

PERSPECTIVE

THE COLD CHAIN DELIVERY OF ORGANS FOR TRANSPLANTATION: FROM RESEARCH LABORATORIES AND INDIVIDUAL ENTHUSIASTS TO PAN- GLOBAL NETWORKS IN 50 YEARS

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ABSTRACT

It is some 50 years since the first published reports appeared of *ex vivo* preservation of organs for transplantation. Over the intervening decades, organ preservation strategies have become one essential component of world-wide clinical transplant services. In the formative years, translational research in organ hypothermic preservation was grappling with the questions about whether static or dynamic storage was preferable, and the practical implications of those choices. Those studies were also informing the newly expanding clinical transplant services. During the middle years, both preservation modalities were practiced by individual group choices. By the 2000's, the shift in donor demographics demanded a re-evaluation of organ preservation strategies, and now a new era of research and development is promoting adoption of new technologies. In this review we outline many important academic studies which have contributed to this successful history, and give profile to the increasing innovative approaches which are being evaluated for the future.

Keywords: hypothermic machine perfusion; isochoric preservation; nanotechnology; organ preservation; organ cryopreservation; oxidative stress; preservation solutions.

INTRODUCTION

Organ transplantation has benefitted countless lives since the early developments seen in the 1960s. It has proven possible to offer life-sustaining therapies, in many cases, a more generalised restoration of health for those suffering from chronic end organ failure which

had proved to be intractable clinical problems up until that time. Organ transplantation is now an accepted practice globally, which permits intervention across all major organ systems where otherwise life-threatening diseases can be addressed. By replacing the defective organ physiology with the normal biochemical pathways encoded in the cellular components

within new grafts, an holistic health improvement can be offered which surpasses anything which can be achieved by providing individual drugs and medicines. The development of transplant services has also impacted on many other areas of medical innovation, including surgery, anaesthesia, intensive care support, ethical and managerial health deliveries (1).

Following the first single centre reports of the surgical transplant techniques, the spectacular success of the Boston team in 1953, acted as a catalyst in the 1960's for major clinical centres started to organise resources towards transplantation (2). As one example, a small number of kidney transplants were carried out in London at the Royal Free Hospital under the guidance of Moorhead, Hopewell and colleagues (3), although the outcomes were variable. However, these studies also identified the need for a robust pathway to deliver organs of high quality for transplantation, truly the beginnings of interest in organ preservation and in the cold chain delivery for organs.

THE EARLY DAYS - UNDERSTANDING THE BENEFITS AND CHALLENGES OF APPLYING HYPOTHERMIA

By the late 1960's, organ transplantation was being proposed as a realistic clinical therapy (Table 1). The questions then turned to dealing with logistics. It was already known from background studies in biochemistry and physiology over the previous century that organs could be manipulated outside the body and still retain good activities. It had been shown that in virtually all organs of interest, normal cell activity is fuelled by aerobic metabolism supporting the universal energy fluxes (via ATP turnover) which in turn maintain the myriad of balanced processes (called homeostasis). By removing organs from the body during transplantation, it is immediately obvious that these interacting pathways are jeopardised. Therefore it was intuitive to use cooling to hypothermic temperatures (mostly between 2°C to 10°C) to reduce the metabolic rate. More extensive descriptions of effects of hypothermia on cell metabolism, energy turnover and ultrastructure can be found in previous reports (4, 5, 6).

Renal transplantation was largely the earliest organ system to be addressed for surgical practice (7). The experiences of Calne and

colleagues supported the notion that whilst organ cooling was an important step for the removed kidney, simple surface cooling (by packing the organ in a sterile bag in melting ice) was not sufficient. Efficiency of cooling was improved by infusing cooled diluted blood via the renal artery, and the concept of cold flush preservation was established. However there were problems of intra-organ stasis and poor perfusion from using diluted blood. Other chilled solutions were sought out and for example, in 1968 a chilled infusion of Ringer's lactate solution was used for kidney flush cooling at the Royal Free Hospital in London UK (O N Fernando, Royal Free Hospital retired, personal communication). During the same period Collins and colleagues were developing a novel concept for a wash-out solution, namely that it should mimic where possible the intra-cellular ion contents of renal cells, which would reduce the driving factors for loss of homeostasis during the cold hypoxic state and improve organ preservation (8, 9). Additionally, being a synthetic solution it would reduce any variability in using blood from different sources for flush preservation. Collins solution quickly became widely used in a variety of countries for flush cold preservation (FCP). Other solutions based on balances of ions and buffers were proposed and found clinical uptake (10, 11, 12). However, during the same period of time in the 1960's, there were proponents of the alternative approach to organ preservation, namely hypothermic machine perfusion (HMP).

One of the most famous early practitioners of HMP was Folkert Belzer, working in San Francisco who developed a portable machine for renal preservation (13, 14). Humphries group were also active, and like Belzer reported results for preservation periods by HMP beyond 24 h using perfusates based on diluted blood or plasma products (15, 16). The main obvious differences between FCP and HMP were the ability to supply oxygen during HMP in a dynamic fashion, alongside the opening of the intra-organ vascular compartments, and assessment of potential injury markers or measures of active metabolism in the effluent solution throughout the perfusion period (5, 6). However, the need for sterile, reliable perfusion equipment and pharmacy-grade solutions avoiding blood products, rendered HMP relatively expensive, difficult to scale up, and only a limited number of specialist centres persisted with the technique in the following years.

**THE TARGETS FOR GOOD
PRESERVATION – UNDERSTANDING
THE ISOLATED ORGAN**

With the need to handle organs outside the body, there was a focus put onto the metabolic consequences of the ex vivo state. A consensus was achieved during the 1970's on the main problems of ex vivo organ ischaemia which defined the rapid decline in aerobic metabolism,

leading quickly to a growing disruption of cellular homeostasis i.e. maintenance of the intracellular milieu of ions, solutes and macromolecules which provide the support for cellular functions and ultrastructure, and how FCP might be used to influence this (17, 18). The cells of most organs can survive short disruption of homeostasis (1-2 h depending on the organ and any pre-existing pathologies] but as time passes the combination of damage effects eventually

Table 1. Historical development of different cold chain pathways for solid organs.

Technologies coming online	Author groups	Solid organ	Reference
Early studies on flush preservation (FCP) 1960's	Calne, Pegg & Colleagues	Kidneys cooled by infusing cold blood	(7)
Early hypothermic machine perfusion (HMP) 1960's	Belzer team; Humphries and colleagues	Kidneys on locally developed machines	(13, 15, 16)
Fundamental research mostly HMP; setting the basic scientific principles 1970's	Pegg, Green	Model systems, kidneys, livers, hearts	(28, 29, 30, 31)
Progressive FCP, new understanding of hypothermic injury; production of sterile synthetic solutions 1970's -1980's	Collins team; Ross and Marshall, Brettschneider	Kidneys, livers. Some work in hearts	(4, 6, 8, 9, 11, 12, 22)
2 nd generation preservation solutions; Used for both FCP and HMP 1980's onwards	Southard & Belzer team	Kidneys, pancreas, livers, some small bowel. Some work in hearts and lungs	(5, 14, 25, 27)
Concepts of oxidative stress in organ preservation; use of antioxidants and ion chelators 1980's onwards	Fuller, Green and colleagues; Southard & Belzer; Rauen group	Most solid organ systems	(19, 20, 27)
Upswing in HMP Novel machines, value of reliable oxygenation, transportability, associated disposables 2000's - onwards	Various groups; Dutkowski; Guarerra; Moers; Porte	Model systems; Kidney, heart, liver	(32, 39, 40, 41, 42, 43, 44)
Resurgence in interest of cryo-storage; model systems for organ vitrification; nanotechnologies for warming; high subzero preservation; isochoric cryopreservation 2010's onwards	Taylor group; Fahy group; Bischoff group; Rubinsky group	Model systems; kidney; heart; liver	(50, 60, 61, 62, 63, 64, 65, 66, 67)

becomes irreversible. The large part of basal energy metabolism is concerned with the maintenance of homeostasis, which combines complex, integrated controls of ion distribution, pH, solute content and osmotic potential within the cells. Anaerobic energy metabolism via glycolysis once again can only provide partial support. The issue is further complicated because the metabolic changes predisposes the organ cells to additional damage when oxygen is returned during reperfusion of the transplanted organ, leading to what became the classically-defined 'ischaemia reperfusion syndrome'. This is largely a consequence oxidative stresses from liberation of intracellular transition metals and an inability of subcellular mitochondria to process oxygen metabolism in a normal fashion (19, 20).

The global depression of metabolism induced by cooling is well documented (6, 21) but also is a double-edged sword. All intracellular metabolism is slowed, including both harmful or supportive pathways, and thus there is a cost – benefit ratio which in the end will dictate the organ survival *ex vivo*. The depression by cooling from normal body temperatures to close to 0°C can be understood using the 'Q10' relationship, which is the fall in reaction rates for each 10°C temperature drop (4, 22). For many biochemical reactions, the Q10 between 37°C to 0°C is approximately a factor of 3. Also, the impact of developing hypoxia in the organ *ex vivo* can depress metabolism for example by fall in intracellular pH (4, 6). Nevertheless hypothermia *per se* has a helpful limitation of some few hours, and thus the need became quickly identified to investigate strategies which could extend safe periods to clinically useful times (12 h or more) which allowed organisation of transplant services (14, 22).

In some specialised mammalian systems, prolonged hypothermia can be tolerated (for example natural hibernators (21, 23)). These physiological states are very different to organs removed from the body by FCP where hypoxia may also be a significant factor. In contrast, HMP there is the opportunity to supply oxygen by perfusion, and all the evidence points to a residual aerobic metabolism in clinically-preserved organs if there is sufficient delivery of oxygen (6, 24). In simple terms, hypothermia can be aerobic or anaerobic; even under aerobic conditions there is a global depression of metabolic activities, both catabolic and anabolic. Most attempts to improve organ preservation have thus focused on

managing the rates of molecular deterioration, always present as the underlying trends (24, 25) which in turn dictate the safe storage times in the clinic.

Even from the earliest times of clinical transplantation, the choices for organ preservation had to be either FCP (anaerobic - without oxygen provision) or cooling plus continuous vascular perfusion in HMP (aerobic – with the ability to supply oxygen in a variety of ways). The pragmatic choices made by individual centres were driven by logistics, expertise, availability and costs. Looking back over history, this led to centres choosing one method or the other and sticking with that over many years. It was not until much later that there was a wider debate and evaluation about the values of either FCP or HMP as outlined below.

FLUSH COOLING PRESERVATION – THE ANAEROBIC MODEL

FCP can be visualised as a pharmacological approach to protecting homeostasis during the cold period. The innovations made by Collins *et al.* in solution design were based on their understanding of cooling on cell physiology (8, 9). They applied their knowledge that cooling and hypoxia inhibited the transmembrane active pumping of Na⁺ and K⁺ via the ATPase, leading to influx of Na⁺, loss of K⁺ and associated influx of water (26). By designing a synthetic solution which mimicked as closely as possible the intracellular ion balances, Collins showed that FCP could be successfully used to preserve donor kidneys for several hours ahead of transplantation. Other solutes such as high magnesium and sulphate ions, plus phosphate which provided pH buffering, and glucose were included to target other homeostatic systems. These concepts, in addition to the possibility for sterile manufacture and distribution of Collins' solution encouraged a field of study on organ preservation solutions (OPS), which were a game changer in the establishment of renal transplant services across many countries. Appraising the pharmacology of different solutes and drugs at hypothermia was the key to success (27).

Different formulations of OPS were proposed, for example where citrate was used to replace phosphate as a buffer (12, 28) in what became known as Marshall's Hypertonic Citrate solution and which found wide clinical application in the 1980's. In another OPS, amino

acid buffers were used as the major additives rather than inorganic salts and this became available as Bretschneider's histidine-tryptophan-ketoglutarate (HTK) solution (29). Oligosaccharides proved effective for controlling cell oedema during cold preservation, and solutions such as phosphate buffered sucrose (PBS) (30) were developed. Sucrose based solution was formulated 20 years ago (31) and had some applications for cell preservation. A generalised overview suggests that any of these solutions can be effective for short (periods of up to about 18 hours) preservation depending on the organ under consideration (27).

The summation of this knowledge provided a major step change in the efficacy of OPS following the work by Southard, Belzer and colleagues (6, 32). They followed to some degree the principles of Collins (an intracellular mix of some ions) but introduced other novel solutes which in combination led to the University of Wisconsin (UW) solution. The high molecular weight anion lactobionate was introduced which also possessed properties of buffering and calcium chelation (free ionised calcium being one harmful aspect of cold preservation injury (6, 27)). The osmotic buffer was switched to raffinose, and a colloid (hydroxyethyl starch) was provided to protect the intra-organ vascular compartments – very important at the point where normal blood supply is re-established at transplantation. Lastly, antioxidants (glutathione and allopurinol) were added because there had been much concurrent research identifying oxidative stress as an injury of cold preservation / reperfusion, and adenosine was included to boost substrate availability for renewed ATP synthesis at the point of transplantation, boosting essential aerobic metabolism in the organ (33). Based on successful outcomes in donor organ preservation for both abdominal and (to some extent) cardiothoracic organs, UW solution became adopted in many countries for FCP in the multi-organ donor teams which were increasing globally over the next 20 years. Even today, when FCP is being practiced, UW remains one of the main OPS of choice (25).

Over the same time period, other OPS have been developed with changes in some of the components. For example Celsior is quite similar to UW but with an extracellular ion balance and also contains amino acid buffers and other antioxidants. Polyethylene glycol (PEG) is a colloid with putative extra protection and PEG-35

has been incorporated into the OPS IGL-1 (Institut Georges Lopez-1 solution). For OPS in cardiac preservation, antioxidants have been included in a range of synthetic solutions. Additional specific hypoxic modulators and antioxidants have also been investigated (5, 27).

Thus currently in FCP, there have been many changes proposed to the solutions which have often resulted in specific advantages such as amelioration of early reperfusion injury in the grafted organs, but it is still the case that the safe storage period has not been routinely extended for any organ beyond about 24h. Clinical imperatives such as the age and general health of the donor, and the degree of deranged physiology in the waiting recipient patient all combine to push organ retrieval teams to deliver organs within shorter FCP times if possible (21, 27).

HYPOTHERMIC MACHINE PERFUSION TECHNOLOGY ALLOWING ACTIVE INTERVENTION

The concepts of supporting organs *ex vivo* with an artificial circuit and oxygenation had been developed since the early 1900's, and progressed by the pioneering work of Carrel and Lindbergh [reviewed in (6)]. With the arrival of clinical transplantation in the 1960's, there was already a body of opinion supporting perfusion techniques, allied with cooling, to deliver high quality organ preservation (5, 6). More recently, the acute shortage of suitable organ donors and the inevitable pressure to use organs from sub-optimal (often called expanded criteria) donors, has forced a re-evaluation of HMP, and the development of a new generation of HMP machines and associated OPS, which will be discussed below.

HMP developed at a time when other clinical technologies where controlled *ex vivo* circulation of solutions were being introduced, e.g. cardiopulmonary by-pass technology and renal haemodialysis. The series of articles from Pegg and Green in the 1970's provided many of the important scientific technical details for HMP which are still relevant today (28, 29, 30, 31). These all sign-posted the importance of reliable pumps, tubing sets of sterile, non-thrombogenic materials, suitable synthetic acellular OPS, stable temperature control and oxygenation [12][32]. In depth descriptions of HMP have recently been provided (24, 32), but a summary is provided here for comparison with FCP.

Pumps and pressures for HMP

Mechanical roller pumps were used in early systems, with a pulsatile flow pattern (33, 34) and providing sufficient but low pressures for opening for the capillary beds within the organ. This was based on extensive work by Pegg and Green's group (29, 30, 31, 32, 33), showed that a stable low flows with reduced pressures (31, 32) was optimal since higher flows increased the risk of damage to the vascular endothelium. Recently, atraumatic centrifugal pumps (which have become commonplace in various clinical bypass perfusion systems) were adopted for HMP (5, 35).

OPS for HMP dynamic perfusion

Early studies in HMP used diluted blood or plasma protein solutions as OPS (6, 15). Belzer's Machine Perfusion solution was developed as a variant of the raffinose containing solution which led to the UW formulation, with the main differences being inclusion of gluconate as the major anion, and a different HES fraction as colloid (6, 36, 37). This was designated KPS-1, and is widely applied (6, 38). The KPS-1 base was modified for liver perfusion by adding antioxidants, vasodilators and metabolic intermediates (N-acetylcysteine, L-arginine, nitroglycerin, prostaglandin E1, α -ketoglutarate) to produce Vasosol® (39, 40, 41).

The recent interest in optimally oxygenating HMP has refocused attention to use of red blood cells for their oxygen carrying capacity by applying OPS for erythrocyte dilution. The albumin-based Steen solution with a plasma-like ionic balance, has diluted erythrocytes for cardiac HMP (27, 42), with additional hormones and antibiotic imipenem. Steen solution has also been applied to clinical normothermic oxygenated liver perfusion as erythrocyte diluent (27). In another trial, oxygenation was facilitated using erythrocytes diluted using the colloid gelofusine, supplemented with gluconate, sodium bicarbonate and cefuroxime for liver normothermic perfusion (27, 43).

Dynamic end-ischaemic reconditioning HMP used oxygenated Custodiol-N (based on HTK) in a small clinical trial which also investigated graded rewarming of the stored livers during perfusion (32, 44). Adequate oxygen delivery during perfusion presents opportunities to introduce novel solutes into OPS. A cell-free bovine haemoglobin product has been tested in a human liver perfusion mode (32).

The need for reliable oncotic agents in perfusion is another factor to consider. Diluted blood and blood products were used in early work (15, 16), but seen to have technical problems. Attempts were made to employ serum albumin as the oncotic agent (6, 31) but concerns remained about batch variation and costs of purification.

The step-change in HMP once again followed work from Southard and Belzer who used a synthetic perfusate, based on hydroxyl ethyl starch (HES) as colloid, and the production of the purified HES penta-starch fraction provided a colloid which was found to be suitable for long-term HPP (5, 6, 14).

The need for oxygenation, filtration and monitoring

Given the recognised residual aerobic metabolism in hypothermic organs (6), it was intuitive to plan direct oxygen supply using different oxygenator systems (6, 25, 32). In experimental situations, novel oxygen carriers (such as produced by marine invertebrates) have been added to OPS such as Perfadex® (27, 33) but have not yet been widely employed in the clinic. Clinical end-oxygenated HMP liver systems often use membrane oxygenators in the circuit. Such systems are now in wide clinical use and contain additionally filters to avoid micro-aggregates injury to the organ (32), and real time sensors for perfusion pressure and temperature. Further details can be found elsewhere (32).

FUTURE TRENDS IN ORGAN PRESERVATION FOR TRANSPLANTATION

The demand for robust, successful cold chain delivery of organs for transplantation will continue to grow as more countries engage in this clinical therapy, and different models of donor organ retrieval develop – for example moving to a hub and spoke model with specialist centres taking on organ conditioning and providing high quality organs to their own network of transplant centres [34,35]. Each of the different approaches will require further research as new molecular techniques become available.

Flush cooling and static cold storage

Whilst a lot is known about the effects of the solutes included in current OPS, there may yet be opportunities to better support the static cold organ and prepare it for rapid full functional

recovery (27) . For example, oxygenation of the cold OPS just before infusion has been studied at a research level (36) and specific molecular pathways of protective solutes (27).

Dynamic HMP

The last 5 years have seen an increase in enthusiasm for clinical applications of HMP, supported by wider availability of HMP machines, and a need to deal with problems of donor organs which have experienced hypoxic episodes during donation (32). Results for larger patient cohorts, multi-centre collaborations and randomised trials against static cold storage are all being reported (38, 39, 40, 41, 42, 43). The concept of altering perfusion temperature during perfusion of the same organ are also being investigated (44), along with warm perfusion (45), which may be ways into organ reconditioning as new molecular interventions become available in the coming years.

Subzero organ preservation

There has been a long history of attempts at solid organ cryopreservation but they have all been confounded by the multiple problems of ice nucleation and crystal growth during cooling to and recovery from deep cryogenic temperatures (46, 47, 48, 49, 50) . Much has been learnt about the perfusion technologies required for loading and unloading essential CPA (51, 52, 53) and cooling strategies for the 3-D volumes which organs represent (54, 55, 56) but even with these in depth investigations it has not been possible to establish a clinical programme for frozen organs. However, there has been renewed interest in tackling the problems of organ cryopreservation (57) as the efforts to avoid donor organ wastage have intensified, and new approaches are being brought forward into the 2020's.

(i) Organ vitrification. One way to avoid ice crystal injury is to cool by the process of vitrification, which depends to a large extent on using very high concentrations of CPA which can inhibit ice nucleation. The group of Fahy and colleagues have over some years developed sophisticated technologies for CPA perfusion, cooling and warming, all under careful microprocessor control (58, 59, 60). Partial Success has recently been shown in experimental kidneys after transplantation (61).

(ii) Focusing on organ rewarming by nanotechnology. The size of vitrified organs has been a recognised challenge to organ vitrification for many years (60, 61). In general, surface

warming of such large volumes is a slow process, which gives time for harmful ice recrystallization during warming. Recently the concept of using ultrasound irradiation has been developed to warm vitrified organs throughout their entire volume by nanotechnology and this shows great future promise (62, 63, 64).

(iii) Isochoric subzero preservation. Another way to interfere with ice nucleation is to apply the concept that high pressures can modulate the way water molecules behave. The ice-water transition is accompanied by an increase in volume, so restricting the potential volume change can increase the pressure within the system. This is the technology of isochoric freezing, which again requires special equipment for reliable application, but recently advances have been made in this area which may help organ cryopreservation (65, 66, 67) although this has yet to be studied in the context of organ preservation.

(iv) High subzero limited duration freezing. Interest has also recently been rekindled in application of freezing temperatures down to about -20 C which might provide limited duration of organ preservation or organ cryostasis. The approach also needs careful interactions between CPA and other additives and provide stable storage for a few weeks (50). By avoiding deep cryogenic temperatures many of the technical and physical requirements for long-term cryopreservation may be avoided and thus become easier to apply across clinical services, and important future investigations for this approach are being planned.

SUMMARY

The success of current cold chain approaches to organ preservation continue to be one key component of transplantation services. These have been refined to a level whereby the protocols can be transferred between centres on an international basis, with excellent reproducibility and governance. The earlier studies on organ hypothermia and hypothermic machine perfusion have led to an almost universal consensus that dynamic preservation can greatly improve transplant outcomes given the expansion of acceptance criteria for more marginal donor organs. As of the current time there are some ten clinically approved organ machine perfusion technologies and the potential for additional modalities is increasing – there appears to be a

growing appetite for these technologies in the clinic [68]. The much sought ability to take solid organs below to temperature of ice nucleation for prolonged storage seems tantalisingly within our reach, and reports on organ vitrification with life sustaining function are starting to arise (69). Never-the-less, the fast moving innovations in patient treatment based on organ transplantation continue to encourage new research into better and longer-period preservation of organs outside the body and enhanced technologies for manipulating organs *ex vivo*. The history of organ preservation remains still only partially completed, and the next decade is likely to see yet further advances which will change the face of how transplant services are delivered.

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