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REVIEW ARTICLE

Andrew James McLaren
Peter John Friend

Trends in organ preservation

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P.J. Friend · A.J. McLaren
Nuffield Department of Surgery,
John Radcliffe Hospital,
Oxford, OX3 9DU, UK
e-mail: peter.friend@surgery.oxford.ac.uk
Tel.: +44-1865-221319
Fax: +44-1865-765063

Abstract Organ preservation aims to provide a viable graft with primary function post-transplant. The current basis of preservation for transplantation is static cold storage using specific preservation solutions which minimise cellular swelling and membrane pump activity, thus maintaining cellular ATP levels. The current organ shortage and consequent expansion of donor criteria places even greater reliance on minimising graft injury during preservation. This review focuses on current and future advances in preservation technology. The key areas of advance are additives to preservation solutions, alternatives/adjuncts to preservation solutions including perfluorocarbons. A major area of advance is in the modulation of organs during the storage period. This may be achieved by biochemical additives or genetic manipulation. Machine perfusion technology is improving, and this is discussed together with the recent concept of warm (normothermic) perfusion as an alternative means of preservation. The authors provide an overview over the current methods of organ preservation. Cold storage, effective in the short-term is insufficient for marginal organs, does not allow assessment of viability markers, and provokes ischaemic injury. Potential strategies for minimising ischaemic injury include additives to preservation solutions; the two-layer method with perfluorcarbons and UW solution—at present limited to pancreas preservation; organ modulation; organ preconditioning and genetic modification of organs. In particular, the authors illuminate the potential in a reappraisal of the concept of normothermic perfusion.

Keywords Transplantation · Preservation

Abbreviations *HSP* Heat shock proteins · *ICAM-1* Intercellular adhesion molecule-1 · *I/R* Ischaemia reperfusion · *PFC* Perflurocarbon · *UW* University of Wisconsin solution

The aims of preservation

The purpose of organ preservation in the context of transplantation is to deliver a viable graft to a recipient which will exhibit primary function. Improvements in hypothermic preservation solutions have allowed extended preservation times. This has provided important logistic benefits including, in the case of kidney transplantation, the institution of HLA-based organ sharing across geographical boundaries. However there is an increasing recognition that the limits of conventional preservation have been reached, and future

expansion of transplantation will depend on new approaches. These are the focus of this review.

Cold static storage is an effective means of organ preservation in the short-term. However, increasing cold ischaemia time is associated with non-function in liver [1] and cardiac allografts and with delayed graft function in renal transplantation [2]. Access to an expanded donor pool is increasingly necessary to meet the demand for solid organ transplantation, and further advances may require alternative modes of preservation. This is particularly evident when the use of marginal organs is considered. At present, the classification of certain organs as "marginal" is based on the risks of dysfunction in the context of current preservation technology. However, much of the injury sustained even by a marginal organ is related to the period of cold preservation—if preservation could be improved significantly, a primary component of the risk would be abrogated, rendering the use of these organs a rational option.

Marginal donors

The worldwide shortage of donor organs has resulted in a progressive expansion of donor criteria to include donors that would have been considered unsuitable in the past. This is particularly evident with the reintroduction of non-heart beating cadaveric donation for kidney transplantation in many centres and, increasingly, for liver transplantation. These organs invariably sustain additional warm ischaemic injury. Conventional preservation methods result in primary non-function rates of 4–9% and in delayed graft function rates of 22–84% after kidney transplantation (compared with 1–2% and 7–25% with heart-beating donors) [3, 4, 5].

The limitations of cold ischaemia

It is sometimes assumed that cooling to ice temperature results in cessation of aerobic metabolism. Metabolism is in fact slowed by a factor of 1.5–2 for each 10°C fall in temperature [6]. Metabolism is not arrested, and there is increasing evidence that cooling may not occur quickly during the retrieval process, thus increasing ischaemic injury.

It is instructive to consider the changes that take place during static cold storage. Cellular oedema and swelling occur as various membrane functions, including the Na-K ATPase pump, are affected. The composition of preservation solutions is designed to limit this swelling effect.

Anaerobic metabolism continues at a slow rate, resulting in accumulation of end-products of metabolism—protons, lactate and breakdown products of adenine nucleotides (hypoxanthine). On reperfusion, these by-products contribute to the generation of oxygen free radicals, which are directly damaging to tissues, and act to induce an acute inflammatory response (ischaemia-reperfusion injury). There is also direct damage from cold, with physical alterations in cellular membranes and calcium influx into parenchymal and endothelial cells, leading to tissue damage [7]. The extent of damage from oxygen-free radicals is unclear, and experimental strategies designed to limit free radical production or to scavenge free radicals have not moved into routine use.

These various damaging mechanisms are aggravated because it is not possible to accurately assess their impact on post-transplant graft function until after transplantation. Static cold storage does not allow any assessment of viability markers. This is a major limitation in the context of marginal donor organs. Primary non-function of a liver or heart allograft is a disaster that carries a high mortality. There is also considerable impact on renal recipients with associated morbidity and a high probability of inducing anti-HLA antibodies which will limit future possibilities for retransplantation. A number of potential strategies for minimising ischemic injury with alternative techniques of organ preservation are outlined below.

Hypothermic perfusion

Hypothermic machine perfusion has been used for kidney preservation for many years. Although more complex and expensive than static cold preservation, such systems do have some advantages, particularly in the context of marginal organs. Viability markers include the enzyme glutathione S-transferase and measurement of flow/resistance on circuit. These are helpful in the prediction of graft outcome. Hypothermic perfusion improves short and long-term outcome measures after renal transplantation [8]. However, given short cold ischaemia times (less than 24 h) in clinical practice, the cost and logisitical problems of machine perfusion contrasted with the convenience of static cold preservation has ensured that hypothermic perfusion is used in only a minority of transplant units.

Additives to preservation solutions

Novel preservation solutions may result in improvements in ischaemia-reperfusion injury and/or allow for prolonged preservation times. A range of chemical compounds could potentially be added to preservation solutions in an attempt to achieve this aim.

Superoxide dismutase

The superoxide anion free radical (O^{2-}) has been implicated in the pathogenesis of tissue injury consequent to ischemia/reperfusion in several different organs, including kidney and liver. Superoxide dismutase (SOD), an enzyme free radical scavenger specific for superoxide, has been used successfully to protect organs from structural damage during reoxygenation of ischemic tissue. This compound has been shown, in a clinical trial, to reduce both acute and chronic rejection, although, disappointingly,without impact on the incidence or duration of delayed graft function [9]. This implies that a major impact of I/R injury is priming the graft for acute rejection.

Lazaroids

These are a class of agents which inhibit iron-mediated lipid peroxidation on reperfusion by an unknown mechanism(s) when given to donors prior to organ retrieval. To date, there have been no human trials, but a number of animal studies show benefit [10, 11, 12]. Their efficacy is however limited, and this may be in part due to the inability of lazaroids to block all stages of cell injury, whilst being able to reduce lipid peroxidation. In-vitro studies show that the later events of DNA damage and ATP depletion still occur [13].

Anti-apoptosis agents

Apoptosis is a specific form of cell death characterised by a sequence of nuclear changes and cell phagocytosis without inflammation. Apoptosis has been demonstrated to be a key part of I/R injury with increased levels correlating with cold ischemia time [14].

Specific inhibitors of apoptosis, such as the p53 inhibitor pifithrin-alpa, have been demonstrated to effectively inhibit transcriptional activation of the pro-apoptotic proteins Bax and p21 in a model of renal I/R injury. This inhibition was effective even when given up to 14 h post-injury. Thus, anti-apoptosis strategies may represent effective and highly specific means of limiting I/R injury.

Calcium channel blockers

Under normal conditions, calcium controls and regulates the enzymes of oxidative metabolism within the mitochondrial matrix Calcium accumulation is thought to be a key component of I/R injury by effects on mitochondrial function [15]. Recently, experiments have demonstrated that reperfusion/reoxygenation results in net mitochondrial calcium ion uptake by myocardial cells, contractile dysfunction, and cell death as a result of a loss of energy generation capacity in the mitochondrial respiratory chain [16, 17]. Calcium channel blockers have been used successfully to ameliorate I/R injury in rat livers and in models of myocardial injury in addition to clinical renal transplantation [18, 19, 20].

Perfluorocarbons

These are hydrocarbon molecules in which hydrogen atoms are replaced by fluorine. The resulting liquid is able to dissolve 20–25 times more oxygen than plasma at room temperature. In addition, oxygen release is facilitated by a low oxygen-binding constant. This results in a linear relationship between oxygen saturation and partial pressure, facilitating oxygen release to tissues. Recent studies have utilised perfluorocarbons in the preservation of pancreas grafts using a two-layer method (TLM) [21]. Pancreas grafts are placed in a chamber containing perflurocarbon (PFC) and University of Wisconsin (UW) solution. The PFC is lipophilic and has a higher density, resulting in separation of the two fluids. The pancreas graft floats at the interface between the layers and, by fully oxygenating the PFC prior to storage, the graft can be preserved without continuous oxygenation. During the preservation by the two-layer method, sufficient oxygen is available from the PFC solution to maintain ATP synthesis within the pancreas by means of the direct phosphorylation of adenosine contained in the UW solution [22]. The Edmonton islet transplant group has demonstrated increased islet recovery using this method compared to simple UW cold storage and improved function from transplanted islets isolated in this way [23].

Storage by the two-layer method offers a simple means of supplying oxygen to explanted organs without the necessity for continuous perfusion equipment. Fully oxygenated PFC has been shown to maintain oxygen saturation of more than 85% during 18 h of pancreas storage [24]. To date; clinical utilisation of this method has been reported only for the pancreas, however successful experimental storage has been achieved with canine small bowel and rat heart [25, 26]. It remains to be seen whether larger solid organs will be able to benefit from simple diffusion of oxygen from perflurocarbons.

Organ modulation during cold ischaemia

The period of cold storage can also be used to modulate the graft in a variety of ways in order to ameliorate responses to the organ post-reperfusion. The two main areas of interest are modulation of the alloresponse and reduction of ischaemia-reperfusion injury.

There are a number of potential target molecules for ameliorating ischaemia-reperfusion injury. Intercellular adhesion molecule-1 (ICAM-1) is an antigen-independent mechanism that facilitates adhesion of leukocytes to endothelial cells with high basal levels of expression which are upregulated during I/R injury. Antisense oligonucleotides which effectively block ICAM-1 - Mac-1 interaction have been shown to reduce ischaemia-reperfusion injury in animal models [27, 28, 29]. There is also evidence from a phase I clinical trial in which an anti-ICAM-1 antibody was administered to renal allograft recipients, suggesting that ICAM-1 blockade can limit ischemic damage [30]. Similarly, tissue factor, a key initiation factor for coagulation, is induced on macrophages and endothelial cells by inflammatory and immune responses and can also be successfully blocked by antisense techniques [31, 32]. These strategies have only been used at the reperfusion stage and not during the cold preservation period. It would be of interest to extend the scope of these studies to the storage and even donor retrieval phases.

Normothermic perfusion

Machine preservation of the organ at body temperature, normothermic perfusion, is not a new concept, having been reported originally in 1976 [33]. However, this still represents a complete reversal of current thinking in preservation technology. As discussed above, the problem central to all cold preservation is the failure to permit normal aerobic metabolism.

The maintenance of normal metabolism minimises the accumulation of the substrates for free radical formation on reperfusion. In the case of ischaemically-injured organs (from non-heart-beating donors), the restoration of normal metabolism for a period immediately after retrieval allows regeneration of ATP levels and clearance of metabolites.

Pig livers have been transplanted successfully after 1 h of warm ischaemia and 4 h of normothermic perfusion, in contrast to control animals with cold preservation, in which there were no survivors [34]. Compared with conventional cold-stored livers, isolated warm-preserved livers have been shown to exhibit stable metabolic function (bile and Factor V production), glucose metabolism and galactose clearance [35]. This ex-vivo reperfusion study also showed cold-stored livers to generate higher levels of hepatocellular enzymes in the perfusate and to exhibit more histological damage. Dog kidneys have been successfully transplanted after 2 h of warm ischaemia and 18 h of ex-vivo warm perfusion with clear evidence of resuscitation of function [36]. A more recent report has shown stable liver function, acid-base balance and normal enzyme levels for 72 h in an isolated pig liver perfusion model [37]. If it is possible to translate this to clinical practice and to maintain normal enzyme levels of organs for transplantation, then storage times could be extended well beyond the current limits.

The technique has been successfully applied in human renal transplantation, but only in a limited manner. Valero et al reported a series of 44 kidneys retrieved from non-heart beating donors and transplanted, over a 12-year period. The method of organ preservation prior to retrieval evolved during the course of the series. Initially in-situ cold preservation via a femoral catheter was used. Subsequently, total body cooling with oxygenation was achieved with cardio-pulmonary bypass. This was then developed into warm in-situ perfusion for 1 h using cardiopulmonary bypass prior to core-cooling for organ retrieval [38]. After retrieval, the organs were preserved using conventional cold storage. A significant advantage was demonstrated for the normothermic perfusion group with primary non-function rates of zero compared with 22.5% for conventional in-situ cold perfusion and storage. Delayed graft function rates were also reduced from 55% to 12.5%. Although this was an uncontrolled series, it does demonstrate that normothermic techniques can be applied successfully in the clinical field. Cardiopulmonary bypass has also been used in Japan for organ cooling before retrieval [39, 40, 41, 42, 43].

The results published by Valero et al. are striking, particularly as the duration of warm perfusion was only 1 h and followed by core-cooling. The main reason for core-cooling prior to retrieval is to ensure the retrieval operation could be performed without the systemic anticoagulation normally required with bypass. The recent development of a cardiopulmonary bypass circuit which does not require anticoagulation [44] offers the potential to maintain normothermia until organs are retrieved and may allow a further significant advance. Indeed, combining retrieval by such an approach and warm ex-vivo perfusion would allow transplantation not only without significant ischaemia but also with the avoidance of cooling.

A further advantage of normothermic preservation is that of viability assessment. Because the organ is functioning, rather than quiescent, during the period of storage, it should be possible to define and measure key functional parameters which correlate with post-transplant function. This would be of particular value in allowing more accurate assessment of organs from marginal donors. Work in liver perfusion has shown that normothermic livers can recover bile production, glucose metabolism and clotting factor production [35]. Such viability measures have not yet been validated by the gold standard of post-transplant function.

Overall, whilst offering potential advantages, normothermic perfusion is at present a complex undertaking requiring expertise to set-up, continuous monitoring by trained personnel, and this limits its practical application.

Organ preconditioning

A limited period of ischaemia followed by normothermic reperfusion facilitates protection from further ischaemia as a type of organ pre-conditioning. Ischaemic preconditioning exposes an organ to a brief period of ischaemia, reducing the pathological damage from a subsequent, longer period of ischaemia. This preconditioning effect has been studied most extensively in myocardial tissue and is related to ATP-sensitive potassium channels which open when intracellular ATP levels fall, resulting in changes in the cardiac action potential that prevent further ATP depletion protecting the cell from irreversible impairment of its energy metabolism [45, 46]. The K-ATP channel does not exist in the same manner in renal tissue, however similar intracellular changes in G proteins and protein kinase C do mediate a process of ischemic preconditioning in renal tissue [47].

There is evidence that ischaemic preconditioning is relevant to organ transplantation, as shown in the mouse liver, possibly mediated via regulation of apoptosis. The preconditioning may be short. Clavien's group have demonstrated optimal preconditioning in a mouse liver model with 10 min of ischaemia followed by 15 min of reperfusion—this protocol allowing survival of a subsequent prolonged period of ischaemia [48]. The mechanism for this was an inhibition of apoptosis through down-regulation of caspase-3 activity. Extension of this work into clinical liver surgery has revealed similar effects. A preconditioning period in human livers resulted in increased resistance to ischaemic injury during subsequent liver resection performed with arterial inflow occlusion. Reduction in apoptosis was demonstrated in liver biopsies from preconditioned patients [49].

Preconditioning effects can also be induced by hyperthermia [50] and other chemical stressors for example NO donors, adenosine receptor agonists, endotoxin derivatives, or opioid receptor agonists, which principally induce heat shock proteins. Thermal preconditioning has been shown to reduce leucocyte-endothelial interactions by impairing leucocyte rolling in an animal model which represents a major immunomodulatory pathway [51]. These proteins may represent the final common pathway for multiple forms of stress preconditioning.

Heat shock proteins

Heat shock proteins (HSP) were first identified in *Drosophila* in 1962. These are proteins, induced during a period of sublethal heat exposure, that are protective during a later period of lethal heating [52]. It is now known that HSPs are induced by a variety of factors that stress mammalian cells, including oxygen free radicals injury. After an ischaemic insult, protein synthesis is generally inhibited, but synthesis of HSPs continues and can reach 15–25% of intracellular protein concentration. HSP production after heat preconditioning has been associated with improved outcome in experimental models of pulmonary isografts and renal allografts [53, 54, 55, 56].

The mechanism for this protective effect is unclear. However, a recent study in which HSP-72 levels within renal tubular cells were elevated by either liposomal transfer or heat stress has suggested that HSPs may inhibit renal tubular cell apoptosis by preventing nuclear factor-kappaB activation and subsequent tumour necrosis factor-alpha production [57].

Genetic modification of organs during normothermic preservation

Gene transfer offers the possibility of delivering molecules with protective effects to the donor organ. Potential mechanisms of protection include reduction in ischaemia-reperfusion injury, reduced graft immunogenicity and increased protection against the host immune response.

Successful experimental results have been obtained using adenoviral IL-10, CTLA4-Ig, Fas Ligand and ICAM antisense to modulate the graft [58, 59, 60, 61]. Tolerance induction has been achieved in small animal studies by transfer of MHC Class I and II molecules [62].

However, whilst attractive and effective, at least in small animal models, several problems exist with these strategies. The protocols used require donor treatment often many days before transplantation. This is because the vectors used have very limited transfection efficiency during cold storage and secondly significant amounts of recombinant protein are required for protection to be effected. During conventional cold storage, even if transfection occurs, protein production is likely to be minimal by the time of reperfusion, thereby limiting the therapeutic effects.

By maintaining cell metabolism and division, normothermic reperfusion may allow more efficient transfection. This has recently been demonstrated in an exsanguinous perfusion system using canine kidneys [63]. This study showed that, using an adenovirus, a fluorescent protein was effectively encoded and expressed on vascular endothelium and tubules after 24 h of normothermic perfusion but not after attempted transfection during hypothermic perfusion.

Conclusions

Organ preservation has advanced considerably since Belzer and colleagues developed cold perfusion with

plasma in the 1960 s. The challenges for the future lie in building upon this successful heritage. Clearly, although traditional cold preservation methods have stood the test of time, they are not sufficient to allow continued expansion of worldwide transplant programs to meet recipient needs by means of successful preservation of marginal organs. Some of the novel strategies outlined above will improve existing preservation solutions. However, it is likely that significant advance will require a more fundamental review of the approach to preservation. The technique of normothermic perfusion, whilst technically challenging, offers a number of opportunities to minimise ischaemic injury, to allow organ viability assessment, and to permit organ manipulation during storage.

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