A Comparison of Kidney Preservation Methods by Oxidative Phosphorylation Studies

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A Comparison of Kidney Preservation Methods by Oxidative Phosphorylation Studies

Devprakash Samuel* and Stanley G. Dienst, MD*

An oxygen consumption assay using adenosine diphosphonucleotide (ADP) was performed on canine kidneys to measure the efficiency of oxidative phosphorylation. The purpose of the assay was to establish a respiratory control index (RCI) that could be used to evaluate kidneys preserved by different methods. Of the four methods tested, perfusion yielded the best results after 24-hour preservation. Additionally, four pairs of human cadaver kidneys were evaluated with the same assay. Some correlation existed between the RCI values of the nontransplanted kidney in each pair and the clinical viability of its transplanted mate.

OXIDATIVE phosphorylation is the process by which the energy released in the oxidation of organic foodstuffs is made available for cell metabolism, including protein synthesis. When cells are deprived of oxygen, molecular changes take place that finally result in the loss of oxidative phosphorylation capacity. From these general observations, the ability of renal cells to carry on oxidative phosphorylation has been suggested as a means of evaluating their viability after anoxia (and hypothermia).1-2

Methods currently used for determining the viability of kidneys harvested for transplantation include an assessment of the vascular resistance from measurements of pressure and flow on preservation machines, measurements of lactic acid accumulation determined from the perfusate, determination of the progressive release of intercellular enzymes, particularly lactic dehydrogenase, and measurements of tissue concentration of total adenine nucleotides.3-4 In the ischemically-damaged kidney the values indicated by these tests are abnormal. However, there is substantial overlap between nonviable kidneys and those which recover and have good function.

The aim of this study is to measure tissue oxygen consumption with an oximeter during the oxidative metabolism of succinate. By adding adenosine diphosphonucleotide (ADP) to this system and determining the active phosphorylating capacity of the tissue, it is possible to estimate the immediately recoverable energy production and potential function of the kidney. This work differs from previous studies1-2 in that this biochemical test is used to compare and evaluate several kidney preservation methods.

Materials and Methods

Preservation method

Kidneys were removed from anesthetized adult mongrel dogs and treated by one of the following procedures:

1. Warm Ischemia (N = 5 kidneys). After heparinization and clamping of the renal vasculature, an atraumatic nephrec-
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tomy was performed. The kidney was immediately placed in a thin sterile plastic enclosure, without flushing and left in a 37°C water bath.

2. Collins (N = 4 kidneys) or Ringer's lactate flush (N = 5 kidneys). The nephrectomy was performed as described for the warm ischemia. Immediately after nephrectomy, the kidney was flushed with Collins or Ringer's lactate (approximately 150 ml) at 4°C until the effluent was clear. The kidney was stored in a saline ice slush bath for varying periods up to 24 hours, before cortical tissue samples were excised for the oxygen consumption assay.

3. Perfusion (N = 8 kidneys). Bilateral nephrectomy was performed, and the kidneys were placed en bloc on a Waters MOX-100 Renal Perfusion Console. Cryoprecipitated dog plasma with standard additives was used as the perfusate. Periodic flow, pressure, pH, PCO₂, and PO₂ measurements were obtained over a 24-hour period. Cortical biopsies were taken before the kidneys were placed on perfusion, at 24 hours, and in some cases at 48 hours. Oxygen consumption was determined immediately after taking the biopsies.

Oxygen consumption assay

Oxygen consumption was measured polarographically with a Clark-type oxygen electrode and a Yellow Springs Instrument Biological Oxygen Monitor. To prepare a 10% tissue homogenate, kidney biopsies of 300 to 400 mgm were placed in ice cold 0.25 M Sucrose and 0.01 M Tris-HCl pH 7.4. Sixteen μ moles of sodium succinate was added to 3 ml of a solution containing potassium 115 mEq/L, sodium 10mEq/L, phosphate (HPO₄) 100 mEq/L, chloride 15 mEq/L, bicarbonate 10mEq/L, and magnesium 60 mEq/L. A cuvette containing 2.8 ml of this solution was placed in a water bath at 37°C. Tissue homogenate (0.2 ml) was added to the reaction media. Oxygen consumption was monitored and expressed as μ moles of O₂ per milligram of wet tissue weight per hour. After a steady rate of oxygen consumption was obtained, 0.4 μ moles of ADP was added. The tissue was then allowed to respire in the presence of ADP until oxygen consumption ceased. From these rates, the Respiratory Control Index (RCI) or ratio of the respiratory rate in the presence of ADP to that in the absence of ADP was computed (Figure 1). This index is an exact measurement of the phosphorylation capacity of the mitochondria.⁷

Four pairs of human cadaver kidneys were evaluated for their ability to phosphorylate ADP oxidatively while maintained on continuous perfusion. One kidney from each pair was used for transplantation, and its function after one month was correlated with the RCI values during preservation.

Results

The mean RCI values for the different preservation methods are shown in Figure 2. Warm ischemia produced a rapid drop in the phosphorylation capacity of the kidney homogenate, as reflected by the decreasing RCI values, which fell to 1.0, with no phosphorylation response after three hours. In contrast, the other preservation methods showed sustained phosphorylation capacity over a 24-hour period, with the following mean RCI values: perfused kidneys 1.66 ± .17, Collins flush 1.36 ± .11, and Ringer’s lactate flush 1.13 ± .09. These results suggest that perfusion is the best method of preserving a kidney over a 24-hour period.

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**Calculation of Oxygen Consumption**

\[
\text{RCI} = \frac{\text{Rate O}_2 \text{ Consumption} + \text{ADP}}{\text{Rate O}_2 \text{ Consumption} - \text{ADP}}
\]

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Phosphorylation capacity expressed in terms of mean RCI (Respiratory Control Index) values during various kidney preservation methods. N = the number of kidney biopsies assayed.

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**Fig. 1**

Calculation of oxygen consumption rates and Respiratory Control Index (RCI).

**Fig. 2**

Sustained phosphorylation in kidney preservation.
Kidney Preservation by Oxidative Phosphorylation

In addition, this oxygen consumption assay was performed on four pairs of marginally viable human kidneys evaluated for the Michigan Donor Program. One kidney from each pair was transplanted. The biopsies for the RCI were taken from the other kidney. As indicated on the table, the results show that some correlation may exist between the RCI values of the nontransplanted kidney and the clinical status of its transplanted mate.

The kidney with an RCI of 1.48 is still functioning well, while those with RCI values of 1.13-1.22 never established good renal function after transplantation.

Discussion

The respiratory control of kidney homogenates with and without normothermic ischemia has been previously studied by polarographic measurement of oxygen consumption. However, this early study failed to quantitate the data and correlate the findings with actual kidney transplants. Renewed interest in the energy metabolism of kidney tissue has led several investigators to use total adenine nucleotides (TAN), as postulated by Calman, to predict viability. The use of TAN, however, fails to yield a dynamic measurement of tissue metabolism, and the existence of a relationship between recoverable renal function and TAN levels has not been established.

The theoretical basis of applying the respiratory control index can be explained by the two-fold process of oxidative phosphorylation as it occurs at the cellular level in the inner mitochondrial membrane. Electrons originating from the oxidation of high-energy reduced substrates are ultimately transferred to a low-energy terminal acceptor such as molecular oxygen. This reaction provides sufficient chemical energy for the phosphorylation of ADP to yield adenosine triphosphonucleotide (ATP). This coupled relationship lends itself well to functional assessment for the quantitative measurement of RCI values. In the presence of oxidizable substrate (i.e., succinate), oxygen, ADP, and phosphate, respiration and phosphorylation rates are fast, and mitochondria are in the active state (State 3). In the controlled state (State 4), i.e., in the presence of substrate and oxygen but in the absence of excess ADP, there is minimal respiration in tightly coupled mitochondria. The RCI, or the ratio of the rates in the active and the controlled states is a measure of the degree of control imposed on oxidation by phosphorylation.

In oxidative phosphorylation hydrogen ions are conveyed by molecules of Flavin Adenine Dinucleotide (FAD) to the electron transport chain in the mitochondrial membrane (Figure 3). The transport of H⁺ ions across the inner membrane generates a H⁺ ion gradient, which results in an electric potential that forces H⁺ ions back through the inner membrane spheres and releases the energy for ATP synthesis. The ATP thus generated provides the chemical energy for many metabolic processes, such as the transport of ions and small molecules, and the molecular synthesis needed for maintenance of intracellular structure.

The application of oxidative phosphorylation measurement to the in vitro evaluation of preserved canine kidneys was successful even though the ultimate test of viability (i.e., allotransplantation) was not performed. Our results suggest that those kidneys with RCI values between 1.4-2.0 indicate

<table>
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<th>MEAN RCI VALUES ON FOUR PAIRS OF HUMAN KIDNEYS</th>
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tightly coupled mitochondria with controlled oxidation. Low RCI values below 1.4 can be correlated with a low level of mitochondrial coupling, with an RCI of 1.0 indicating no active phosphorylation (Figure 2). Our results show that at 24 hours several preservation methods will yield RCI values within the active range (1.4-2.0), and that perfusion has significantly higher RCI values than other methods. These findings coincide with other reports in the clinical transplantation literature, which suggest that perfusion of cadaver kidneys for 24 hours is a more effective method of preservation than ice storage. While the phosphorylation capacity of the perfused kidneys (RCI 1.66 ± .17) at 24 hours indicates that the tissue is metabolically active, this may not be the only factor contributing to the metabolic requirements of the preserved kidney. Other factors, such as the TAN levels of the tissue preserved and ionic concentration gradients, may play an equally important role.

These findings using oxidative phosphorylation to evaluate different preservation methods were extended to the clinical transplant setting, and the predictive ability of the RCI was analyzed in four pairs of marginally viable human kidneys (See Table). Although we are not able to present statistically significant evidence of the relationship between RCI values and the success of transplantation at this time, the results suggest that a correlation may exist between RCI and immediate posttransplant function. Other factors, such as the TAN levels of the tissue preserved and ionic concentration gradients, may play an equally important role.

Conclusions

1. Three nonperfusion preservation methods and preservation by perfusion have been evaluated in the canine model. When the Respiratory Control Index (RCI) was used to measure the phosphorylating capacity of kidney tissue homogenate, kidneys on perfusion yielded the best results after 24-hour preservation.

2. Four perfused human cadaver kidneys were shown to have RCI values which correlated with eventual clinical function. This finding suggests the possible value of this assay in determining viability and immediate posttransplant function of human cadaver kidneys.

References


