Non-invasive monitoring of renal haemoglobin oxygenation kinetics following hypothermic storage and transplantation

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Damage incurred during ischaemia and reperfusion (IR) is a major cause of dysfunction in transplanted organs. The limiting factor for reduced function during perfusion after transplantation is still of debate. It is established that reperfusion defects- the so called no reflow phenomenon- contribute to IR injury in kidneys. However, the dynamic relationship between reperfusion kinetics and tissue damage is still unclear. Current techniques to assess whole organ perfusion are invasive and depend upon injection of microspheres (necessitating tissue destruction) or measurements of radioisotope clearance.

Our objective was to determine whether renal IR damage following storage and tranplantation is related to the kinetics of reperfusion and haemoglobin oxygenation. We have correlated histological, functional and physiological parameters with haemodynamics and tissue oxidation as judged by cyt aa_3 the terminal enzyme of the respiratory chain (RC). The kinetics of change in total haemoglobin concentration are indicative of changes in perfusion following transplantation.

These measurements were made using near infra-red spectroscopy (NIRS) in transplanted rabbit kidneys. NIRS enables monitoring of concentration changes of HbO_2, Hb and cyt aa_3 [1]. The principle depends upon the fact that NIR light (700-1000 nm) penetrates tissue and allows absorption measurement of oxygen-dependent chromophores. Information is therefore obtained about the supply of oxygen to the tissue and intracellular oxygen utilisation [2].

We designed 2 experimental groups: in Group 1 kidneys were harvested but not stored before transplantation (95-100% viability post transplant); and in Group 2- 72 h stored kidneys in which 20% would be viable post transplant. Near maximal differences should therefore be apparent [3].

The surgical procedures were as described in the accompanying paper. In Group 1, freshly nephrectomized left kidneys were flushed with 30 ml 1-2°C HCA and autografted immediately into the right renal bursa using standard microsurgical techniques; Group 2 kidneys were flushed as in Group 1 and stored on ice (1°-2°C) for 72 h before autografting. NIRS measurements were made using a NIRO monitor (Hamamatsu). NIR optrodes were placed on either side of the kidney. NIRS parameters were continuously monitored pre-and post-reperfusion in both groups for up to 6 h.

Reperfusion of a flushed kidney resulted in a large increase in the haemoglobin concentration and oxygenation, the latter being pO_2 dependent. In a closed system, changes in oxy and deoxy haemoglobin should theoretically be equal and opposite. However if the total haemoglobin concentration changes then the two rates will not necessarily correspond and will be a function of the tissue (not systemic) pO_2.

We have found that the kinetics of Hb oxygenation were fundamentally and significantly different between the 2 Groups. In Group 1, the rate of increase of HbO_2 was greater than the rate of increase of Hb (4/6 kidneys), whilst in 2 kidneys the rate was equal. In Grp 2 the magnitude in Hb increase was always (6/6 kidneys) greater than the HbO_2 increase, consistent with greater extraction of oxygen in the more ischaemic tissue. This is a rapid phenomenon, not to be confused with the medullary perfusion defect (congestion), which develops at a slower rate.

Fig.1.

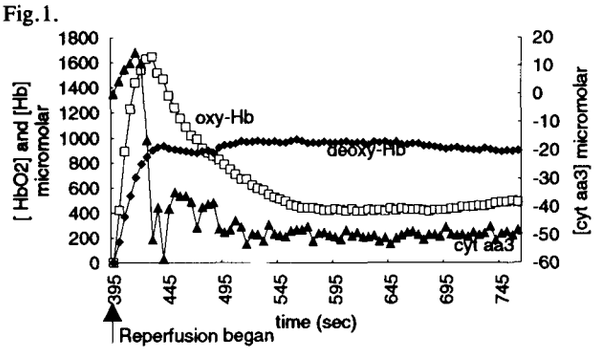

Fig.1 Plot showing change in concentration of HbO_2, Hb and cyt aa_3 with time of reperfusion after transplantation.

In Group 1, reactive hyperaemia-an overshoot of HbO_2,- followed by a reduction to normal levels accompanied by a reduction of cyt aa_3 was measured in all 6 kidneys. In Fig. 1 is shown a plot of change in concentration of HbO_2, Hb and cyt aa_3 following reperfusion of a Group 1 kidney. The initial stages of reperfusion resulted in a large increase in $[HbO_2]$ and in [Hb], a net increase in the oxygenation index $[[HbO_2]-[Hb]]$, and no measurable change in the redox state of cyt aa_3. However as the HbO_2 level fell (end of hyperaemic phase) cyt aa_3 became reduced. This was not observed in 4 out of 5 Group 2 kidneys.

In Group 1, there was a significant change in the redox state of cyt aa_3 (6/6 kidneys) on reperfusion. This correlated with minimal oedema, good organ function and good respiratory chain function (as described in the accompanying paper). In Group 2 there was no significant cyt aa_3 change compared to baseline which correlated with severe cortical oedema, inadequate respiratory chain function and poor long term viability.

In Group 1 kidneys hyperoxidation of cyt aa_3 correlates with hyperaemia but also with rapid oxidation of NADH, the enhancement of ATP synthesis which has also been reported during the first stages of reperfusion following brief ischaemia [4,5]. The single Group 2 kidney which exhibited similar cyt aa_3 response to Group 1 kidneys also showed good respiratory chain function and had only moderate oedema. There was a consistent correlation between the NADH and cyt aa_3 response and morphological appearance.

These preliminary studies indicate that NIRS can be used to measure differences in the rates and magnitudes of haemoglobin oxygenation between the two groups. The initial deoxygenation of haemoglobin which was observed in Group 2 is consistent with a rapid off loading of oxygen, a function of low tissue pO_2. Despite this, no significant cyt aa_3 change was observed. This indicates that respiratory chain damage preceded the slower onset of secondary ischaemia resulting from medullary congestion. Our results demonstrate that NIRS is able to provide information on the kinetics of reperfusion in the transplanted kidney.

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