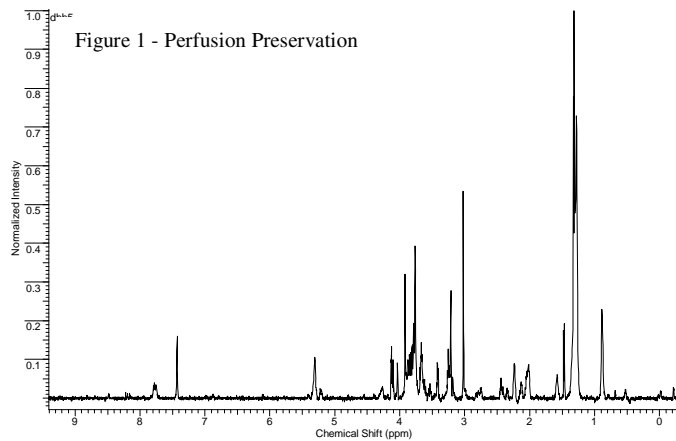
Group	Left Atrium	Left Ventricle	Left Ventricle MAS
Static Storage	5.1±1.3	3.1±.7	3.9±.5
Perfusion Preservation	1.2±.4*	0.7±.2*	2.4±.3*

* - p <.05 versus static storage

Results

As in our prior study, total analysis time for MAS was brief, less than 15 minutes. Significant differences in the lactate/alanine ratios were noted between static and perfusion preservation strategies, confirming our previous findings. These differences were greatest in LV extract samples. Left atrial and LV MAS MRS data were more variable. See Table 1. The later analysis was limited by the predominance of fatty acid methyl protons in the analyzed spectral region. Examination of MAS spectra suggested that the peak peaking partial least squares

algorithm utilized in our previous model may be a more powerful application for MAS data. See Figures 1 and 2 below.



Conclusions

Perfusion preservation of human hearts maintains oxidative metabolism after long-term storage. ¹H MAS MRS in particular may allow for rapid evaluation of minute tissue samples to identify metabolic differences that arise from varying cardiac preservation methods, and may provide clinicians with important information on the status of a donor heart without deleterious extension of the ischemic interval. Further studies will be required to test whether preservation of oxidative metabolism pre-transplantation translates into satisfactory reperfusion cardiac function after implantation.

References

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