In Vivo Monitoring of Respiratory Chain Dysfunction following Renal Storage and Transplantation

MAUREEN S. THORNILEY, NICK J. LANE, SANJIV MANEK and COLIN J. GREEN

Section of Surgical Research, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex, HA1 3UJ, U.K.

Damage incurred during ischaemia and reperfusion (IR) is a major cause of dysfunction in transplanted organs. It has been shown in freshly grafted livers and kidneys that the capacity to resynthesise ATP, rather than the immediate post-ischaemic ATP level determines the return of function [1]. Return of function may therefore relate to preservation of inner mitochondrial membrane integrity during reperfusion of ischaemic tissue. In this study our objective was to correlate *in vivo* measurements of respiratory chain function with histological and functional parameters and explore the possibility that surface fluorimetry might be useful for predicting the likely viability of an organ before it is transplanted into patients.

Chance *et al* [2] reported on direct *in vivo* measurements of fluorescence from intracellular pyridine nucleotides in hypoxic brain and kidney. More recently, fluorescence measurements have been made of the reduction rate from NAD^+ to NADH, the energetically unfavourable reverse reaction, and therefore more critically dependent upon the integrity of the respiratory chain, in livers on an *ex vivo* circuit. NADH can also be correlated with other respiratory chain components, such as cyt aa_3, the terminal electron carrier in the respiratory chain (Fig.1). Cyt aa_3 can be measured using the non-invasive method of near infra-red spectroscopy (NIRS) [3]. In this study, measurements of NADH and cyt aa_3 have been made in unstored and stored transplanted rabbit kidneys. The effect of sodium pentobarbitone, thought to directly inhibit complex 1, on NADH levels was determined.

Female NZW rabbits (2.5 kg) were sedated with an i.m. injection of ketamine (50 mg/kg) and xylazine (8 mg/kg), trachaeotomized and artificially ventilated with a 50:50 oxygen:nitrous oxide mixture for periods of up to 8 hr. Anaesthesia was maintained by continuous i.v. infusion of ketamine:xylazine (50mg:8mg/kg/hr). Continuous measurements of pO_2, BP, core temperature, $EtCO_2$, FiO_2 and ECG were made. Intermittent blood gas samples were taken for pCO_2, pO_2, pH, and HcT determination. After 6 hr of reperfusion, rabbits were killed with an i.v. infusion of sodium pentobarbitone (200 mg/kg). In Group 1, freshly nephrectomized left kidneys were flushed with 30 ml hypertonic citrate solution (HCA) at 1-2°C, and autografted immediately into the right renal bursa using standard microsurgical techniques. In Group 2, kidneys were flushed with HCA as in Group 1 and then stored for 72 hr (0°-4°C) before autografting.

SF measurements were made using a Perkin Elmer LS 50 with a fibreoptic probe placed gently on the surface of the kidney. Emission spectra of NADH (400-600 nm) were obtained by excitation at 366 nm. Measurements (both discrete and continuous) were made of the intensity and rate of change of NADH emission in response to: (i) reperfusion after transplantation and (ii) infusion of sodium pentobarbitone in both groups.

NIRS measurements of cyt aa_3 were made using a NIRO monitor (Hamamatsu), for up to 6 hr, in which optrodes were placed on either side of the kidney.

Reperfusion of Group 1 kidneys resulted in oxidation of NADH by 70% to 100% in all kidneys (Table 1) and thereafter values oscillated within this range. Sodium pentobarbitone infusion resulted in rapid formation of NADH, to pre reperfusion levels within 1 min as expected for the inhibition of NADH dehydrogenase. In Group 2 kidneys the magnitude and rate of oxidation of NADH was considerably less upon reperfusion than in Group 1. However 20 min after perfusion 100% of the NADH had become oxidised. Sodium pentobarbitone infusion resulted in a decreased rate and magnitude of NAD^+ reduction within 20 min of monitoring, suggesting either a lesion possibly at the level of complex 1, or substrate deficiency.

Table 1. Correlation of Respiratory Chain Function and Morphology

Grp No	cyt aa3 (μM) Max change	% NADH oxidised Reperfusion	% NADH reduced Inhibitor	Severity of cortical oedema
1.1	-58.25±0.85		95	mild-moderate
1.2	-65.25±0.21			
1.3	-60.0±0.51	72	76	mild
1.4	-49.7±0.22	96	69	absent
1.5	-62.0±0.34	70	70	absent
1.6	-103.05±1.4	87	70	mild
1.7		72	70	
1.8		88	100	
1.9		95	80	
2.1	nsd	96	0	severe
2.2	-16.5±0.24	67	82	moderate
2.3	nsd	96	0	severe
2.4	nsd	84	8	severe
2.5	nsd	86	0	moderate
2.6		100	0	
2.7		100	37.5	

* Maximal change in the 10 min post perfusion (mean ±sem of 6 time points); preceded by a lag phase of up to 3 min in which there was no significant difference (nsd) from baseline variation (0.75±0.197).

Reperfusion of Group 1 kidneys also resulted in a reduction of cyt aa_3. It is established that cyt aa_3 is not maximally oxidised *in vivo* and values between 20 and 50% reduced have been reported [2,5]. The results presented agree with the view that cyt aa_3 could have become rapidly hyperoxidised, and we are observing the change reverting to baseline. The cyt aa_3 response correlated with good histological appearance, and the usual response to sodium pentobarbitone (ie NADH formation).

In Group 2 there was no significant change in the cyt aa_3 level compared to baseline values in 4/5 kidneys and this correlated with lack of NADH formation with sodium pentobarbitone and poor histological appearance. Interestingly there was no inflammatory infiltrate in any specimen, so it is unlikely that neutrophil activation was responsible for the observed damage.

We believe that non-invasive measurements of NADH and cyt aa_3 could provide a valuable indication of organ viability if measured on an *ex vivo* circuit prior to transplantation.

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