A Magnetic Resonance Spectroscopy Technique for Evaluating Lung Preservation Strategies

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Background: The optimal conditions for preserving lungs harvested for transplantation are not established. Lungs are usually stored inflated with oxygen at profound hypothermia (4°C), although some data suggest that lesser degrees of hypothermia may be advantageous by allowing cellular metabolism to continue. Also, lungs are typically stored inflated with 100% oxygen, which may support ongoing oxidative metabolism in the stored organ (1). However, other investigators believe that a reduced oxygen concentration within the alveoli of the harvested organ may reduce oxygen-mediated free radical injury. We previously demonstrated that strategies aimed at supporting cellular metabolism led to increased graft oxidative metabolism and resulted in improved reperfusion lung function (2). However, temperature and alveolar oxygen content were kept constant during these experiments. The purpose of the current study was to apply magnetic resonance spectroscopy techniques to evaluate the effect of storage temperature and lung oxygen content on metabolic indices of lung allograft preservation over both medium and extended storage intervals and to examine the relationship of these events to cellular preservation.

Methods:

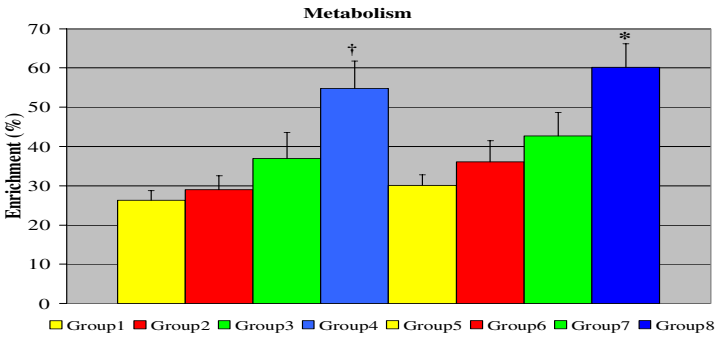
Experimental Design: Rat lungs (n=6-8 per group) were harvested and stored in modified low-potassium dextran preservation solution supplemented with carbon-13 labeled pyruvate and glucose for 6 and 24 hours. Eight groups were established as follows: Lungs were inflated with room air, stored at 4°C for 6 or 24 hours (Groups 1 and 5); room air, 10°C (Groups 2 and 6); 100% oxygen, 4°C (Groups 3 and 7); or 100% oxygen, 10°C (Groups 4 and 8) – see Table. After the preservation interval, lungs were freeze clamped in liquid nitrogen. Pulmonary tissue was extracted with perchloric acid and lyophilized. A portion of the each extract was reconstituted in D_2O and high-resolution ^1H MR spectra (MRS) with and without ^{13}C decoupling were obtained on a 14.1T Varian Inova spectrometer. Oxidative metabolism was quantified by enrichment of TCA cycle intermediates with exogenous substrate as described by Jones et al. (3). Additional samples of the extracts from each lung were analyzed by high performance liquid chromatography (HPLC) to measure intracellular levels of high energy phosphates [adenosine tri-phosphate (ATP), adenosine di-phosphate (ADP), and adenosine mono-phosphate (AMP)]. Cellular energy charge was calculated as [(ATP + ½ ADP)/(ATP + ADP + AMP)]. Stored lung viability after the end of the storage interval was assessed in tissue sections from other (non-frozen) lung samples by trypan blue exclusion and TUNEL assays as measures of necrotic and apoptotic cell death.

Statistics: Data for all groups are reported as the mean ± SEM. Groups were compared by one-way analysis of variance. Differences between groups, when present, were determined by the Student-Newman-Keuls method using SigmaStat® statistical software. A p-value of less than 0.05 was considered significant.

Results: After 6 hour storage, ATP levels, energy charge, and cell death were not different among groups. However, TCA cycle enrichment was significantly higher in Group 4 (100% oxygen, 10°C) suggesting increased aerobic metabolism under these conditions. All 6 hour groups had less than 10% of nonviable cells (p=NS). In contrast, after 24 hour storage, ATP levels and energy charge were lowest in Group 8 (100% oxygen, 10°C). A trend towards increased pneumocyte death (necrosis + apoptosis) was observed in this group (p=.09). Best preservation parameters (highest ATP levels and lowest cell death) after 24 hour storage were obtained in Group 7 (100% oxygen, 4°C). See Table and Figure below.

Group	O_2 (%)	Temp (°C)	Storage Interval	ATP (µmol/g dry wt)	Energy Charge	Cell Death (%)
1	21	4	6 Hours	4.7 ±.7	.81± .02	6.9± .9
2	21	10	6 Hours	3.9 ±.9	.68± .07	9.1 ±2
3	100	4	6 Hours	5.0 ±.6	.79 ±.01	4.8 ±.8
4	100	10	6 Hours	5.3 ±.9	.82 ±.02	6.7 ±2
5	21	4	24 Hours	1.8± .7	.55 ±.06	11± 1
6	21	10	24 Hours	1.9 ±.7	.55± .08	13 ±2
7	100	4	24 Hours	4.4 ±.6*	.77 ±.04*	11± 2
8	100	10	24 Hours	0.7 ±.2	.41 ±.02	16 ±2

* - p<.05 vs Groups 5,6,8



† - p<.05 vs Groups 1,2, * - p<.05 vs Groups 5,6

Conclusion: The optimal preservation strategy for lung transplantation has not been established and a variety of preservation solutions, preservation temperatures, and lung inflation conditions are used clinically. In part, this is due to the absence of an efficient method to rapidly assess differing preservation parameters. The present study provides information from a small animal model where metabolic, biochemical and histologic indices can be readily acquired and compared.

These data suggest that after a moderate storage interval (6 hours), similar cellular energetic parameters are achieved over a range of conditions with differing degrees of hypothermia and alveolar oxygen concentration. MRS enrichment data suggest that preservation at 10°C with 100% oxygen can increase aerobic metabolism during storage which may support energy generation within lung tissue. However, these same conditions appear to have deleterious effects when applied over extended (24 hour) periods of lung preservation, where ATP loss and increased cell death are observed. For extended preservation, deeper hypothermia and higher alveolar oxygen content appear more effective in preserving harvested lungs. Further evaluation, including functional assessment of reperfused lungs, will be needed to confirm these findings, but this technique may offer a rapid and inexpensive method for testing multiple preservation conditions.

References:

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