Improving liver preservation
't Hart, Nils Arnaud
Chapter III

New Solutions in Organ Preservation

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In the late 1960s Belzer et al. and Collins et al. demonstrated successful kidney preservation using continuous hypothermic machine preservation and simple cold-storage. Since then, many investigators have tested numerous methods and solutions to be used in organ preservation. Over the last 20 years a number of methods and solutions were developed to preserve the viability of donor organs before, during, and after transplantation. Significant improvements in suppression of rejection and treatment of postoperative complications were accomplished and consequently graft viability improved. These major achievements in organ transplantation resulted in an increase in graft and patient survival. However, despite increased survival rates, we are still counting preservation times in hours rather than in days. In this respect, the method of preservation and the preservation solution are of major importance to bridge the time span between procurement and transplantation and remain vital issues for further improvement of organ viability.

Many factors determining organ viability prior to transplantation are donor and preservation related. Ideally, organs should be retrieved in perfect condition and preserved in the most ideal cold-storage preservation solution. Each potential graft is however, affected by several deteriorating mechanisms. Cadaveric organs originate from either brain dead donors or non-heart-beating donors. The state of brain-death is characterized by hemodynamic instability, an autonomic catacholamine storm and is often accompanied by an increased length of hospital stay after transplantation and with a non-ideal nutritional status of the donor. Non-heart-beating donors are subjected to an uncontrolled or a controlled cardiac arrest, i.e. ventilator switch of procedures, that result in a detrimental period of warm ischemia.

To date, the clinically most popular and applied technique in organ preservation is static cold-storage (CS). Negative effects of hypothermia, however, limit the duration of preservation expressed as the cold-ischemia-time (CIT) and needs to be counteracted.

Thus, progress in preservation can only be achieved with an adequate understanding of mechanisms involved in ischemia-reperfusion injury. The important components in a preservation solution are directed towards the physical and the biochemical environment. Most components acting on the physical environment counteract edema formation. Other agents act on the biochemical environment and minimize potentially harmful precursor release such as hypoxanthine, which can be converted to xanthine, forming reactive oxygen species during reperfusion. This review describes a number of prominent pathophysiological mechanisms that are of interest during hypothermic conditions and subsequent reperfusion. The components that counteract negative effects related to cold and warm ischemia are summarized and a comparison is made between important components in different preservation solutions. Finally, a number of preservation solutions is discussed.
Pathophysiological effects of organ preservation

Effective organ preservation is still based upon the principle of lowering the metabolic rate of the organ. Reducing the organs core-temperature below 4 °C results in a decrease in metabolism to approximately 5-8 % in the majority of cells and inactivates enzyme-mediated reactions\textsuperscript{5,6}. The inactivation of most enzyme reactions protects the cell and lowers the use of high-energy purine nucleotides. During organ procurement, a rapid vascular perfusion cools down the organ\textsuperscript{7}, removes the blood to prevent clotting and subsequently obstruction of the microvasculature\textsuperscript{8} and allows an equilibration between the cold storage solution and the tissue\textsuperscript{4,9}. Despite a rapid onset of hypothermia, hypothermia remains a suboptimal condition due to the onset of cold-ischemia. Also blood components that remain in the donor organ during cold-ischemia will have a detrimental effect on tissue viability after reperfusion\textsuperscript{10}.

Hypothermia induced cell swelling

Histological prominent alterations in cellular structure during hypothermia are cell swelling\textsuperscript{11} and formation of protruding pockets. The mechanisms underlying these structural changes are impaired activity of membrane bound sodium/potassium ATPase and impaired excretion of water from the cell. During hypothermia, sodium is no longer actively excreted but tends to enter the cell due to intracellular proteins. These anionic proteins create an osmotic force resulting in a passive transport of sodium across the cellular membrane. The hyperosmolar intracellular environment subsequently results in an influx of water into the cell. The osmotic driving forces as well as the impaired sodium/potassium ATPase both result in cell swelling. Counteracting these mechanisms is an important feature of CS solutions. Organ preservation with CS requires the definition of three fluid compartments in preventing cell swelling and interstitial edema formation. These compartments are the intravascular, interstitial and intracellular compartments and require the definition of components which are capable to maintain an optimal balance. Impermeants and saccharides can achieve homeostasis of the intracellular water content. Homeostasis of the interstitial compartments is achieved by counteracting an hydrostatic force during the initial wash-out using colloids. The intravascular fluid compartment does not need an effective component in static cold-storage.

Compromised membrane function

During hypothermic organ preservation changes in cell morphology and the development of characteristic protruding pockets are observed\textsuperscript{12}. These changes are not only due to hypothermic cell swelling. A second mechanism is the compromised integrity of the cytoskeleton due to increment of the calcium concentration of the cell\textsuperscript{13,14}. Increasing cellular calcium concentration results from either inactive ATPase
or depletion of ATP\textsuperscript{15,16}. Calcium efflux from the cell consequently decreases, resulting in increased cytosolic calcium that results in activation of phospholipases\textsuperscript{17} and ultimately cell death\textsuperscript{6,15}. Enhanced phospholipase activity results in a fusion of the inner and outer membrane of the mitochondria and generation of holes in the cellular membrane\textsuperscript{6}. To prevent this mechanism agents that interfere with the calcium-calmodulin cascade which activates phospholipase A can be added to the preservation solution. Calmodulin is localised in cytosol and is activated by binding calcium to one or four sites\textsuperscript{15}. Each binding site results in a conformational change of its apha-helical structure and these changes effectuate enzym reactions. Effective agents in preventing calcium mediated injury are calcium-entry blockers such as Verapamil\textsuperscript{17} or other mediators preventing the activation of the Ca-calmodulin complex itself, e.g. trifluoperazine\textsuperscript{6}.

**ATP usage and cellular acidosis**

Under hypothermic conditions, the metabolic rate is decreased. With each 10 °C decrease, the metabolic rate diminishes 1.5 to 2.0 fold. At 0-4 °C, there is still some degree of metabolic activity, causing an ischemic/anoxic cascade leading to cellular injury and finally to cell death\textsuperscript{6}. The ongoing metabolic rate rapidly depletes cellular ATP content. The inability to phosphorylate ADP and thus regain energy prevents the use of ATP in energy requiring processes. Dephosphorylation of ADP to AMP and further to adenosine, is the energy source that is used alternatively\textsuperscript{18}. Under anaerobic conditions, adenosine is not re-phosphorylated to form ATP but is degraded to inosine and hypoxanthine\textsuperscript{19,20}. During reperfusion the accumulated hypoxanthine can easily leave the cell and is lost in the intravascular space\textsuperscript{21}. Without the presence of oxygen, anaerobic glycolysis occurs to generate high-energy phosphate bonds. During this process, metabolism of 1 mol glucose only yields 2 mol ATP versus 38 ATP molecules in aerobic circumstances. Unfortunately, during anaerobic glycolysis two lactic acid molecules are formed as well. The ongoing metabolism and the increasing amount of lactic acid contribute to a progressive acidosis\textsuperscript{6,22,23}.

Acidosis results in activation of phospholipases and proteases that induce lysosomal lysis and eventually lead to cell death\textsuperscript{6,23}. The depletion of cellular energy results in inactivation of the energy dependant, membrane bound sodium/potassium pump and inactivation of the calcium/magnesium pump\textsuperscript{6}. Due to the formation of membrane leaks, mitochondria are now no longer able to couple the electron transport chain with phosphorylation processes. To prevent a decrease in pH an adequate buffering capacity of the preservation solution is mandatory.

**Prevention of precursor formation of Reactive Oxygen Species**

An important issue in organ preservation is the generation and role of toxic Reactive Oxygen Species. A prominent generator of ROS is xanthine oxidase. During ischemia,
xanthine dehydrogenase is converted to xanthine oxydase by proteolytic enzymes\textsuperscript{24,25}. Xanthine oxidase uses oxygen as an electron acceptor instead of NAD that is used during aerobic glycolysis\textsuperscript{26,20}. Under normal conditions oxygen is reduced in the mitochondrial electron transport chain (ETC) since it has an electron-seeking potential. Oxygen is reduced to water, by dehydrogenases, quinons and cytochrom-C. However, small amounts of molecular oxygen leak out of this tetravalent pathway and enter a univalent pathway. In the latter case free radicals (\(O_2 \cdot^\cdot \) and \(HO\cdot\)) are produced. These ROS are potent oxidizing and reducing agents and are intracellularly detoxified by super-oxide-dismutase (SOD), catalases and peroxidases with water as the end product\textsuperscript{27}. The formation of ROS occurs during reperfusion\textsuperscript{28} and is a combination of preservation and reperfusion injury. The detrimental effects of ROS, however, can be minimized using an effective preservation solution that also includes antioxidants.

To bridge the inevitable timespan between organ retrieval and transplantation effective preservation is mandatory to allow maximum organ viability after reperfusion. Currently used preservation solutions were designed to minimize the negative effects of hypothermia. Essential constituents for effective preservation are the sodium-potassium ratio, the addition of impermeants, an adequate buffering capacity, and the use of scavengers of oxygen free radicals and inclusion of ATP-precursors\textsuperscript{29,30}. Table 1 shows individual components related to the physical and biochemical environment in different preservation solutions. In the next paragraph a number of specific components of preservation solutions is discussed.

**Table 1:** Effective Components of Cold-Storage Solutions

**Table 1a:** physical-environment

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<tr>
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<th>EC</th>
<th>PBS</th>
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<th>HTK</th>
<th>UW</th>
<th>Celsior</th>
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<td>Na</td>
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<td>Citrate</td>
<td>Histidine</td>
<td>Phosphate</td>
<td>Phosphate</td>
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<td>Mannitol</td>
<td>Raffinose</td>
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<td>Lacto-</td>
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**Table 1b:** biochemical-environment

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<th>UW</th>
<th>Celsior</th>
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<td>Mannitol</td>
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<td>Ketto-glutarate</td>
<td>Adenosine</td>
<td>Glutamate</td>
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</table>
Important components in preservation solutions

In the last three decades several solutions for preservation were developed. The first breakthrough in improving organ preservation using the cold-storage technique was the development of the Collins solution. This solution was used for kidney preservation in the late 1960s\(^6\). From 1970 until 1986, the most frequently used solutions were: Eurocollins-, Collins-, Sacks- and Marshall’s hypertonic citrate solution (HOC). Prominent differences between the four solutions used in this era are the electrolyte composition and iso- or hyperosmolarity of the solution. In Eurocollins solution, which is a Collins solution modified by the Eurotransplant Organization, magnesium was omitted due to induced formation of crystals during shelf storage. Subsequent viability testing over the years revealed no differences in kidney function comparing Eurocollins and subsequent Collins-modifications. Eurocollins, Sacks’ and HOC are hyperosmolar solutions and hyperosmolarity was proven to be equally effective in organ preservation compared to the iso-osmolar Collins solution\(^31\). A major step in organ preservation followed in the 1980’s, when in 1986 the UW cold storage solution was developed by Belzer and Southard. Its development lead to a wide spread revival of interest in organ preservation\(^32,33\). The new UW cold-storage solution was based upon concepts derived by Belzer from his vast experience with machine preservation. For the first time this University of Wisconsin-cold storage solution (UW-CSS) allowed extended experimental preservation times of pancreas\(^34\), kidney\(^9\) and liver\(^11\). Following these experiments UW was successfully introduced into the clinic\(^35-37\). In Table 2 the currently used average clinical preservation times are indicated per organ as well as the experimental experience. After its introduction, the UW-CSS became the most tested preservation solution as many investigators intended to unravel which of its components are essential. Some of its components are now considered to be key components for newer modifications. In the next paragraphs a number of steps towards the development of the UW-solution are described.

Table 2: Current Status of Clinical and Experimental Cold-Storage Preservation

<table>
<thead>
<tr>
<th>Organ</th>
<th>Clinical</th>
<th>Experimental</th>
<th>Optimal CS solution</th>
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<tr>
<td>Kidney</td>
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<td>120 hours</td>
<td>&lt; 24 hours: UW, HTK, PBS &gt; 24 hours: UW</td>
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<td>96 hours</td>
<td>UW, (HTK)</td>
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<td>Liver</td>
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<td>48 hours</td>
<td>UW, HTK</td>
</tr>
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<td>Small Bowel</td>
<td>8 hours</td>
<td>48 hours</td>
<td>UW</td>
</tr>
<tr>
<td>Lung</td>
<td>8 hours</td>
<td>24 hours</td>
<td>EC, UW, Pap</td>
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<tr>
<td>Heart</td>
<td>6 hours</td>
<td>24 hours</td>
<td>CU, HTK, UW</td>
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Electrolyte composition: key components originally defined

In the early years of organ preservation it was believed that a high sodium/potassium ratio of the preservation solution was effective in preventing cell swelling. These intracellular type solutions copy high intracellular potassium and low sodium concentrations as opposed to the extracellular fluid compartment. The exchange of fluids between the cellular, intercellular and vascular compartments was thought to be reduced by creating an intracellular sodium/potassium ratio in the vascular space. In the concept of cellular electrolyte concentration and cell swelling the so-called Donnan equilibrium plays an important role. The balance between extracellular sodium ions and intracellular protein anions creates the Donnan equilibrium that prevents cell swelling. Initially cold-storage preservation solutions were developed using this equilibrium to determine their electrolyte composition.

The intracellular type solutions were long considered to be mandatory for maintaining cell viability. It was assumed that due to the inactivity of sodium/potassium ATPase under hypothermic conditions a high sodium/potassium ratio in the extracellular fluid compartments would prevent sodium and chloride to enter the cell. Therefore, sodium would not contribute to the generation of an osmotic force inside the cell and compromise the cell membrane potential. Over the last decade, however, it was shown that both intracellular and extracellular type solutions are equally effective. Heart and pancreas preservation even seem to benefit from extracellular type solutions. An additional beneficial effect of extracellular type solutions is improved wash-out of blood during organ procurement. Hence, a potassium induced vasospasm resulting in increased vascular resistance can be prevented, contributing to a better graft function. In general the concept has now become to use extracellular-like solutions for preservation in multi-organ procurement.

Buffering capacity

Another important feature of preservation solutions is the prevention of cellular acidosis. Frequently used buffers are phosphate buffers, histidine or citrate, as shown in Table 3. Adequate buffering is of major importance in preventing cellular acidosis as well as activation of phospholipases. Although phosphate buffers are effective and frequently used, histidine can be a suitable alternative. The choice of buffer should be based upon its secondary effects, e.g. chelation of calcium or inhibition of enzymes, since the buffering capacity is sufficient in both phosphate and histidine buffers. In addition to its function as a buffer, Histidine is also an inhibitor of matrix metalloproteinase (MMP) and an impermeant.
Table 3: Composition of CS Preservation Solutions

EC: EuroCollins, PBS: Phosphate-Buffered-Sucrose, HTK: Histidine-Tryptophan-Ketoglutarate,
UW: University of Wisconsin Cold Storage Solution, HOC: Hypertonic Citrate, HES: hydroxyethyl starch,
ROS: reactive-oxygen-species

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Impermeants

During hypothermic preservation cell swelling occurs due to the intracellular osmotic force, generated by proteins and anions. To counteract this effect, powerful impermeants are added to preservation solutions. These agents are ideally localised in the intravascular or interstitial space and thus reduce cell swelling by generating an osmotic force outside the cell. Effective impermeants in preservation solutions are saccharides and negatively charged anions that prevent cell swelling. Molecular size and effectiveness of saccharides are related with respect to prevention of cell swelling, with the larger saccharides being more effective. The most effective saccharide is raffinose (594 mW), followed by sucrose (342 mW), mannitol (182 mW) and finally glucose (180 mW). Initially glucose was commonly used in cold-storage solutions, but is replaced by larger molecular size saccharides. Smaller sized sugars such as glucose and mannitol, are able to pass cellular membranes and are therefore less effective in preventing cell swelling. Since small saccharides pass membranes they are able to contribute to a shift in osmotic force from the interstitial side towards the intracellular side of the cell membrane. Furthermore, intracellular glucose is used in an anaerobic environment as substrate to produce ATP and lactate. The increase in cellular lactate induces lactate-acidosis that is the main reason why glucose is not longer in use as an impermeant. Another saccharide is mannitol and is only slightly larger than glucose. Although mannitol passes through cellular membranes, it is still used since it has a secondary beneficial effect as an oxygen radical scavenger. The larger saccharides sucrose and raffinose are used in Phosphate Buffered Sucrose (PBS) solution and in UW-solution respectively. Both saccharides do not pass cellular membranes.

Non-saccharide impermeants reduce cell swelling by electrochemical force. These impermeants are anions, such as citrate, gluconate and lactobionic-acid. Size and charge determine the effectiveness of these anions, with lactobionic-acid (358 mW) as one of the most powerful ones, while chloride and gluconic-acid are less effective in preventing cell swelling. A number of experimental studies have discussed the importance of lactobionic-acid in preservation solutions for abdominal organs. It was originally included in the UW solution based on tissue slice studies of the pancreas. It turned out to be important for several other abdominal organs as well. The success of lactobionic-acid in UW-CSS raised the question whether it was possible to simplify preservation solutions with lactobionic-acid as a key component. In pancreas preservation a simple lactobionic-acid based solution resulted in similar survival rates compared to the original UW-CSS. Moen et al. demonstrated that the use of lactobionic-acid in combination with electrolytes in an extracellular composition was as effective as the original UW-CSS with a high potassium content. The same effect was confirmed by Sumimoto et al. who also included histidine to buffer a lactobionate based solution: histidine-lactobionate. Following the same concept, Collins et al. showed also good results with his sodium-lactobionate-sucrose solution. Southard et al,
finally, postulated that the beneficial effect of lactobionic-acid is a result of its relatively large molecular weight of 358 Daltons leading to an inability to pass cellular membranes. In addition lactobionic acid is also a strongly charged anion with a membrane protective effect.

**Colloids in preservation solutions**

Colloids are effective ingredients in preservation solutions because they can not pass the cellular membrane. Static CS solutions, however, usually did not contain any colloids. In the past, Belzer and Southard reasoned the addition of a colloid in static CS as they intended to develop one preservation solution suitable for both CS as well as continuous hypothermic machine perfusion techniques (MP). In solutions used in MP, a high colloidal substance is routinely included to counteract extravasation of fluids due to hydrostatic pressure. Besides the practical approach of designing one solution for static as well as dynamic preservation methods, an additional consideration was used. Interstitial edema formation during the donor hepatectomy or nephrectomy and in situ blood wash-out is prevented with colloids in static CS-solutions. Another reason was the variability between outcomes of abdominal and thoracic organs after cold-storage preservation in colloid containing and in non-colloid containing solutions.

The necessity of the diafiltrated hydroxyethyl starch (HES) has been the focus of several studies and subject of many debates over the past years. The reason for Belzer and Southard to include HES was based on their experience with continuous machine preservation techniques, where HES prevents edema formation due to hydrostatic pressure. Thus in MP the presence of HES in the perfusion solution is important, however it is questionable whether HES is necessary in CS solutions. In the early nineties it was reported that the importance of HES differed between organs and that HES was not essential for short-term liver or kidney preservation, but is important in pancreas and heart preservation. In rat liver preservation with UW, Howden et al. showed results that did not differ with or without HES in the UW-CSS. As many groups before, Howden et al. used transaminases, bilirubin and albumin as parameters to determine liver function and reflect liver viability. More recently, protein synthesis rate has been used as a more refined parameter for liver viability. This study revealed, unexpectedly, that inclusion of HES in UW-CSS supported protein synthesis in the liver. In their paper Neveux et al. described that HES was important for maintaining hepatic metabolism as well as reduce the content of interstitial fluids. In addition, Calmus et al. showed the detrimental effects of proteolysis after transplantation. Finally the most prominent and beneficial effects of colloids, included in preservation, are seen after long-term cold ischemia times and transplantation. Thus, a preservation solution applicable for all organs as well as for both preservation methods, CS and MP, can still benefit from the inclusion of an intravascular colloid.

While edema formation is prevented by colloids, this is not the sole beneficial action
of colloids in preservation solutions. Since the dispute about HES as an important component in CS preservation, other colloids, such as dextran were tested for their efficacy.55,58. Another beneficial effect of some colloids is their ability to reduce aggregate formation of blood cells. This effect results in an optimal wash-out of blood prior to procurement.55,59. It is however not clear by which mechanism colloids influence the initial wash-out of organs during the donor procurement operation. Different colloids are tested for cryopreservation techniques for blood products and it is shown that HES, albumine, dextran or polyethylene glycol do not influence the coagulation system.60. Thus colloids, as they are in use for preservation solutions, do not alter the coagulation system and bloodcell aggregation is likely to be caused by another mechanism. In contrast to dextran, the use of conventional UW with HES, does not show a complete wash-out of the organ. We recently showed in our laboratory that HES in the UW solution increased formation of red blood cell aggregates. The mix of blood with the original UW-CSS induced increased aggregation compared to UW without HES and compromised the initial blood wash-out. This phenomenon can finally explain the slower wash-out with UW and also the sometimes initially patchy reperfusion of the donor organ. We were able to demonstrate that the viscosity of the UW-solution, can not entirely explain both effects.

**Scavengers of reactive oxygen species**

Reactive oxygen species (ROS) production can partly be prevented by inhibition of ROS producing enzymes. Xanthine oxidase, an enzyme generating ROS precursor is inhibited by allopurinol.54,61. Although its action during an ischemic period is not fully understood, allopurinol is shown to be a beneficial component during preservation, possibly as a result of less ROS formation during the reperfusion phase. Liver and pancreas preservation with CS solution lacking allopurinol did not affect animal or graft survival in contrast to kidney preservation. Allopurinol is an effective component for kidney function although it does not affect recipient survival.28,62. Long term effects however might be influenced, improved short term kidney function is therefore sufficient to advocate allopurinol to be included in preservation solutions.

Another common component preventing ROS induced injury, is glutathione (GSH). This naturally occurring tripeptide consists of glutamic acid, cysteine and glycine.63. The main mechanism involved in the specific function of detoxification of ROS is the oxidation of GSH to GSSG, which is catalyzed by the enzyme glutathione peroxidase. The half-life of GSH is however limited to eight days at 4 ºC during shelf-storage. During storage, GSH content decreases, and oxidizes to GSSG, loosing a hydrogen group and forming a disulfide bridge with a second GSH peptide.64. Some authors even mention a half-life of just one day.65. Experimental research proved GSH to be important for liver graft and animal survival beyond 24 hours preservation.64,66. Vreugdenhil et al. therefore recommended a supplementation of fresh GSH to the solution, just prior to use and
organ retrieval. Other authors have published data suggesting that there is no beneficial effect of supplementation of GSH. These authors, however, have looked at preservation times less than 24 hours, while, GSH supplementation is especially of importance for medium term or long term preservation beyond a 24 hours period.

**Secondary effects of important components**

In addition to their primary task in a preservation solution, some components have a beneficial and useful secondary effect preventing injury and maintaining organ viability. Both, lactobionic-acid and histidine are chelators of calcium. Chelating calcium effectively prevent the uptake of calcium in the cell, and therefore are important in decreasing calcium activation of hydrolytic enzymes. Calcium is also a strong trigger for smooth muscle contraction. The initial vasoconstriction during organ wash-out is due to the potassium content of the preservation solution. High concentrations of intravascular potassium result in hyperpolarisation of cell membranes and an influx of calcium into the cell through voltage-gated calcium channels. By subsequently chelating intravascular calcium, a net shift of calcium outside the cell occurs, which results in a decrease in intracellular potassium content. High concentrations of cytosolic calcium can therefore result in vascular constriction. This could explain the observation in kidney transplantation in rats, where a reduction of potassium resulted in an increase in flush rate. Also, some authors have been able to demonstrate that preservation results improve by using calcium channel blockers, such as verapamil and nifedipine.

Glutathione (GSH) is an effective component in preventing ROS induced injury. Another function of GSH is improving the capacity of the cell to regenerate ATP through maintaining cellular membrane integrity. It also acts as a sulphhydryl buffer and participates in transport mechanisms of amino acids across the cellular membrane. Upadhya et al. demonstrated that MMPs, especially the gelatinases, play an important role in preservation injury. When activated, gelatinases will cause a rounding and detachment of endothelial cells from the connective tissue matrix due to lysis of the anchors of the endothelium. This effect is due to the capability of gelatinases to digest fibronectin. When UW and Eurocollins were compared, liver effluent had a significant decreased gelatynolitic activity when UW was used as preservative. When HTK was compared to UW, HTK turned out to be a superior MMP inhibitor in in-vitro experiments. The components that inhibit MMPs are primarily GSH, but also lactobionic acid, histidine and citrate. All components are ligands for heavy metals acting as co-factors in MMP’s, possibly explaining their effectivity on MMP inhibition.

Effective cold-storage preservation depends on the right choice of solution components on the basis of their primary as well as secondary effects on organ viability. In this respect, lactobionic-acid and GSH are interesting examples. These components are included in UW-CSS for thei potential as impermeant and their antioxidant effects.
Since new insights in preservation it is now known that their effectiveness also participate in refined mechanisms to prevent ischemia and reperfusion injury. Thus, the revival in the interest in organ preservation since the development of the UW solution, has not only resulted in the determination of its essential components and more effective preservation but also in the recognition of new mechanisms that play a role in cytoprotection and organ viability.
Outlook

Over the past years the renewed interest in organ preservation has led to better insights in organ preservation and also the formulation of a new preservation solution. In 1994 the Celsior solution was introduced\textsuperscript{78,18}, following the concept of the UW solution. Celsior was originally developed for CS preservation of the heart\textsuperscript{78}. Later it was also shown to be effective in other organs and it is now clinically used for heart\textsuperscript{79}, lung and liver\textsuperscript{72,80} preservation. The results of these studies show that Celsior is equivalent to the UW solution in experimental heart\textsuperscript{7,81} preservation, probably due the high sodium-low potassium concentrations in the Celsior solution. Lung preservation with Celsior also showed good results, however, whether these results are due to the electrolyte composition or due to the low viscosity of Celsior remains unclear\textsuperscript{82}. Similar results between UW and Celsior in kidney\textsuperscript{83}, pancreas\textsuperscript{84} and small-bowel\textsuperscript{73} preservation look promising. For liver preservation, the UW solution appears to be the superior preservation solution\textsuperscript{85} although the results are not conclusive yet\textsuperscript{86,87}.

Celsior was designed along the same specifications as the UW solution. The promising results of this new UW-look-a-like are therefore not astonishing. The three principles for effective cold-storage preservation in UW were: First, a rapid cooling of the organ, with the solution effectively removing the blood from the vasculature and allowing an equilibration between the cold-storage solution and the tissue, second, the solution should prevent hypothermia induced cell-swelling, and third, the solution should prevent cellular acidosis\textsuperscript{9}. Additional effects of both UW and Celsior preservation solutions are the prevention of ROS precursor formation, inhibition of MMP activity and the promotion of ATP formation. Menasche \textit{et al}. has adressed the prevention of cell swelling, reduction of oxygen derived free radical formation and prevention of contractures\textsuperscript{78}. Celsior is an extracellular-type solution and contains mannitol instead of raffinose, glutamate instead of adenosine and no colloid. It can be speculated that the first promising results are due to its low viscosity resulting in a better wash-out during procurement of the graft and the extracellular-type electrolyte composition which is beneficial for heart and pancreas preservation and does not invoke vasospasm.

A definite answer whether Celsior will succeed the UW solution as the standard preservation solution in transplantation must await the results of a clinical multicentre trial.

The development of the UW Cold Storage Solution has resulted in renewed interest in organ preservation. This interest has focused on cell and organ viability and a better understanding of mechanisms underlying compromised cellular functions. Understanding important mechanisms in static CS results in new insights and will lead to improved organ preservation to reduce a persistent enemy in transplantation: ischemia-reperfusion related injury. The other topic that has been somewhat neglected are donor related risk factors. Not only age or systemic illnesses of the donor are detrimental to
graft outcome after transplantation, but also the mechanism resulting in the death of the donor and the unphysiological state and management after cerebral injury are of importance. The heart-beating and non-heart-beating donors are well-known examples. The onset of brain death results in a massive release of catacholamines contributing to a systemic alteration of the immune system. In addition to ischemia-reperfusion injury and allore cognition contributing to delayed graft function and transplant dysfunction, certain mechanisms may have been initiated before the preservation period started. The state of cerebral injury or critical illness might compromise graft function via neuro or neuro-humoral mechanisms and medication protocols.

With the development of the concept of UW-CSS and its modifications the limits of cold-storage preservation are probably reached. Organ preservation by static cold-storage, even though some extra beneficial components could be added to newer solutions, merely slows and ameliorates extra corporeal ischemic and hypoxic damage, rather than reverse damage. To further improve organ viability other ways to overcome donor related factors and ischemia-reperfusion injury have to be explored. Cytoprotection and pretreatment during brain death and prior to retrieval may be beneficial. Also, conventional cold-storage should be reverted to continuous machine perfusion, either hypo or normothermic, to reduce catabolism and support anabolic metabolism. Continuous perfusion will also allow intervention and addition of potentially beneficial chemical agents and might be a better mode to overcome the bridge between organ procurement and transplantation. Forty years after its first use, continuous machine perfusion could thus regain its major role in organ preservation and might be capable in combination with early intervention in the donor by cytoprotection to repair or even prevent ischemic damage.
Reference List


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