

Organ reperfusion and preservation

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1. ABSTRACT

Organ transplantation is one of the medical success stories of the 20th century. Transplantation is, however, a victim of its own success with demand for organs far exceeding supply. The ischemia/reperfusion injury associated with organ transplantation is complex with interlinking cellular pathways and cascades. With increasing use of marginal organs and better understanding of the consequences of ischemia/reperfusion, enhanced organ preservation is required. Traditional static cold preservation cannot prevent ischemia/reperfusion injury, the low temperature itself is damaging and viability testing is limited. Donor preconditioning techniques to enhance organ preservation in advance of retrieval are starting to show convergence on several key pathways (HO-1 and cell apoptosis). Microdialysis and bioimpedance techniques may allow viability assessment during cold storage. Hypothermic machine perfusion has a role to play, particularly in preservation of kidneys from non-heart-beating donors although results of clinical trials are awaited. Normothermic preservation offers benefits over cold storage (at least experimentally) by avoiding damage induced by low temperature, minimising ischemia/reperfusion injury and allowing resuscitation of damaged organs. Normothermic preservation is likely to increase as the average quality of donor organs declines and clinical trials are needed. In the long term, normothermic preservation may be used, not just to resuscitate organs, but facilitate organ immunomodulation.

2. INTRODUCTION

Organ transplantation has become an accepted treatment for many end-stage organ failure states. Kidney transplantation is highly successful with 5 and 10 year survival far surpassing anything possible on dialysis (1). Second and third kidney transplants are not uncommon (2) and live-donor kidney transplantation programs are increasing (3). Liver transplantation is the accepted treatment for end-stage liver disease (4) and in addition both split liver transplantation and live-donor transplantation are developing rapidly (5). Both heart and lung transplantation programs are well established worldwide and although results for lung transplantation have yet to match that seen in kidney or liver good five and ten year survival figures can be obtained (6, 7). For the diabetic population whole pancreas transplantation is increasingly able to offer escape from insulin dependence and reversal or stabilisation of diabetic complications (8). For a highly selected group of patients with irreversible intestinal failure, small bowel transplantation offers increasingly good results (9).

Despite the undoubted success of organ transplantation many challenges have yet to be overcome. Transplantation by necessity requires a period of ischemia. With the need for organ transportation and organ matching many hours may elapse before an oxygen rich circulation can be restored (10). During this period of ischemia preservation of the organ is essential and has

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conventionally relied on refrigeration to reduce the cell metabolism, combined with instillation of a specialised preservation solution. The principles underlying cold preservation are the slowing of metabolism (by cooling) and the reduction of cell swelling by the inclusion of an impermeant in the specialised preservation solutions. Metabolic activity is not halted at ice temperature, it is reduced by 10-12 fold (11), with anaerobic cellular metabolism continuing. This leads to an accumulation of metabolic products including the breakdown products of ATP. The major component of organ damage occurs not during cold preservation, but at the time of reperfusion. There is a rapid metabolism of accumulated metabolic products leading to the production of toxic molecules, particularly reactive oxygen species. The degree of damage during cold preservation is therefore inevitably in proportion to preservation time.

In addition to damage caused by oxygen free radicals reperfusion also initiates an inflammatory response with the infiltration of the graft by leukocytes and concomitant leukocyte-mediated tissue injury (12). The release of inflammatory mediators, cytokines, extravasation of neutrophils and the recruitment of additional leukocytes results in a cascade causing both local and systemic inflammatory tissue injury. The exact cellular mechanisms underlying this process remain unclear despite extensive work by laboratories around the world.

In the early days of transplantation, where donors were selected with great care to ensure optimal graft function, these limitations of cold preservation could be tolerated. The situation now is different. The numbers waiting for transplantation far exceeds the availability of organs. Death on the waiting list is now an increasing likelihood and there is a pressing need to expand the donor pool and use all available organs. Organs with pre-preservation damage are at a greatly increased risk of primary non-function or delayed graft function, but in order to meet demand, extended criteria donors, once thought unsuitable for transplantation, are now actively considered. With such donors every effort must be made to avoid any further injury during transplantation. If organ preservation could be improved and ischemia/reperfusion injury lessened, the understandable reluctance to use marginal donor organs would be reduced, and many more transplants could be performed.

There is increasing recognition of the consequences of ischemia/reperfusion injury. For many years, the importance of preservation injury was under-recognised in kidney transplantation. While delayed graft function in heart, lung or liver transplantation is often fatal in kidney transplantation it was accepted as a consequence of pushing the boundaries of both donor selection and cold storage. Delayed graft function (DGF) was thought to have little or no long-term consequences but now an important association between DGF and poor outcome is recognised (13). In kidneys with DGF there is a higher rate of chronic allograft nephropathy and five-year graft survival is reduced. The longer-term effects of acute tubular necrosis (the underlying injury in DGF) are a cause for concern with

the increasing use of kidneys from non-heart-beating donors. These kidneys are removed after circulatory arrest and experience an inevitable period of warm ischemia before cold preservation. Thus with the increasing use of marginal organs from extended criteria donors and the need to avoid delayed graft function, organ reperfusion and preservation has, once again, become a hot topic.

A bewildering array of strategies exists to reduce ischemia/reperfusion injury and enhance organ preservation. Pharmacological manipulations of preservation solutions to reduce damage during cold ischemia and more importantly at the time of reperfusion have proved successful in the experimental setting but few have been introduced clinically (14). Reperfusion in the absence of leukocytes or with inhibition of the key players in the injury cascade has proved successful but again use in the clinical setting is limited (12). The use of hypothermia during reperfusion has proved successful in non-transplant ischemia/reperfusion injury (15-22) including cerebrovascular and myocardial models but has yet to be applied to organ transplantation. Ischemic preconditioning in which a brief non-lethal ischemic interval confers protection for a subsequent period of extended ischemia has proved successful in several organ systems (23-25). Organ preservation techniques have advanced from simple static storage to machine perfusion with encouraging results in the field of non-heart-beating donor kidney preservation obtained using hypothermic machine perfusion preservation (26).

Normothermic organ preservation, perhaps the most exciting development in the field of ischemia/reperfusion injury and organ preservation simultaneously addresses both problems and combines much of the above ideas (27). Normothermic organ preservation sees the ischemia/reperfusion injury occur in a controlled ex-vivo setting and by restoring a near physiological circulation extended organ preservation is possible. Importantly this technique opens a whole new avenue in organ preservation that is particularly relevant to the use of extended criteria donors. Organ resuscitation and viability testing using normothermic preservation is certainly possible and in the longer-term gene transfer and immunomodulation of the organ during warm preservation may revolutionise transplantation.

This review seeks to briefly identify the current status and limitations of clinical organ preservation using static cold storage. New strategies in limiting ischemia/reperfusion injury and enhancing organ preservation will be considered (Figure 1). Donor preconditioning by pharmacological, gene transfer and ischemic or heat shock preconditioning are potential interventions to reduce preservation-related injury before the organ has even been retrieved. Many additives to cold preservation solutions have been shown to be beneficial in protecting the organ at reperfusion and these will be briefly reviewed. The emerging techniques of microdialysis and bioimpedance analysis have been used to predict graft viability and may prove to be very important in static cold storage where until now it has not been possible to assess

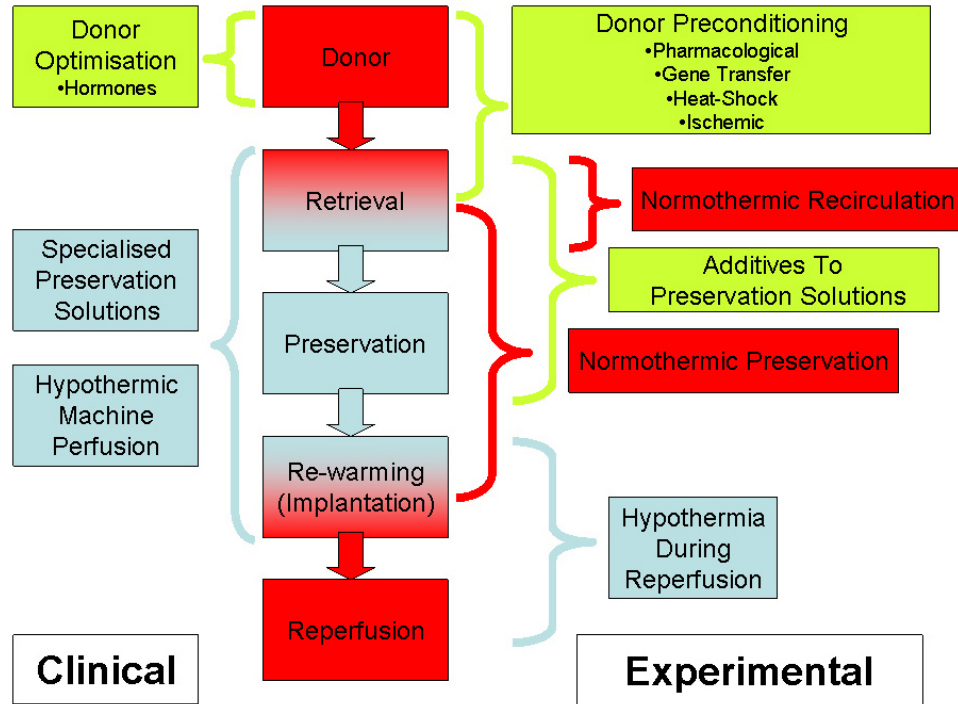


Figure 1. Timeline demonstrating phases of organ damage and strategies to reduce ischemia/reperfusion injury and enhance viability.

function. Hypothermic machine preservation will be discussed and then normothermic recirculation and normothermic preservation techniques will be reviewed.

3. ISCHEMIA/REPERFUSION INJURY

Organ damage can occur during retrieval, preservation, re-warming (during implantation) and at the point of reperfusion. By flushing the organ with a hypothermic preservation solution and then storing the organ at near ice temperatures it is hoped that metabolism will be sufficiently reduced to avoid exhaustion of energy supplies during the storage interval, aiding recovery of function on reperfusion.

During cold storage metabolism continues in the form of anaerobic metabolism resulting in an accumulation of lactate and the breakdown products of adenosine nucleotides including hypoxanthine. The increased lactate concentration lowers cellular pH and confers protection from cell death. On reperfusion microvascular dysfunction is one of the key initiators of ischemia/reperfusion injury (28). Endothelial cells are activated and produce less nitric oxide and more oxygen free radicals. Mitochondrial injury appears to be one of the key components of ischemia/reperfusion injury (29). During ischemia the mitochondrial anti-oxidant mechanism is impaired and intra-mitochondrial calcium concentration is increased. With the return of oxygen comes an over-production of superoxide by the mitochondria swamping the already decreased anti-oxidant system. This increase in reactive oxygen species results in protein, lipid and DNA damage

and ultimately activates pathways to cell death from either apoptosis or necrosis. This process of cell death is further enhanced by a correction of cellular pH (30). Mitochondrial permeabilisation in response to ischemia/reperfusion releases the stored calcium activating proteases, nucleases and phospholipases (31) and this together with the release of cytochrome c triggers cell death. The exact mechanism of this cell death appears to be dependent on the residual levels of ATP in the cell with complete depletion of ATP leading to necrosis in contrast to apoptosis when some ATP remains (32). Inflammatory mediators are released in response to the imbalance between superoxide and nitric oxide. Platelet activating factor, tumour necrosis factor and a host of interleukins are released. Upregulation of cellular adhesion molecules for leukocytes such as intercellular adhesion molecule-1 and P-selectin occurs, and this, together with mechanical and vasoactive changes in the microvasculature results in trapping, adherence, and extravasation of activated leukocytes (28, 33). This leukocyte extravasation leads to yet further tissue damage from the release of reactive oxygen species, proteases and inflammatory mediators.

4. STATIC COLD PRESERVATION – CURRENTS STATUS AND LIMITATIONS

Organ preservation by cold storage relies upon the use of specialised preservation solutions which contain agents designed to combat ischemia/reperfusion injury by targeting the injury pathways. Clinical organ preservation has advanced little since the breakthrough introduction by Belzer and Southard in the late 1980's (34) of University of

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Wisconsin solution (UW) for static cold storage. UW contains 13 components thought to enhance organ preservation including the colloid hydroxyethyl starch to prevent cell oedema, adenosine to supplement ATP recovery on reperfusion and allopurinol to act as inhibitor of xanthine oxidase. Despite evidence to suggest that some additives in UW may not be required (35) and indeed do more harm than good (36) it remains the gold standard for multi-organ retrieval (37). UW static cold preservation is a very effective technique allowing prolonged cold storage of organs. For the kidney 24 hours and the liver 18 hours of cold preservation would be considered acceptable by most units.

UW solution has been joined by a host of alternate organ preservation solutions each modified to suit a particular niche market and each thought to confer advantages over UW. Histidine-tryptophan-ketoglutarate solution is a low viscosity solution initially developed for cardiac transplantation that has now proved a cheaper and acceptable alternative to UW in multi-organ preservation and perfusion (10, 38, 39). Celsior, an extracellular-type, low-potassium, low-viscosity preservation solution that was developed to act not only as a storage medium, but also as the perfusion fluid during initial donor heart arrest (40), has now been shown to be effective in kidney (41), pancreas (42), and liver preservation (43, 44). Polysol, a new modified derivative of UW with a high sodium/low potassium composition, polyethylene glycol instead of hydroxyethyl starch and over 60 additives has recently proved effective in both machine perfusion of non-heart-beating donor livers (45) and cold storage of steatotic livers (46). A similar, but less complicated solution, again utilising a high sodium/low potassium composition and polyethylene glycol, Institut Georges Lopez Solution (IGL-1), has recently proved effective for renal preservation in both the experimental (47) and clinical setting (48). In addition IGL-1 has shown promising results in a very small trial in clinical pancreas transplantation (49) and in experimental liver transplantation of both healthy (50) and steatotic livers (51). For lung transplantation Perfadex, a low potassium, dextran/glucose solution, has been used for cold storage of lungs (52) and PBS-140, a simple phosphate buffered sucrose solution, has proved effective in renal preservation both during cold and warm ischemia (53-55).

Despite the renewed interest in cold preservation solutions there is increasing evidence that simple cold storage has three fundamental limitations. First is the damage caused by the cold itself, second is the difficulty in assessing function and predicting viability during cold storage and third is that ischemia/reperfusion injury is inevitable. Currently the success of any strategy to improve organ preservation solutions can only be assessed after the organ has been transplanted and with marginal organs one simply has to hope that it has not been too severely damaged during storage.

There is good evidence that cold and warm ischemia result in a different pattern of injury and that their effects are synergistic (56). In the liver cold ischemia affects the sinusoidal endothelial cells while warm

ischemia mainly damages the hepatocytes (11, 57). The injury to the sinusoidal endothelial cell is the key injury in early graft failure (58, 59) and indeed the cold ischemic insult has a later influence on graft survival (60). Cold is a direct trigger to hepatocyte and sinusoidal cell apoptosis (61). For all organs hypothermia inactivates the Na^+/K^+ ATPase pump maintaining cell electrolyte concentrations hence cell oedema occurs during cold storage and is only partially overcome by impermeants in specialised preservation solutions (62). Using the cardiac glycoside, ouabain, which specifically inhibits the ATPase system, Martin *et al* were able to determine the presence or absence of cation transport and the relationship between temperature and ATPase activity in porcine liver and kidney slices (62). In liver slices ATPase activity was noted at 20°C but not at lower temperatures unlike kidney slices where activity persisted at 10°C. At 4°C there was no ATPase activity in either tissue. In kidney proximal tubular cells hypothermia alone has been shown to cause a marked injury (63) which appears to be aggravated by commonly used preservation solutions (64).

5. ORGAN PRESERVATION – FUTURE DIRECTIONS

5.1. Donor preconditioning

Donor preconditioning is a strategy to protect organ prior to retrieval for transplantation. Essentially preconditioning can take the form of either a pharmacological intervention, gene transfer or a physical intervention such as ischemic preconditioning or heat-shock preconditioning. These techniques are beginning to move from the experimental arena to clinical trials.

5.1.1. Pharmacological interventions

The optimum management of heart-beating organ donors already sees the administration of a variety of drugs aimed at maintaining a stable cardiovascular function in the donor for transplantation. With brain death comes a “catecholamine storm” with a rapid rise in circulating norepinephrine, epinephrine and dopamine followed by a fall in levels to below baseline accompanied by a falling cortisol, free triiodothyronine, insulin and antidiuretic hormone (65). Such hormone imbalance leads to haemodynamically unstable donors thus a degree of generalised “preconditioning” is already seen in clinical practice with the use of hormone replacement therapy in the form of triiodothyronine, vasopressin, and methylprednisolone (66). The mean number of organs transplanted from donors treated with this three drug regime was found to be 22.5% higher than if no hormone correction was attempted with the most striking difference seen in the age >40 donor population. However hormone replacement merely corrects the endocrine imbalance caused by cardiac death whereas “true” preconditioning agents are specifically aimed at improving the condition of the target organ and decreasing susceptibility to ischemia/reperfusion injury.

The drugs tested to date are widely varied and have had differing degrees of success. Their selection has often been the result of positive effects in reducing

ischemia/reperfusion injury or organ damage in other contexts. In liver transplantation promising results have been obtained in both animal and clinical trials involving administration of the hydrophilic bile salts ursodeoxycholic or tauroursodeoxycholic acid (67, 68). Donor pre-treatment either by enteral administration three hours in advance of retrieval or more conveniently by intra-portal infusion during retrieval resulted in histological evidence of ischemia/reperfusion injury. Hydrophilic bile salts are thought to be potent inhibitors of apoptotic pathways and helpfully have a very well established safety profile having been used for many years as an oral treatment to treat cholelithiasis. Donor pre-treatment with an adenosine deaminase inhibitor has provided successful in reducing the number of rolling leukocytes in a rat perfusion model and significantly improving survival in a transplant model (8/10 vs. 1/13 after 44 hours of cold preservation) (69). During liver ischemia vasoactive adenosine is deaminated leading to less vasorelaxation and poor perfusion. In China the traditional herbal remedy, Panax notoginseng saponins (PNS) has been shown to have anti-apoptotic properties and be protective for rat liver grafts (70). This protection appears to be the result of upregulation of anti-apoptotic Bcl-2 and decreased caspase 3 and tissue necrosis factor alpha. N-acetyl cysteine, a precursor of the antioxidant glutathione (and an anti-oxidant itself), commonly used to treat acetaminophen induced liver injury, was recently trialled as a preconditioning drug in liver transplantation. Despite evidence that N-acetyl cysteine can reduce hepatic ischemia/reperfusion injury administration to the donor during retrieval failed to confer any protective benefit to the graft. In the setting of renal and non-heart-beating lung transplantation N-acetyl cysteine donor preconditioning appears to be more effective (71, 72). A decrease in allantoin and trimethylamine-N-oxide which are sensitive markers of renal ischemia/reperfusion injury was noted (although creatinine was not significantly different). In the lung, early injury, as assessed on a reperfusion circuit, was significantly attenuated when nebulised N-acetyl cysteine was used either, 10 minutes prior, or 15 minutes after cardiac death. Indeed donor preconditioning for lung transplantation is being actively explored by several groups and appears to be possible with other agents – nebulised prostacyclin analogue iloprost (73) or intravenous glycine (74).

Heme oxygenase-1 (HO-1) is one of the key players in tissue response to stress and by breaking down the pro-oxidant heme to biliverdin, carbon monoxide and iron it has a significant cytoprotective effect (75). The products of HO-1 themselves have an anti-oxidant, anti-apoptotic effect through complex pathways and administration of these agents in low concentrations can significantly reduce ischemia/reperfusion injury. Likewise an upregulation of HO-1 is also beneficial and is likely to be one of the key mechanisms mediating the effect of heat shock preconditioning (see later). HO-1 induction is also successful when gene transfer is used (see later). The use of HO-1 downstream products, carbon dioxide and biliverdin, in the protection of organs for transplantation has been extensively reviewed recently and will not be discussed further (76, 77).

5.1.2. Gene transfer preconditioning

Gene transfer preconditioning is more complicated than simple pharmacological preconditioning, usually requiring administration of the agent 24 to 48 hours in advance of retrieval. This raises significant logistical and ethical problems, certainly with cadaveric donors (78). Again many of the chosen treatments have already proved successful in ameliorating ischemia/reperfusion injury in other contexts or pre-transplant models and most centre on reducing cell death by apoptotic pathways.

Working in the field of lung transplantation the Patterson group based at Washington University Medical School have published a series of papers charting their use of adenovirus transfection of rats with genes conferring protection from ischemia/reperfusion injury after transplantation (79-84). Using either intravenous or endobronchial administration 24 hours prior to retrieval, grafts have been protected and survived 18 hours of cold storage. Successful transfection experiments include the genes for interleukin-10, tumour necrosis factor inhibitor, endothelial nitric oxide synthase, interleukin-1 receptor, heat shock protein-70 and nuclear factor kappa beta suppressor gene.

In the liver adenovirus transfection with the anti-apoptotic gene Bcl-2 has been successful in reducing ischemia/reperfusion injury (85). 48 hours after transfection, mice livers were successfully transplanted with a lower enzyme rise and better histology. This gene encodes an integral outer mitochondrial membrane protein that blocks the apoptotic death of some cells such as lymphocytes. It is capable of blocking cytochrome c release and caspase activation (86). In an obese Zucker rat model Amersi *et al* successfully transfected the HO-1 gene intravenously 24 hours prior to liver retrieval (87). Rats transplanted after 4 hours of cold storage were followed for 100 days. Over expression of HO-1 increased survival from 40% to 80% and improved liver function with decreased T-cell infiltration. Similar encouraging results were obtained in a renal transplant model in which HO-1 transfection occurred at the time of retrieval and involved a prolonged cold storage interval of 24 hours (88). Again HO-1 expression was protective. Caution in the use of exogenous HO-1 induction protocols comes from a recent study in human liver transplantation in which donor organs that had a high endogenous HO-1 level prior to transplantation suffered more reperfusion injury and had a worse outcome than those with low initial levels (89). Whether this was because the organs with initial high levels could not mount a further increase in HO-1 is not clear. The authors conclude that HO-1 may be either cytoprotective or cytotoxic depending on the level and strategies to modulate HO-1 should be targeted at increasing expression in the post transplant phase.

5.1.3. Ischemic preconditioning

Ischemic preconditioning sees the application of a short period of sub-lethal ischemia conferring protection from a subsequent more prolonged ischemic insult. The brief ischemic episode triggers an adaptive stress response

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making tissue more resistant to further damage. Initially described in myocardial tissue (90) this process has been identified and utilised in many other tissues (91). The underlying cellular mechanism behind ischemia preconditioning is complicated involving the release of vasoactive compounds, activation of intracellular cascades and activation of cytoprotective mechanisms. Ischemic preconditioning in the setting of transplantation sees a brief period of organ ischemia induced by clamping of the vasculature followed by reperfusion and organ retrieval.

Ischemic preconditioning has been extensively investigated in liver transplantation models. A recent large animal study yielded disappointing results (23). Canine livers exposed to 10 minutes of surgical ischemic preconditioning prior to 48 hours of cold storage failed to show any difference from controls when transplanted. Similarly disappointing results were found by Koneru *et al* in one of the first clinical trials with no evidence of benefit following 5 minutes of hepatic ischemic preconditioning (92). This failure may however be related to the short conditioning ischemic episode. Recently a more positive result has been obtained using a 10 minute episode with decreased platelet deposition, decreased neutrophil infiltration, better AST and lactate levels and less acute rejection seen in preconditioned livers (93). In kidneys ischemic preconditioning has proved successful in animal models (24, 94). 15 minutes of ischemia followed by 10 minutes reperfusion protected grafts stored for 42 hours and then transplanted (24).

5.1.4. Heat-shock preconditioning

Heat-shock preconditioning involves the application of a brief sublethal period of elevated organ temperature in order to induce a protective response prior to a prolonged ischemic insult and has been successfully used in a transplant model (95). In a rat model Matsumoto *et al* raised the whole animal body temperature to 43°C for 15 minutes. 48 hours later the liver was retrieved and after 8 hours of standard cold storage function was assessed using a normothermic reperfusion circuit or transplantation. The grafts treated by heat-shock preconditioning demonstrated increased levels of heat-shock protein-70 in both the hepatocytes and sinusoidal endothelial cells and had less evidence of apoptosis. The enzyme rise on reperfusion was less and in the transplant model 60% of the heat-shock protected grafts survived in comparison to none of the untreated. Using a similar model (20mins at 42°C, 24 hours in advance) grafts can be protected during prolonged cold storage (44 hours) with improved bile production, decreased enzyme rise and better histology on transplantation (96). Significantly this effect of heat-shock preconditioning appears mediated by the HO-1 pathway with the beneficial effects of heat-shock preconditioning inhibited by cobalt and tin protoporphyrins which are potent inhibitors of HO-1. Heat-shock preconditioning has yet to be used in clinical transplantation.

5.1.5. Limitations of donor preconditioning

Many of the preconditioning techniques discussed above require administration of agents to the donor well in advance of retrieval which is not usually

feasible. There are ethical implications associated with preconditioning particularly in the administration of treatment before certification of death (78) and current legislation would prevent the use of preconditioning in many countries. Many preconditioning strategies have a systemic effect and all organs will receive the preconditioning treatment. Given that multi-organ retrieval is desirable and frequent, it will be important to demonstrate that any preconditioning effect is not only beneficial to the original targeted organ but also not detrimental to any other organs.

5.2. Viability testing during cold storage

In an effort to address the problem of predicting viability during storage microdialysis has been used to assess the function of livers during cold static preservation in both animal and human liver transplantation (97, 98). Microdialysis enables monitoring of chemical events taking place in the interstitial fluid and involves the insertion of a thin microdialysis catheter into the tissue connected to pump perfusing the interior of the catheter with a physiological fluid. The semi-permeable membrane at the distal end of the catheter functions like a dialysis membrane with diffusion of interstitial fluid across the membrane into the perfusion fluid, which is continuously collected in microvials. Real time analysis is possible giving a snapshot of the tissue state. The advantage of microdialysis is the possibility to detect early signs of tissue ischemia. When the supply of glucose and oxygen is inadequate, it is followed by an instant increase in the ratio of lactate to pyruvate which is a well-known marker for tissue ischemia.

Using a porcine model in which livers were subjected to 30 or 60 minutes of warm ischemia then preserved cold for 6 hours prior to transplantation Nagayama *et al* demonstrated that microdialysis hypoxanthine levels during cold preservation could predict graft survival (97). Animals were followed for 7 days and hypoxanthine levels correlated well with graft ATP levels and ALT rise at reperfusion as well as animal survival. The microdialysis technique has recently been reported in human transplantation (98-100) and importantly was able to predict graft function. In a series of 15 liver transplants, 6 had impaired graft function (AST>2000 IU/L). This group had a significantly higher interstitial lactate level in the donor and during backtable preparation. Once reperfused it took longer (18 v 8 hours) for the lactate levels to recover. This is a very promising observation that could have major implications for predicting graft function in cold preserved livers and offers the possibility of viability testing marginal donor organs.

Electrical bioimpedance (electrical impedance of biological samples) allows monitoring of the physiological and morphological condition of living tissues and has been suggested as an indicator of anoxic cellular oedema and inter-cell uncoupling in cold preserved organs (101). Recently this technique has been applied to organs for transplantation undergoing cold storage to establish if bioimpedance can be used as a viability marker. Rat kidneys subjected to 45 minutes of warm ischemia were

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compared to heart-beating donor kidneys during 24 hour preservation in UW. Formal transplantation was not attempted but function tested using an ex-vivo reperfusion circuit. Tissue ATP level, histology (not significant) and reperfusion function were different between the groups. However ATP and histology requires invasive biopsies whereas bioimpedence is non-invasive. One of the bioimpedence parameters was consistently different between the groups and increased with preservation time. This could signal a potential role for bioimpedence analysis during preservation in predicting graft function.

5.3. Additives to cold storage solutions

Given the multifactorial problem of ischemia/reperfusion injury there are many potential targets for pharmacological intervention by the addition of drugs to the cold preservation solution. With so many pathways involved it is understandable that no one drug has proved completely successful but many can reduce ischemia/reperfusion injury. UW, and the commercially available alternatives to UW, discussed above incorporate several of the best performing additives – Polysol has over 60 (45).

Pharmacological manipulation of preservation solutions in the field of liver transplantation has been extensively reviewed (14, 102, 103). Unfortunately few drugs have been tested by formal transplantation studies. Of those that have, four of the most promising are a cocktail of tissue growth factors (104, 105), the p38 mitogen-activated protein kinase inhibitor FR167653 (106, 107), the colloid polyethylene glycol (108, 109) and trimetazidine which reduces ATP depletion and intracellular calcium accumulation (110).

Addition of a cocktail of substances to UW including insulin-like growth factor, nerve growth factor, bactenecin and substance P gave significant improvements in post transplant function of canine kidneys despite an extremely prolonged (six day) preservation period (105). This group then demonstrated similar survival benefits in porcine liver transplantation using the same cocktail with higher post transplant ATP levels and less haemorrhagic necrosis (104). Using a non-heart-beating donor heart transplantation model p38 mitogen-activated protein kinase inhibitor FR167653 proved beneficial when added to Celsior (106) and in liver preservation after prolonged (30 hour) cold storage using UW (107). In the liver transplant model 10 day survival, ALT rise and histology were all improved by the inhibitor suggesting that p38 mitogen-activated protein kinase activation is indeed an important part in the ischemia/reperfusion injury cascade. Polyethylene glycol (PEG), a high molecular weight compound that can reduce cell swelling by exerting a positive osmotic effect and reduce the immune mediated response post reperfusion, has been used as an additive to a simple preservation solution (109). PEG inhibits the early inflammatory response due to ischemia/reperfusion, improves renal function, and may prevent the progression of interstitial fibrosis in the long-term autotransplanted pig kidney. Trimetazidine, an anti-ischemic cytoprotective agent, added to standard UW improved function and

protected the vulnerable renal medulla in porcine kidneys subjected to 48 hours of cold storage (110). Recently this group has combined PEG and trimetazidine and demonstrated additive protection of the renal medulla (111) and in a porcine transplant model with 16 week follow-up improved graft function and less interstitial fibrosis (112).

5.4. Hypothermic machine perfusion

Hypothermic machine perfusion of organs for transplantation is not a new technique (113). Kidneys are connected to an ex-vivo perfusion circuit pumping a specialist preservation solution and maintained for extended periods until transplantation. Renal perfusion flow and pressure together with analysis of perfusate parameters have been investigated as potential viability markers. The ability to predict graft function offers a real advantage over traditional cold storage. As early as 1967 Belzer and his colleagues were successfully preserving canine kidneys for up to 3 days using pulsatile perfusion with oxygenated plasma at 8-12°C (114). Kidney transplant units adopted machine perfusion during the 1980's. However the benefit of hypothermic machine perfusion was hotly debated in the literature with most studies showing no major differences in short term survival but several suggesting that early function was better in the machine perfused group. These early studies involved good quality organs and tended not to involve any oxygen delivery system. As the demand for organs has increased and the use of non-heart-beating donors has become increasingly common, machine perfusion of kidneys has become more prevalent. In a large non-randomised study from the US, where machine perfusion is most widely used, 985 renal transplants were reviewed and pulsatile hypothermic machine preservation was found to be associated with improved early graft function and reduced effects of warm ischemia (115). Recently Wight *et al* performed a meta-analysis looking at the cost effectiveness of machine perfusion of kidneys (116). Their review suggests that taking all kidneys into consideration machine perfusion has a relative risk of delayed graft function of 0.804 compared to standard cold storage. In the longer term it would appear that machine perfusion is better for function and more cost efficient. With regard to markers of viability the meta-analysis suggested that flow may be an indicator but that studies are underpowered for a definitive answer. The perfusate marker alpha-S-transferase may be a better test with 93% sensitivity and 33% specificity. In an effort to answer the question of which preservation method is best for non-heart-beating donor kidneys an open randomised controlled multi-centre trial (PPART) began recruiting in August 2006 and the results are eagerly awaited.

In liver transplantation hypothermic machine perfusion remains an experimental technique and differs from kidney preservation in that most circuits involve oxygenation of the liver perfusate. In 2003 Lee *et al* demonstrated survival transplantation of rat non-heart-beating donor livers (30 minutes warm ischemic time) preserved using 5 hours of oxygenated machine perfusion in comparison to simple cold storage (117). Hypothermic machine perfusion is capable of “recharging” a liver after

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standard cold storage in a rat model with 3 hours of perfusion rescuing function when tested by ex-vivo reperfusion (118). In a larger porcine model using 24 hour preservation period machine perfusion proved better than cold storage at maintaining viability and function when tested using an ex-vivo reperfusion system (119). This result has yet to be translated into formal transplantation.

5.5 Normothermic preservation

In contrast to cold storage the underlying principle of normothermic preservation is to provide a normal physiological environment for the organ (27). By perfusing the organ with normothermic oxygen rich medium ischemia is prevented and this there is no ischemia/reperfusion injury to impair graft function. In an ideal world with perfect normothermic preservation complete replication of the physiological environment would allow unlimited preservation duration. Normothermic preservation of any organ for transplantation is more technically challenging than cold storage, requiring specialist skills and complex apparatus. Normothermic preservation has, however, seen a resurgence of interest in the last 10 years as donor organs continue to decline in quality. Avoiding ischemia/reperfusion injury in such marginal organs from extended criteria donors is highly desirable. In addition normothermic preservation offers the opportunity to assess function in the ex-vivo setting. Non-functioning or poorly functioning organs can be identified and discarded removing the risk to the donor. In this sense normothermic preservation overcomes the three weaknesses inherent in traditional static cold storage by avoiding ischemia/reperfusion injury, avoiding cold injury and allowing viability assessment. Extended preservation using normothermic preservation is feasible raising the possibility of semi-elective transplantation and more time to prepare the recipient.

Normothermic preservation has been used in the experimental preservation of kidneys, heart, lung and liver but has yet to break through into clinical use. Results from heart and lung preservation are encouraging and require further development. In the field of kidney transplantation, although preservation related primary graft dysfunction may seem to be less important, in fact the benefits of normothermic preservation may be just as great given the increasingly recognised long-term consequences of delayed graft function.

Normothermic preservation is, however, much more than a good preservation technique for the true power of this technique is not simply to preserve organs but to recover function. Hence normothermic preservation should perhaps be re-branded as normothermic resuscitation. By restoring function in marginal donor organs the donor pool could be greatly expanded.

The resuscitation of non-heart-beating donor kidneys using warm perfusion allowed successful transplantation despite a prolonged cold storage interval (120). Canine kidneys were subjected to 30 minutes of warm ischemia and 24 hours of cold storage and then were either directly autotransplanted or first warm-perfused for 3

hours before autotransplantation. The perfusion solution, termed "Exsanguinous Metabolic Support", is a complex proprietary solution based on cell culture medium but containing over 60 ingredients including pyridoxilated bovine haemoglobin as an oxygen carrier. A higher rate of immediate function, lower 24 hour post transplant creatinine levels and a lower peak creatinine in the warm perfused group translated into enhanced survival of the recipients (90% versus 73% at 14 days). It is possible that the inflammatory neutrophil-mediated cascade that accompanies ischemia/reperfusion is avoided and the presence of free radical scavenging compounds in the perfusate could potentially ameliorate the reperfusion oxidative insult. In 2002 this group demonstrated the superiority of their normothermic preservation technique compared to hypothermic machine perfusion in kidneys exposed to prolonged warm ischemia (121). In contrast to the hypothermic preserved kidneys or kidneys transplanted immediately following a warm ischemic insult, warm preserved kidneys were life sustaining with normalisation of creatinine post-transplant on day 9.

Normothermic preservation of kidneys has also been achieved using a blood-based perfusion system by the Nicolson group in Leicester, England (122). In keeping with the importance of leukocytes in mediating graft injury following ischemia/reperfusion this group has recently demonstrated improved renal haemodynamics and function during normothermic preservation using leukocyte-depleted blood (123). Just as in hypothermic machine perfusion there is an important association between perfusion pressure and the quality of preservation and graft function (124).

Normothermic preservation has been extensively investigated in liver transplantation where it has been used in two forms – normothermic recirculation and normothermic preservation. Normothermic recirculation involves the arterial and venous cannulation of the donor and the reinstitution of an effective circulation by means of a cardiopulmonary bypass circuit prior to organ retrieval. This technique has been pioneered by the Barcelona group of Valero and Garcia-Valdecasas (125). In a porcine model normothermic recirculation was used for 30 minutes to improve the energy status of a liver damaged by 20 minutes of warm ischemia. The circuit was then used to progressively cool the whole animal over 20 minutes to 15°C. Organ retrieval and transplantation were then performed with survival assessed at 5 days. Normothermic recirculation was found to reduce the ischemia-reperfusion injury and viability predictions could be made from analysis of hepatic blood flow and oxygen extraction ratios (126). Normothermic recirculation was then used to treat livers subjected to a maximum of 40 minutes of warm ischemia. After 6 hours cold storage the livers were transplanted and survival was 100% when 20 minutes of warm ischemia was used, falling to 70% at 30 minutes and only 50% at 40 minutes. In comparison to a control group (subjected to 20 minutes of warm ischemia and immediate total body cooling) normothermic recirculation clearly offered a survival benefit but all the grafts were complicated by ischemic necrosis of the biliary tree.

Organ reperfusion and preservation

Not unlike the situation in cold preservation drugs have been added to the perfusate to reduce ischemia/reperfusion injury - L-arginine, a substrate of nitric oxide synthase (127) and S-adenosyl-L-methionine (128). Recently the Barcelona group have studied the effect of adenosine (129). During normothermic recirculation hepatic adenosine levels rose and xanthine levels fell. Adenosine appears to exert protective effects via adenosine A2 receptors and isolated adenosine A1 receptor activation proves detrimental.

In contrast to normothermic recirculation, normothermic preservation of the liver involves standard organ retrieval and then ex-vivo perfusion using a specialised circuit. With a high pressure arterial and low pressure portal supply, normothermic perfusion of the liver is more complicated than kidney perfusion. In the early 1990's several groups investigated the use of normothermic perfusion in the resuscitation of livers for transplantation (130, 131). In 2001 Schon *et al* published a landmark paper in which formal transplantation studies demonstrated that, for the heart-beating donor normothermic preservation was as effective as cold storage and more importantly for the non-heart-beating donor, normothermic preservation offered a substantial survival benefit (132). In a porcine model heart-beating and non-heart-beating donor were transplanted directly, after 4 hours cold storage or after 4 hours of normothermic preservation. In the heart-beating series there was no significant difference between the groups but in the non-heart-beating series the difference was dramatic. Of six animals, 5 survived if transplanted directly, none if cold storage was used and all six survived when 4 hours of normothermic preservation was employed. Survival after 1 hour of warm ischemia is a result that has yet to be bettered.

The normothermic preservation circuit designed by Schon *et al* was complex, requiring a dialysis system. In Oxford the Friend group developed their own normothermic perfusion circuit which utilised the inherent ability of a healthy liver to regulate its own acid-base status. This circuit, assembled from standard cardiopulmonary bypass components, consisted of a centrifugal pump, a membrane oxygenator and a heat exchanger. Livers were provided with nutrients, bile salts and prostacyclin. The circuit allowed perfusion of the liver via the hepatic artery and portal vein at physiological pressures and flows. From 2001 the group published a series of papers documenting their progress in maintaining heart-beating donor livers in a viable condition ex-vivo, demonstrating improved preservation over 24 hours in comparison to cold storage and achieving successful extracorporeal porcine liver preservation for 72 hours. (133-135)

Next this group addressed the role of normothermic preservation for livers from non-heart-beating donors (136). Livers exposed to 1 hour of warm ischemia were either stored cold or resuscitated with 24 hours of normothermic preservation. After a rewarming ischemic period of 45 minutes (to mimic implantation) liver

function was tested on a fresh ex-vivo circuit. The warm preserved livers functioned well with continuing synthesis and substrate utilisation and displayed histological features of viability. 24 hour cold preservation resulted in livers with little evidence of metabolic or synthetic function. In the setting of non-heart-beating donor livers the addition of a period of cold preservation prior to normothermic preservation is extremely detrimental (137, 138) indicating that for normothermic preservation to become a realistic option in clinical practice, the circuit will need to be portable in order to remove the need for a period of preliminary cold storage. Alternatively the energy state of the liver will have to be regenerated prior to retrieval in order to maximise the chance of the liver surviving during cold ischemic transport to the recipient centre. This suggests a role for normothermic recirculation.

The most exciting work in normothermic preservation of organs is the possibility of "organ culturing" and in particular inducing repair of ischemic tissue ex-vivo (139). If normothermic preservation can restore metabolic function it should be possible for de-novo protein synthesis and tissue repair to occur during preservation. After 2 hours of warm ischemia kidneys were perfused for 24 hours in the presence or absence of fibroblast growth factors. These factors are known to enhance kidney differentiation and have a role in recovery of function after renal injury. During perfusion gene transfection of the kidneys with adenovirus expressing green fluorescent protein was performed and subsequent histology revealed positive expression of this exogenous protein. This confirms that ex-vivo perfusion is sufficient to allow de-novo protein synthesis and in addition analysis revealed upregulation of a DNA repair protein, reduced expression of a marker of cytoskeleton damage and less apoptotic cells in the kidneys perfused with growth factors. These differences translated into better graft function – and the kidneys perfused without growth factors failed to sustain life. Thus it would appear that normothermic preservation could open the door to genetic modulation of an organ prior to transplantation. The genes used in gene transfer preconditioning could be delivered with subsequent protein expression to reduce ischemia/reperfusion injury. The therapy would be organ specific and require no donor pre-treatment. Even more revolutionary would be the use of normothermic preservation to allow immunomodulation of the organ by transfection with genes responsible for the down regulation of immune response or induction of tolerance.

6. DISCUSSION

With the increasing demand for organ transplantation it is clear that organ preservation must evolve to reduce the ischemia/reperfusion injury and improve organ viability. Although cold preservation solutions are effective in preserving high quality organs long enough for transplantation to succeed, the real challenge for the next decade is to develop technology to allow marginal donor organs to be resuscitated. This may include restoration of energy charge, pharmacological intervention and gene transfer technologies. Perfusion

preservation is likely to become increasingly important with normothermic preservation offering the greatest potential for organ resuscitation and modulation. Many approaches have already been established and there is the exciting prospect of the application of these new techniques in clinical trials during the next few years.

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