Preservation Solutions for Solid Organ Transplantation

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Abstract: Solid organ transplantation was one of the greatest medical advances of the 20th century. Current preservation technology falls short of maintaining organs *ex vivo* in perpetuity. This review examines the biochemical basis of organ degradation in response to ischaemia, preservation solution composition and potential future organ preservation technology.

Key Words: Organ transplantation, preservation solutions, machine perfusion preservation, hypothermia.

INTRODUCTION

Solid organ transplantation was one of the greatest medical advances of the 20th century and now offers the opportunity to replace not only failed organs, unable to perform their normal physiological function, but also as a treatment for cancer (eg. hepatocellular carcinoma) or congenital structural abnormalities (eg. cardiac defects). In the early days immunological barriers were the major obstacles to successful transplantation, but now calcineurin inhibitors (eg. cyclosporine) and antibody therapies mean that the great majority of organ transplant recipients do not reject organs acutely. The immediate problem facing clinical organ transplantation in the 21st century is supply and demand with unprecedented rates of growth (Table **1**), in the face of similar or even falling cadaveric donor numbers.

A considerable number of potential organ donors die out of hospital or shortly after reaching hospital from trauma, cardiac arrest or stroke (non-heart beating donors, NHBD) [1, 2]. This "warm ischaemia", that is to say, intra corporeal time at body temperature without adequate sanguineous perfusion, considerably reduces the cold storage period and, in most cases, makes the organs unusable. In general, each minute of warm ischaemia is equivalent to an hour of cold ischaemia [3]. As a result kidneys can be successfully transplanted after 40 minutes of warm ischaemia combined with around 24 hours of cold ischaemia [4-6]- enough time to transport the organs from a retrieving centre to a potential organ recipient (Table **2**). For other organs the warm ischaemic tolerance is far more limited and the necessary addition of cold ischaemia makes clinical transplantation a more difficult proposition [1, 7-10].

Considerable research efforts are now directed at improving current organ preservation technology to allow for the resuscitation of these damaged organs in the laboratory [11-18] and potentially increase the pool of donors available for organ transplantation.

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PRINCIPLES OF ORGAN PRESERVATION

The elusive goal of organ transplantation has been to maintain organs *ex vivo* in perpetuity [3, 4]. Implantation of a non-viable essential organ (heart, lung, liver) rapidly leads to death of a recipient in the absence of re-transplantation. The prime purpose of organ preservation solutions in this situation is therefore to ensure that the organ will function immediately after implantation (primary function). For non-essential organs, like the kidney and pancreas, a period of recovery (delayed graft function) can be tolerated by the recipient with continuing dialysis or the administration of insulin and hence longer periods of ischaemia are tolerable clinically. If the graft never recovers (primary non-function) a second operation is usually required to remove it, often with dire implications for the recipient.

The principles of organ preservation are therefore to prevent ischaemia-reperfusion injury (IRI) by the use of appropriate biochemical, pharmacological, hormonal and immunological interventions during retrieval, transport, implantation and reperfusion of the graft. This review will address the background of organ preservation highlighting the biochemical processes underlying ischaemia-reperfusion injury, composition of current preservation solutions and future developments concentrating on the use of preservation solutions and their role during retrieval and storage of a graft for transplantation. Complementary contemporary reviews are available examining the pathophysiology of IRI [19-21], immune function [22], machine perfusion [23] and donor management [24].

ISCHAEMIC INJURY

Ischaemic injury is a cascade of cellular events consequent to hypoxia. This is complicated, in solid organ transplantation, by further considerations of structure and function. After cessation of blood flow the haemostatic pathways rapidly lead to intra-vascular occlusion [25]. Anaerobic metabolism continues providing energy to power the principal cellular homeostatic processes (Na^+-K^+ ATPase) maintaining intracellular oncotic pressure [4]. However, the lactate thus produced, overwhelms the intra-cellular buffering systems and acidosis supervenes [26]. Hypothermia mobilises cal-

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Table 1. Patients on the Active Transplant Waiting List in Two Countries (August 2005)

	USA†	% Change over 10 years *	UK‡	% Change over 10 years *
Kidney	67,210	+ 120 %	5,549	+ 35 %
Heart	3,113	- 10 %	126	N/A
Lung	3,403	+ 80 %	280	N/A
Liver	17,901	+ 220 %	300	+ 120 %
Pancreas	1,726	+ 70 %	129	N/A

* Estimates from available data

† Data abridged from United Network of Organ Sharing (UNOS) (www.UNOS.org)

‡ Data abridged from UK Transplant (www.uktransplant.org.uk)

cium sequestered in mitochondrial, sarcoplasmic and endoplasmic reticular sources as well as the extracellular milieu [27]. The rise in intra-cellular calcium ion concentrations destabilises lysozyme membranes leading to leakage of catabolic enzymes including phospholipases [28] and proteases [29]. Catabolic enzymes, whilst relatively suppressed by acidic pH and hypothermia are still active and generate free fatty acids from membrane phospholipids. Free fatty acids and the ongoing depletion of ATP induce the mitochondrial pore transition [20, 30]. After longer periods of ischaemia ATP generation fails secondary to a combination of glycolytic substrate depletion and membrane associated enzyme denaturation with rapid cell swelling [31, 32]. The final observation in lethal ischaemia injury is cell membrane rupture [33]. As would be expected the rate of injury is greater at higher temperatures, but it may also be subtly different. Various researchers have suggested that vascular endothelial cells and parenchymal cells have differing sensitivities to warm and cold ischaemia [34-38]. These observations, however, are often complicated by the use of different species and organs.

Table 2. Potential Clinical Viability of Human Organs for Transplantation After Storage at 4°C with no Primary Warm Ischaemia (Abridged from [131])

Organ	Number of Hours (approx.)
Heart	6
Lung	8
Small Bowel	12
Liver	20
Pancreas	20
Kidney	72

REPERFUSION INJURY

Even in the absence of lethal injury, organ failure can still occur at reperfusion with oxygenated normothermic blood. Cellular regeneration of ATP in a graft is hampered by a combination of factors. Vasospasm, secondary to disturbances in the balance of endothelin 1 and nitric oxide biosynthesis by endothelial cells [36], can be prominent and impair oxygen delivery. Blood flow is further compromised by necrotic cells blocking capillary lumens, intra-vascular thrombosis and interstitial oedema extrinsically compressing blood vessels ("no re-flow") [39]. Mitochondrial dysfunction, secondary to activation of the membrane permeability transition pores during ischaemia [40], further depletes ATP stores and lethally injured cells undergo apoptosis or necrosis depending on cellular ATP levels [41]. Where viable endothelial cells in the microcirculation are still present, the ischaemic injury induces immunological adhesion molecules (ICAM's and selectin's) to be expressed on the luminal membrane [19, 20, 42, 43]. Leukocytes with the appropriate receptors then marginate and enter the organ degranulating on contact with necrotic material or bacterial contamination [19, 44, 45]. Mitochondria, and to a lesser extent, phagocytic cells generate a large wave of reactive oxygen species (ROS's) (OH^-, H_2O_2, 02^-) which overwhelm depleted antioxidant defences causing lipid peroxidation [19]. The characteristic feature of severe reperfusion injury is vascular endothelial cell death leading to graft thrombosis [46, 47].

Despite the accumulation of scientific knowledge in the arena of IRI few interventions have been shown to make much clinical difference and randomised controlled trial evidence of single intervention efficacy in human transplantation remains limited.

ORGAN PRESERVATION

Prior to Donation and During Retrieval

"Brain stem dead" donors often undergo a period of cardiovascular instability, which may be associated with metabolic and endocrine abnormalities. Correction of these derangements can significantly improve the outcome of organs transplanted from these sources. Thyroxine (in the form of T3), vasopressin, steroids and nor-adrenaline have all been shown to improve the quality of organs retrieved [24].

During cadaveric retrieval, just prior to cross clamp of the aorta, unfractionated intravenous heparin is given to prevent intra-vascular clotting with the onset of stasis [48]. At cardiac asystole crushed ice or cold water are then placed in the body cavities and vascular flushing with chilled fluid initiated. The speed and efficiency of this flush appears to be affected by the viscosity [49] and temperature [50] of the perfusate. When correctly performed these manoeuvres can rapidly cool solid organs to under 10 °C [3] - a temperature

at which most enzyme systems are suppressed about 12 fold (van't Hoff's rule) [4] but damaging crystal formation (ice, adenosine) is not possible [51]. When warm ischaemia has been unavoidable, thrombolysis [25, 52] can successfully re-open microvascular beds and permit effective intra-vascular cooling. Using cold anti-coagulated blood as a cooling fluid has been shown to extend kidney preservation times to only 12 hours [53]. With this medium the organs rapidly thrombose as a result of endothelial injury at reperfusion [47]. Removing all cellular and humoral blood components and replacing it with a sterile biochemical preservation solution greatly increases the possible preservation times and, when recognised, was a major advance in organ preservation technology [54, 55].

Storage and Reperfusion of Organs

Collins produced the first cold storage solution that successfully stored kidneys on ice before transplantation [55]. Prior to this Belzer had used a pump to perfuse canine [54] and human [56] kidneys with "cryoprecipitated" (lipoprotein free) plasma for up to 72 hours before transplantation. The pump was large, bulky and expensive requiring constant supervision. As a result static cold storage with Collins solution, with its various modifications (Eurocollins [57]), was standard clinical practice for the next 20 years, until Belzer produced the "gold standard" solution for organ transplantation University of Wisconsin (UW) solution (Table **3**) [58]. This development owed a considerable debt to good fortune rather than impeccable chemical design- Dr Belzer later confessed to a policy of including "the kitchen sink" in his quest for optimal organ preservation [59].

The 1990's have seen an explosion of further preservation solutions, mostly based on UW, and currently over 20 are available for organ transplantation with differing chemical profiles and clinical evidence base. This review will concentrate on the chemistry of six solutions used for thoracic and abdominal organ preservation (Table **3**).

Cation Ratios

Collins solution [55, 57] reversed the normal intra-/extracellular ratio of sodium and potassium ions- the rationale being to reduce the work of the principle energy consuming cellular process (plasmallema Na^+-K^+ ATPase). Since then relatively normal sodium/ potassium ratios (Table **3**) and reduced total cation concentrations (HTK) have highlighted that sodium/ potassium balance may not be as critical as first thought [60].

High potassium ion concentrations causing membrane depolarisation have been associated with vascular smooth muscle and endothelial dysfunction in certain experimental and perhaps clinical settings [61, 62]. In this respect the low

Table 3. Preservation Solution Composition

Solution name	Organs used for‡	Energy Substrate	*Na^+	*K^+	*Mg^{2+}	*Ca^{2+}	pH (at 25°C)	Buffer	Osm†	Colloid	Impermeants	Anti-oxidants
University of Wisconsin	H, K, L, P, SB. HPP (K)	Adenosine	30	125	5	N/A	7.4	Phosphate	325	HES	Lactobionate/ Raffinose	Allopurinol/ Glutathione
Histidine-Tryptophan-Ketoglutarate	H, K, L, P.	Ketoglutarate	15	10	4	0.015	7.02-7.2	Histidine	310	N/A	Mannitol	Histidine/ Tryptophan/ Mannitol
Belzer's machine perfusion solution	HPP (K and L)	Adenine	100	25	5	0.5	7.4	HEPES	320	HES	Gluconate/ Ribose	Allopurinol/ Glutathione
Celsior®	H, K, L, P Lung	Glutamate	100	15	13	0.25	7.3	Histidine	320	N/A	Lactobionate/ Mannitol	Histidine/ Mannitol/ Glutathione
Marshall's Hypertonic Citrate	K	Citrate	28	26	41	N/A	7.1	Citrate	486	N/A	Mannitol	Mannitol
Perfadex®	Lung	(Glucose)	N/A	6	0.8	0.5	7.4	THAM	295	Dextran	Glucose	N/A

* mmol/L

† Calculated approximate osmolality (mOsm/L)

‡ H- Heart, K – Kidney, L – Liver, P – Pancreas, SB - Small Bowel

HPP (K)- Hypothermic Perfusion Preservation (Kidneys)

N/A- (Not Applicable)

HES- Hydroxyethyl-starch

HEPES- 4 -(2-hydroxyethyl)-1-piperazineethanesulphonic acid

THAM- Tromethamine; 2-Amino-2-(hydroxymethyl)-1,3-propanediol

(Glucose)- Glucose is primarily used as an impermeant but has been shown to be metabolised in particular situations (c.f. Energy depletion)

potassium containing solution Perfadex has been popularised as particularly attractive for preservation of vascular endothelial rich lung tissue [63, 64].

Magnesium appears to be essential for the heart [65], assist in ATP regeneration at reperfusion [66] and may mediate some of its beneficial effects by blocking mitochondrial calcium cycling [40].

Energy Depletion

Despite the hypothermia cellular metabolism does continue and respiration can be encouraged by the provision of substrate. With the notable exception of the lung [67], stimulating respiration during ischaemia and hence hydrogen ion generation goes against the central tenants of organ preservation [4]. However, most solutions provide an energy substrate to allow for rapid ATP regeneration at reperfusion (Table **3**). There has been a general move away from the use of glucose and citrate as buffers and impermeants during liver storage as these are taken up and metabolised by hepatocytes to lactate [4] and succinate [68] respectively. The kidney also appears able to metabolise these as substrates after extended periods of preservation: part of the reason why Marshall's solution is generally considered inferior to UW for kidney preservation [6].

Hypothermic perfusion preservation technology (machine perfusion, HPP) is thought to be effective by improving the supply of substrates, removal of metabolites and preservation of microvascular patency [69]. In other words it maintains the structure and function of an organ by ensuring the "bathing" medium for parenchymal cells, distant from the surrounding fluid, is continuously mixed. The addition of oxygen to the perfusate also helps to maintain a low level of aerobic metabolism and can be considered essential for liver and lung perfusion [17, 18, 70]. The main problems with HPP relate to its cost and requirement for monitoring, but in "marginal" situations, where there are doubts about organ viability, HPP can offer distinct advantages. Perfusate enzyme levels (glutathione-*s*-transferases) and perfusion variables (eg. vascular resistance and flow) have been shown to be reasonably good surrogate markers of graft function after implantation [71, 72].

pH buffering

The optimal pH of the preservation solution for cold storage of organ grafts appears to be the physiological normal of 7.4 or perhaps slightly more acidic [73]- changing the pH of UW resulted in a significant deterioration of experimental graft function [74]. Intracellular acidic conditions appear to favour cell survival by inhibiting activated proteases and phospholipases during ischaemia [75]. Reducing the pH further, however, leads to cell death during reperfusion- the so-called "pH paradox" [76]. As oxygenated blood flows through the graft and oxidative phosphorylation resumes membrane pumps act to correct the intra-cellular acidosis and sodium excess, inadvertently triggering these enzymes to breakdown proteins and lipids. Mitochondrial acidosis also compromises the electron transport chain and impairs energy regeneration at re-oxygenation [20]. To this end buffering $[H^+]$ is one of the key attributes of the preservation solution (Table **3**). HTK, with its high concentration of histidine appears particularly adept. Fig. **1** highlights the comparative pH buffering differences between several different presservation solutions.

Calcium and Reperfusion Injury

Zimmerman and Hulsmann highlighted a similar "paradox" problem with calcium ion concentrations long before the effects of pH were recognised [77]. In Langendorff type rat heart preparations removal of calcium from the perfusion medium for as little as two minutes resulted in diastolic dysfunction, hypercontracture and myocardial cell necrosis when physiological calcium was re-introduced. Although this idiosyncratic observation is not directly comparable with the pathophysiological process of IRI, it highlights the detrimental effects of removing calcium completely from the preservation solution on cytoskeletal proteins and mitochondria at reperfusion [78]. The effect does not appear limited to cardiomyocytes either, with reports of similar reperfusion injury in vascular smooth muscle cells [79], vascular endothelial cells [80], hepatocytes [81] and renal tubular cells [82] preserved in calcium free media. On the other hand supraphysiological cytoplasmic levels of calcium occur, even in the absence of added calcium to the preservation solution, as a result of sarcoplasmic release of calcium during cooling of organs with preservation solutions [29]. The consensus has therefore been to provide a lower level of calcium ions than serum in most preservation solutions (c.f. Table **3**). Recent work has challenged this paradigm with the observation of excellent preservation of kidneys damaged by warm ischaemia using calcium-free UW. When Lindell transplanted kidneys exposed only to cold ischaemia, calcium containing Belzer MPS permitted a quicker recovery of renal graft function when compared with UW [83]. Interestingly, after a warm ischaemic insult was added, kidneys perfused with UW survived *more* often (86%) than their Belzer-MPS perfused comparators (25%). The authors speculated that the lack of calcium and iron-chelating properties of lactobionate (c.f. free radicals/ impermeants) were responsible for the differences in organ preservation. This would suggest that, at least for kidneys, the optimal calcium concentration of the preservation solution differs depending on the temperature of the injury.

Colloids and Impermeants

In crude terms the role of colloids and impermeants are to minimise interstitial and cellular oedema respectively [4]. During the "*in situ*" and "back table" flush to remove blood, hypertonicity and the presence of a colloid in a perfusion solution improve clearance of the microcirculation. This may be as a result of reducing individual red cell volumes in the tight capillary lumens, as well as total parenchymal cell volume and extrinsic lumen compression [3]. After retrieval colloids are unnecessary during cold storage of solid organ grafts [84].

Various high molecular weight saccharides have been used as impermeants to sustain the oncotic gradient across the cell membrane and prevent oedema. The original impermeant was glucose (MW 180.16) (Collins solution) which, as mentioned previously, was metabolised even under hypothermic energy deficient conditions and less than satisfactory in liver preservation. Other saccharides with less

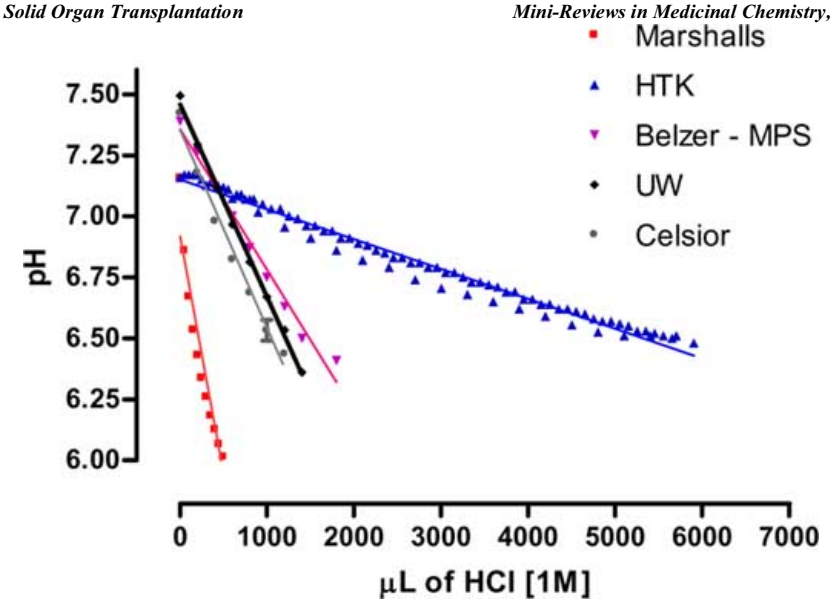

Fig. (1). Effect of acid addition on solution pH of 5 preservation solutions at 6°C [Wilson CH, unpublished data].

Footnote: Simple bench titration experiment in which the appropriate volume of hydrochloric acid (HCl) was added to 100ml of preservation solution and the pH measured.

membrane permeability are the mainstays of impermeant technology in the 21st century. UW pioneered the use of lactobionate (MW 358.3) and raffinose (MW 504.46) of which lactobionate, despite being the smaller molecule, appeared more effective when the two were compared in an experimental liver perfusion [85] experiment. Gluconate (MW 196.16), mannitol (MW 182.17) and sucrose (MW 342.30) have all also been used in various solutions (c.f. Table **3** and [86]). Lactobionate remains the impermeant of choice having been successfully used in preservation solutions other than UW [87], being critical to the success of UW [88] and for its proposed iron chelation properties [83] (c.f. Reactive oxygen species).

Colloids are essential during HPP to maintain an intraluminal vascular oncotic pressure and prevent excessive interstial oedema [4]. Belzer's original colloid was cryoprecipitated plasma [89], which he refined down to albumin alone. This gave an unpredictable response during clinical transplantation as a result of protein denaturation; hydroxyethyl starch (HES) (50kDa), a stable non-toxic, oncotic agent was therefore chosen for the preparation of UW solution and proved effective [90]. Since then other groups have tried substituting HES with other colloids, notably dextrans [91] and poly-ethylene glycol (PEG- 20kDa) [92]. The impetus for a substitute has been both commercial, HES being the object of patent law, and scientific- HES appears to have hyperaggregating effects on red cells in the microcirculation [93]. Dextran (40 kDa), the most widely investigated replacement, reduces vascular resistance in machine perfusion systems, when compared with HES, from a combination of reduced viscosity and anti-thrombogenesis [94, 95]. These characteristics are particularly beneficial for vascular endothelial preservation and were the impetus behind it's inclusion in Perfadex [96, 97].

As a colloid PEG has been used both clinically and experimentally to scavenge free radicals [92] and appears to have further beneficial effects including modifying the rejection response [98]. Currently no commercial perfusion fluid uses PEG as a constitutive ingredient although two solutions underdevelopment for HPP incorporate the colloid [99, 100].

Reactive Oxygen Species

Equation 1

Superoxide ($2\ O_2^{\cdot-}$) + Hydrogen ions ($2\ H^+$) $\xrightarrow{SOD}$ Hydrogen Peroxide (H_2O_2) + Oxygen ($3\ 0_2$)

Equation 2

Hydrogen Peroxide (H_2O_2) $\xrightarrow{Catalase}$ Oxygen (O_2) and Water (H_2O)

Equation 3 (Haber Weiss)

$O_2^{\cdot-} + H_2O_2 + H^+ \xrightarrow{\text{Metal Catalysts}} O_2$ + Hydroxyl Radical ($HO^{\cdot}$) + H_2O

Equation 4

$O_2^{\cdot-} + Fe^{3+} \longrightarrow Fe^{2+} + O_2$

Equation 5 (Fenton)

$Fe^{2+} + H_2O_2 + H^+ \longrightarrow Fe^{3+} + HO^{\cdot} + H_2O$

Schematic Equation 6

Lipid (R–CH=CH-CH_2-CH=CH-CH_2-CH=CH-R') + Hydroxyl Radical ($HO^{\cdot}$)

↓

Alkyl radical($^{\cdot}$R-CH=CH_2)+Lipid aldehyde(0=CH-CH=CH-R')+Malondialdehyde(O=CH-CH_2-CH=O)

Equation 7

$$O_2^{\cdot-} + NO \longrightarrow NO_3^{\cdot} \text{ (Peroxynitrite)}$$

Oxygen free radical (reactive oxygen species, ROS) generation in IRI probably comes from three sources. The xanthine oxidase system during ischaemia producing hydrogen peroxide, uncoupled and overactive mitochondrial respiration at reperfusion and activated neutrophils after these effector cells have been marginated into the reperfused tissues [21, 43, 101]. The superoxide ion is the primary free radical produced by NADPH oxidase in lysozymes and the electron transport chain in mitochondria [20]. This undergoes rapid coupling with hydrogen ions in the presence of superoxide dismutase (SOD) to form hydrogen peroxide (Equation 1). Hydrogen peroxide degenerates to water and oxygen under the influence of catalase (Equation 2)[102].

During longer periods of IRI and in the presence of transition metal catalysts, both of these degradative enzymes can be overwhelmed causing a build-up of the hydroxyl radical *via* the Haber Weiss reaction (Equation 3). In the IRI cascade free iron becomes available from both intracellular sources [103] and extracellular haemolysis to enable hydroxyl radical generation *via* the Fenton reaction (Equation 4 and 5) [102]. This radical is responsible for a considerable proportion of the ROS mediated damage during IRI [104]. Lipid peroxidation is the classical mechanism of ischaemic cellular injury, although hydroxyl radicals have the potential to oxidise proteins and DNA directly as well. This process forms malondialdehyde, alkyl radicals and lipid aldehydes (Schematic Equation 6) and damages cells by changing the structure and function of membranes and membrane associated proteins. Malondialdehyde has therefore been widely used as an indicator of ROS mediated IRI in experimental organ transplantation [96, 105-107] and efforts to prevent its accumulation by reducing free iron, consumption or inhibition of superoxide and hydrogen peroxide abound [102, 104, 105, 108, 109].

Another source of free radicals is the nitric oxide system [19, 110]. Two isoforms of nitric oxide synthase with clinical relevance to IRI have been investigated. Constitutive endothelial nitric oxide synthase (*e*NOS) expression produces low level background NO at the endothelial smooth muscle interface and appears to be protective after ischaemia preventing platelet and leukocyte adherence to endothelial cells and improving blood flow by direct vasodilation (endothelial derived relaxing factor) [35, 36, 111]. Work from our own group has highlighted the difference that preservation solutions can make to endothelial dependent relaxation of vascular tissue after warm ischaemia [112]. The inducible form (*i*NOS), which can be up regulated on vascular smooth muscle tissue, and in macrophages, produces relatively high levels of NO [19]. This concentration of NO is capable of reacting with superoxide to form the peroxynitrite radical (NO_3 , Equation 7): a free radical capable of both nitration and oxidation reactions damaging membranes, proteins and DNA [110]. Cold ischaemia appears to have relatively few effects on NOS isoform expression, unlike warm ischaemia, which causes a distinct shift towards *i*NOS production and *e*NOS loss [36]. There is still debate about the relative merits of *i*NOS function during reperfusion with the demonstration of its critical import to the maintenance of vascular integrity after long periods of ischaemia [14].

UW solution contains two anti-oxidant systems capable of reducing ROS generation. Allopurinol, a xanthine oxidase inhibitor and reduced glutathione, a thiol containing amino acid, are both baseline constituents [84]. Neither of these agents are essential to maintain the therapeutic efficacy of UW solution and studies have shown that the glutathione rapidly becomes oxidised during storage [108]. To combat this Celsior [87] includes an oxygen absorber sachet in the packaging surrounding the solution which, it is maintained by the company, ensures more than 80% of the glutathione remains in the reduced form for 2 years (personal communication; Genzyme Inc., Massachusetts, USA). This has been shown to HTK contains ROS absorbing amino acids (tryptophan and histidine) [113], whilst mannitol is particularly effective at absorbing hydroxyl radicals [114]. Unlike other solid organs the lung is stored inflated with 100% oxygen permitting ongoing oxidative phosphorylation and ATP maintenance [67, 115]. Dextran (40kDa) does have some ROS scavenging ability but the absence of a more traditional anti-oxidant system from Perfadex suggests that this aspect of preservation solution technology is not as crucial to prevent pulmonary reperfusion injury [106].

Experimental addition of agents to reduce ROS mediated damage to preservation solutions has been an area of research in solid organ transplantation. As would be expected, catalysers of ROS degradation (catalase and SOD) have been successful at reducing hydroxyl radical generation and lipid peroxidation when used in highly controlled and refined artificial laboratory models (eg. [102]). Clinical trials with SOD in kidney transplantation suggested a long term benefit of reducing oxidant stress at the time of graft implantation on the vascular endothelium with major reductions in acute and chronic rejection [46], but made no impact on delayed graft function rates. Other anti-oxidants including ascorbic acid [105, 107], vitamin E [17], N-acetyl cysteine [116], glycine [33, 117] and desferrioxamine [109] have also been added to preservation solutions and improved experimental graft function, but doubts remain over their clinical efficacy.

Other Additives

Along with ROS scavengers- vasodilators, anti-coagulants, antibiotics, amino acids, steroids and growth factors have been added to preservation solutions.

Calcium channel (L -Type) blockers (Benzothiazepines and dihydropyridines) have been tested relatively successfully in experimental transplantation (verapamil) [118], but without evidence of effect in formal human trials (nicardipine) [119]. The postulated benefits include reduction of vascular smooth muscle contraction during cooling and reperfusion, as well as prevention of a calcium paradox type injury. Prostaglandin E_1 is a baseline constituent of Perfadex with evidence to suggest that it improves both distribution of preservation solution during cooling and ventilation/ perfusion matching at implantation [120, 121]. Nitric oxide donors (sodium nitroprusside, glycerol tri-nitrate) have been added to certain preservation solutions in an attempt to prevent "no-reflow" and appear to have certain benefits in organs

rich in endothelial tissue such as the liver and lung [97, 116]: the potential for generation of peroxynitrite radicals being a theoretical complication.

The classical anti-coagulant added to preservation solutions is heparin [48]. In its unfractionated form, this glycosaminoglycans molecule is not only capable of anti-coagulation, but also absorbs circulating chemokines preventing leukocyte migration [122].

The most common growth or trophic factor added is insulin, although this is not critical component of the original UW formulation. Recently, the Wisconsin group have trialled a new formulation of UW containing multiple trophic factors, including epidermal and neurone growth factor, in animal and liver renal transplant models with considerable success [16, 123].

Amino acids appear to be particularly good at improving viability of hepatocytes providing a metabolisable source of energy and exerting anti-proteolytic effects [124]. The newer preservation solutions based on cell culture technology place considerable emphasis on these to mitigate IRI [15, 17] utilising combinations of amino acids that have anti-oxidant (eg. glycine [33]), buffering (histidine) and NOS protective (L-arginine) effects [125].

Rinse Solutions

A significant clinical concern with using high potassium preservation solutions is the potential for inducing ventricular fibrillation during reperfusion of solid organs *in vivo*. Even with low potassium solutions, after long periods of storage with cellular necrosis, there may be several hundred millilitres of a proton and potassium rich solution ready to be flushed into the circulation of a critically ill patient. The pre-implantation "rinse" is therefore particularly crucial for larger organs like the liver. Clinical practice differs with some centres using either a further dose of low potassium preservation solution [126], saline and albumin [127], or a specialised solution: Lemasters's "Carolina Rinse" solution being the mostly widely trialled in hepatic transplantation [128]. As an aid to preservation the "rinse" also clears the microcirculation of debris, replenishes anti-oxidants and provides further substrates for energy regeneration at reperfusion.

ORGAN PRESERVATION IN THE 21ST CENTURY

The current crisis in organ transplantation has led to a resurgence of interest in using non-heart-beating donors (NHBD's) and developing organ preservation technologies to not only assess and maintain viability, but also with the potential to resuscitate organs. This has entailed a move away from cooling and cold storage with crystalloid solutions to cell culture technology and revisiting sanguineous perfusion.

To date successful transplants have been performed, using conventional preservation solutions, with kidney [1], liver [7], pancreas [8] and lung grafts [9] from NHBD's and experimental work on using NHBD hearts is under way [11]. The post-operative results of NHBD transplants are generally poorer with a greater incidence of delayed and primary non-function of grafts when compared with their "heart-beating" counterparts. As an immediate solution some centres with active programs are therefore examining the use of cardio-pulmonary bypass [1, 129] or extra-corporeal membrane oxygenation machines [12, 13] to reduce or eliminate the warm ischaemic time before retrieval rather than just simple intra-vascular cooling with preservation solutions.

In the medium term various novel preservation solutions for use with HPP systems will be available for clinical transplantation. Organ Recovery Systems™ have produced machines capable of HPP with hearts and pancreata using a Belzer-MPS solution. Guarrera's group have modified Belzer-MPS with various additives (ketoglutarate, L-arginine, N-acetyl cysteine, glycerol tri-nitrate and prostaglandins) and successfully machine perfused human livers [70] and kidneys [116]. A Dutch group have developed an oxygen driven HPP circuit (6 to 10°C) utilising Polysol®, an enriched cell culture medium with PEG as the colloid, to maintain liver and kidney viability in experimental models beyond that of traditional Belzer-MPS [17, 100].

The most ambitious groups have set about perfecting solutions for normothermic perfusion of organs, a particularly challenging endeavour considering that at these temperatures bacterial growth will be significant and substrate depletion requires close monitoring and manipulation. The high oxygen demands mean that specialised carrier molecules are also required. The great advantages of these proposed systems is the ability to overcome the harmful aspects of hypothermia [130] on cellular physiology and being able to use normal organ function (urine or bile production, oxygenation) as markers of organ viability rather than surrogate enzyme parameters. Imber *et al.* have managed to maintain livers extracorporeally producing bile for over 40 hours using dilute oxygenated heparinised blood at 39°C [15]. This required priming the perfusate with amino acids, glucose, calcium chloride and bile acid precursors on a regular basis. Brasile *et al.* demonstrated the potential of subnormothermic perfusion to improve the quality of organs damaged by warm ischaemia [14, 130]. This group's "exsanguineous metabolic support" system uses an acellular enchriched cell culture medium, oxygenated bovine haemoglobin and perfusion at 32°C to actually improve the viability of retrieved organs when compared with hypothermic storage. After 48 hours of perfusion, changing the perfusate every 4 hours, the canine kidneys displayed immediate function on re-implantation [14, 130].

CONCLUSIONS

Modern organ preservation technology would be alien in its complexity and yet familiar to those who pioneered the field in the 1960's and 70's. From the initial experiments using blood products for machine perfusion, through simple potassium based electrolyte solutions the technology has moved full circle back to blood based perfusates for normothermic perfusion.

The future would now appear to be metabolic rescue of marginal NHBD organs, although it must be remembered for those organs which can be safely removed from live donors that this form of preservation will be unnecessary and possibly even counterproductive. Considerable evidence has now accumulated that organs injured by hypoxia at different temperatures require differing electrolyte environments to maintain viability. It may be that the two technologies can conti-

nue to be developed in tandem, allowing for better targeting of financial resources and ultimately better utilisation of scarce donor organs for transplantation.

ABBREVIATIONS

ATP	=	Adenosine Tri-Phosphate
Belzer – MPS	=	Belzer's Machine Perfusion Solution (Sodium Gluconate)
HEPES	=	4 -(2-hydroxyethyl)-1-piperazineethanesulphonic acid
HES	=	Hydroxyethyl-starch
HPP	=	Hypothermic perfusion preservation
HTK	=	Bretshneider's Histidine-Tryptophan-Ketoglutarate
IRI	=	Ischaemia-Reperfusion Injury
MW	=	Molecular Weight
NHBD	=	Non-Heart Beating Donor
NO	=	Nitric Oxide
NOS	=	Nitric Oxide Synthase
ROS	=	Reactive oxygen species
PEG	=	Poly-Ethylene Glycol
SOD	=	Superoxide Dismutase
THAM	=	Tromethamine; 2-Amino-2-(hydroxymethyl)-1,3-propanediol

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