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REVIEW

Machine perfusion or cold storage in organ transplantation: indication, mechanisms, and future perspectives

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Summary

Most organs are currently preserved by cold storage (CS) prior to transplantation. However, as more so called marginal donor organs are utilized, machine perfusion has regained clinical interest. Recent studies have demonstrated advantages of pulsatile perfusion over CS preservation for kidney transplantation. However, it remains unclear whether there is a significant benefit of one preservation method over the other in general, or, whether the utilization of particular preservation approaches needs to be linked to organ characteristics. Proposed protective mechanisms of pulsatile perfusion remain largely obscure. It can be speculated that pulsatile perfusion may not only provide nutrition and facilitate the elimination of toxins but also trigger protective mechanisms leading to the amelioration of innate immune responses. Those aspects may be of particular relevance when utilizing grafts with suboptimal quality which may have an increased vulnerability to ischemia/reperfusion injury and compromised repair mechanisms. This review aims to enunciate the principles of organ perfusion and preservation as they relate to indication, aspects of organ protection and to highlight future developments.

Introduction

Optimized graft viability and quality under conditions of prolonged ischemia represent the primary goals of organ preservation. With the current increased utilization of more marginal donor organs, alternative preservation and perfusion concepts have regained interest. Organs with an increased sensitivity to injury such as those procured from donation after cardiac death (DCD) or extended criteria donation (ECD) donors may particularly benefit from optimized organ preservation. Furthermore, recent evidence has linked organ injury and quality at the time of transplantation with the activation of innate and adaptive immune responses [1]. Indeed, marginal organ grafts have been associated with increased rates of delayed graft function (DGF) and acute rejection rates [2,3].

To date, the predominant organ preservation method used by most centers is static cold storage (CS) [4]. Principles of CS preservation are based on the suppression of metabolism by hypothermia. By flushing the organ, blood is removed and replaced with an appropriate preservation solution. The concept of machine perfu-

sion (MP) which had already been developed prior to the routine utilization of CS preservation provides an attractive alternative method. Principles of MP are based on a controlled continuous or pulsatile circulation of a perfusate that eliminates toxic metabolic products, provides critical nutrients and oxygen. In theory, MP may protect, at least in part, from injuries related to ischemia/reperfusion, thus providing an improved graft quality.

History of organ preservation

The first recorded attempt of perfusing an isolated organ has been reported as early as 1849 by Loebel [5]. In the 1930s, Alex Carrel cultivated and perfused organs with the help of small pumps in collaboration with the aviator Charles Lindbergh [6]. This concept was continued and refined by Folkert Belzer *et al.* in the early 1960s [7] with work on hypothermic perfusion techniques for the preservation of kidneys. Initially, whole blood had been utilized as a perfusate. Further research demonstrated that microfiltered cryoprecipitated plasma (CPP) allowed longer preservation times. By 1967, the combination of continuous perfusion and hypothermic storage brought organ preservation to a new level: using oxygenated CPP and pulsatile perfusion, canine kidneys were successfully preserved for 72 h as described by Belzer *et al.* in their landmark publication [8]. Unfortunately, the clinically available pumping devices at that time were extremely user-unfriendly and difficult to transport.

By 1971, a miniature portable preservation pump had been developed facilitating clinical utilization. This device had been utilized clinically for several years as the Mini-Belzer unit and the majority of kidneys in the 1970s were preserved by MP. However, large-scale studies comparing MP and CS failed to prove the advantage for kidneys preserved by MP [9–12]. Of note, more potent immunosuppressants had been introduced into the clinic during this time, leading to an improved transplant outcome. By the mid 1980s, the majority of kidneys were preserved by CS. Collins and co-workers were the first to develop a simple, yet effective CS solution for organ preservation in 1969 [13]. Further research supported by the Eurotransplant Foundation led to the modified Euro-Collins solution (EC) in 1976. In the 1980s, Belzer *et al.* developed the University of Wisconsin solution (UW). Thereafter, UW gradually replaced EC as the preservation of choice supported by several studies demonstrating improved organ viability under conditions of prolonged cold ischemia when organs were preserved with UW [14,15]. Meanwhile, Bretschneider from Germany introduced the Histidin–Tryptophan–Ketoglutarate (HTK) solution in 1980. HTK was initially designed as a cardioplegic solution for open-heart surgery. However, the solution had also shown beneficial effects in the preservation of abdominal organs [16,17].

Comparative analysis of preservation solutions

University of Wisconsin solution

The proper osmotic concentration of UW is obtained by the combination of metabolically inert substrates such as lactobionate and raffinose. Hydroxy-Ethyl Starch (HES) is added as a colloid and ATP precursors (adenosine) and oxygen radical scavengers (glutathione and allopurinol) are used as important supplements.

Cold storage with UW provides satisfactory short- and long-term preservation outcomes [14]. Drawbacks include its high viscosity which prolongs the duration of perfusion while compromising the microcirculation [18]. UW's high potassium levels may cause vasoconstriction and may contribute to the hyper-aggregation of HES [19,20]. Washout-solutions such as Carolina Rinse mitigate this problem [21,22], however, are currently only rarely utilized clinically. Importantly, UW is currently the most widely used CS preservation solution. Two different types of UW solution are utilized for MP and for CS. In UW-G utilized for MP, lactobionate is replaced by gluconate while the higher potassium level of the conventional UW is reduced to achieve a consistency with an extracellular solution [23,24].

Histidin–Tryptophan–Ketoglutarate

Histidin–Tryptophan–Ketoglutarate solution was introduced in 1980 by Bretschneider and was originally designed as a cardioplegic solution [25]. Key characteristics and theoretical advantages as compared with UW consist of effective buffering by histidine, membrane stabilization by the aminoacid tryptophan, and the supply of ketoglutarate for anaerobic metabolism. HTK has a low viscosity, which may allow for an improved microperfusion [4,26]. Low pressure perfusion and high volume (not less than 6 l) have been recommended when utilizing HTK.

Eurotransplant conducted a multi-center randomized prospective trial in renal transplantation comparing UW and HTK at the beginning of the 1990s. This trial remains so far the only prospective comparison of those two perfusion solutions and showed no significant difference for DGF and 3-year graft survival [27]. Interpretation of those data, however, needs to consider that the quality of organs currently utilized for transplantation has changed as more ECD and DCD organs are transplanted today. More recent studies have not consistently demonstrated an advantage of one perfusion solution over the other [28–30] and in general it had been felt until recently that

UW and HTK have comparable preservation capacities if preservation times do not exceed 24 h.

Economic aspects have been of importance when comparing the utilization of HTK and UW. HTK is less expensive than UW on a per liter basis; however, increased volumes are required [26]. A single-center analysis revealed overall higher costs when UW was used although HTK perfusion required higher perfusion volumes [31].

In a most recent retrospective multi-center study, Stewart *et al.* analysed the United Network for Organ Sharing (UNOS) database and compared deceased donor kidney transplants preserved either with HTK or UW solution [32]. HTK preservation was independently associated with a 20% increased risk of graft loss in this study. The same group has also recently reported compromised graft survival following liver and pancreas preservation with HTK [33,34]. It is important to point out that those studies have not been adjusted for suboptimal (less than the recommended 6 l) HTK perfusion volumes.

Celsior

In 1994, Celsior was developed initially only for its application in heart transplantation [35]. This solution combines the osmotic efficacy of UW (lactobionate, mannitol) and the potent buffering ability of HTK (histidine). After demonstrating favorable effects in heart transplantation, utilization of the solution had also been proposed for abdominal organs [36]. To study the effects in kidney preservation, few groups from Europe compared Celsior with UW and demonstrated similar rates of DGF and graft survival [37–40].

IGL-1

Institute-George-Lopez (IGL-1), a rather new preservation solution, was developed by a group in France. It combines the advantageous effects of UW and Celsior. Polyethylene glycol binds to cell and tissue surfaces, thus stabilizing the underlying surface from cell interactions. A modification of the inherent immunogenicity of the donor tissue as a consequence of ischemia/reperfusion injury has been suggested when utilizing IGL [41]. Badet *et al.* [42] demonstrated a reduction in DGF as compared with kidneys preserved with UW. However, a recently published multi-center study showed no significant difference in DGF when IGL-1 was compared with UW [43].

Polysol

Polysol has been recently introduced with the goal of facilitating the successful transplantation of ischemically damaged organs. Therefore, many components such as amino acids, vitamins, potent buffers and antioxidants have been added to support metabolism under hypothermic conditions [44]. The solution has also been tested during MP [44,45]. A study by Schreinemachers *et al.* [46] demonstrated a superior graft survival as compared with UW in a porcine renal autotransplantation model. Further reports by the same group showed beneficial effects of Polysol also in kidneys damaged by warm ischemia [47]. While current reports on Polysol seem promising, more clinical data are necessary to demonstrate efficacy and benefits.

Clearly, prospective clinical studies reflecting the current utilization of marginal organs are necessary to determine the superiority of one CS preservation solution over others. Until then, UW solution will continue to be used by the majority of transplant centers for organ preservation.

Machine perfusion

Principles and mechanisms

The principle of MP is based on preserving the organ in a 'better environment'. Hypothermic machine perfusion (HMP) slows down metabolism, thus reducing oxygen requirements and ATP depletion. Circulation of the perfusate is achieved by a device that generates either a continuous or a pulsatile flow. MP provides, at least in theory, a continuous supply of nutrients with or without oxygen while toxic substrates and free radicals produced during CS can be eliminated. MP may also decrease vasospasm and provide additional parameters such as flow and resistance to evaluate organ viability. Furthermore, MP may provide an opportunity to improve organ quality by using pharmacologic and gene transfer therapies in real time [48]. Moreover, MP maintains the hemodynamic stimulation on the vasculature of the organ, which plays a critical role in vascular function under normal physiologic conditions. Notably, this potential benefit of MP remains poorly understood.

Less-than-optimal organs are currently increasingly used in order to meet the rising demand for organ transplantation. Those grafts have usually a compromised quality and are more prone to an ischemic insult. As a result, primary nonfunction and DGF occur at higher rates with the use of these less-than-optimal organs [2,3,49]. Moreover, DGF is associated with an increased frequency of acute rejections and a poorer long-term outcome. More recent retrospective clinical data have suggested superior outcomes following MP, particularly when utilizing organs with inferior quality [48,50,51].

While advantages of MP have been recognized by recent clinical studies, mechanisms involved remain unclear. Notably, pulsatile flow has been associated with the expression of flow-dependent, vasoprotective

endothelial genes [52,53]. In particular, the expression of one of these genes, Kruppel-like factor 2 (KLF2) may play a critical role in protecting the endothelium, potentially through the inhibition of pro-inflammatory responses, thus curtailing the activation of the innate immune system [54–56]. Moreover, flow-mediated KLF2-dependent programs are also critical for the production of vasodilators, specifically endothelial-derived nitric oxide, and the expression of anti-thrombogenic genes (e.g. thrombomodulin) (Fig. 1) [56].

Interestingly, there has been some controversy over the impact of distinct types of flow generated by the devices: Two studies from Japan showed comparable outcomes with either pulsatile or continuous perfusion [57,58]. However, studies by others demonstrated improved microcirculation and organ function when pulsatile perfusion was utilized [59–61]. Further studies in this area are critical as it is now known that different types of flow (in particular shear stress) exert distinct effects on vascular endothelial gene expression and function.

Clinical and experimental studies

Several experimental and clinical studies have compared MP with CS preservation [9,62–66]. Early studies reported no significant differences in DGF or graft survival when comparing MP and CS preservation. However, it is important to recognize that those studies have been retrospective, had not been randomized and reflected a heterogenic patient population.

More recent studies have been better powered, reflect an updated presentation of current clinical organ quality and represent recent advancement in immunosuppression. In 2003, a meta-analysis and systematic review of the current literature by Wight *et al.* [67] demonstrated a 20% reduction in DGF when HMP was utilized. Schold *et al.* [48] reported on the effects of organ perfusion with an analysis of the Scientific Registry of Transplant Recipients in the United States. The authors reported that MP led to an increased utilization of ECD kidneys and a lower incidence of DGF. However, this study was unable to demonstrate improved long-term graft survival. More recently, Matsuoka *et al.* [50] reported that MP reduced the incidence of DGF and enhanced the utilization of ECD kidneys. The authors also stated that decreasing rates of DGF may lead to lower overall costs, a conclusion which supports a concept of comparable cost-effectiveness when utilizing pulsatile or CS preservation previously also supported by others [68]. Most recently, Moers *et al.* have published the first multi-center, prospective, randomized high-volume clinical study comparing machine- and CS perfusion. This study randomly assigned one kidney from 336 consecutive deceased donors to HMP and the contralateral kidney to static CS preservation. Recipients were followed for over a period of 1 year. The study showed a significant risk reduction for delayed graft failure in the HMP group (20.8% with HMP vs. 26.5% with CS). One-year graft survival had significantly improved if kidneys were preserved by MP (94% vs. 90%) [69]. As this study had originally not been sufficiently powered for a

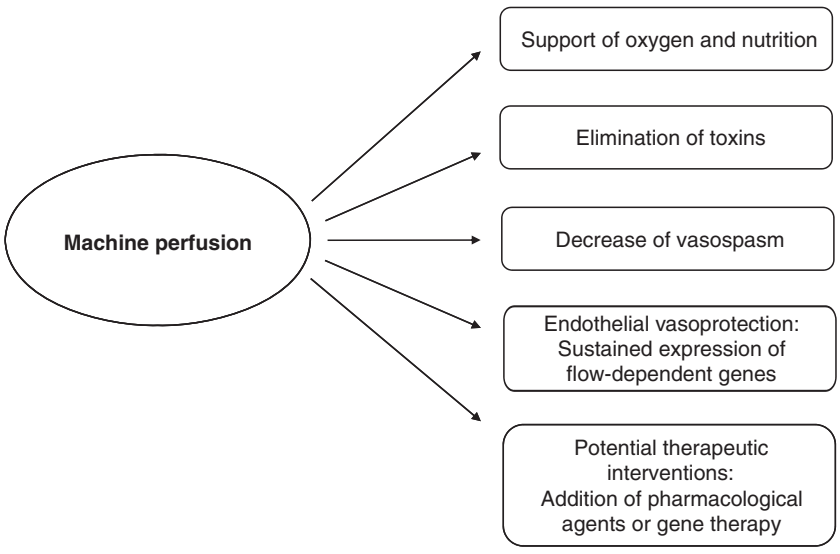

Figure 1 Protective mechanisms of pulsatile perfusion remain largely unknown. In theory, toxins are eliminated while nutrients are supplied. Protective endothelial genes expressed during pulsatile perfusion may play a role. Future research may explore additional therapeutic interventions when utilizing machine perfusion.

subgroup analysis, the same group presented an updated analysis at the American Transplant Congress (ATC) in Boston (2009) in which they extended the volume of marginal kidneys. In this updated analysis, the authors communicated improved transplant outcomes and reduced rates of DGF also in kidneys from donors after cardiac death (DCD) preserved by pulsatile MP. However, improvements at this time were not significantly better in a subgroup analysis of kidneys from elderly (>65 years) donors. Very short ischemic times in this trial may have mitigated beneficial effects of MP in kidneys from elderly donors.

Overall, the recently published data suggest an advantage for HMP over static CS (Table 1). Therefore, it is expected that MP will receive a wider clinical awareness and application in the near future.

Future perspective and challenges

Pulsatile perfusion offers the opportunity for alternative preservation approaches such as normothermic machine perfusion (NMP). This concept has already been reported in 1976 by Fuller *et al.* [70]. During NMP, an organ is perfused and preserved at, or, close to body temperature (37 °C). This preservation method yields several advantages as compared with HMP. In theory, normothermic perfusion at body temperature allows the organ to maintain a physiologic metabolism, thus minimizing the accumulation of toxic substrates and free radical formation. It has been suggested that NMP may be particularly relevant in organs with extensive warm ischemic injuries. In animal experiments, canine kidneys were successfully transplanted after 2 h of warm ischemia and 18 h of NMP with significantly improved recovery of renal function. Notably, all kidneys of this trial procured by static CS demonstrated primary nonfunction [71].

In a recent clinical study, Valero *et al.* demonstrated that NMP reduced primary graft dysfunction of kidneys from DCD. Over a 12-year period, 44 DCD kidneys were perfused by NMP using a cardiopulmonary bypass followed by body core-cooling prior to organ procurement. The results showed a significant reduction in primary nonfunction and DGF in comparison to conventional preservation techniques [72]. NMP may also allow a more advanced assessment of organ viability during preservation which, at least in theory, may improve transplant outcome [73].

The application of pulsatile perfusion for the preservation of extra-renal organs has not been extensively explored so far. Liver transplants may require perfusion of both, portal vein and hepatic artery. In previous experimental studies isolated perfusion of the portal vein or retrograde perfusion via the hepatic vein yielded comparable outcomes. However, the perfusion via the hepatic artery alone was less beneficial [74]. Moreover, sinusoidal endothelial cells and the biliary tract are critical targets of ischemia/reperfusion [75] and may require modified perfusion pressures. Animal models have shown benefits of oxygenated HMP as compared with CS in liver transplantation [24,76] and Guarrera *et al.* have shown in a pioneering effort, promising clinical results as reported at the ATC, Boston, 2009.

Early experimental data are also available for pancreas transplants. Again, organ-specific aspects need consideration as the pancreas is a low-flow organ and therefore potentially more susceptible to barotrauma during pulsatile perfusion. Cardiac preservation by MP has recently shown encouraging results in animal experiments. Longer preservation times, reduction of ischemic injury and improved early ventricular function upon reperfusion have been noted [77,78]. However, some groups reported on edematous swelling associated with high flow rates, inadequate perfusion pressures and nonoptimally adapted compositions of the perfusate [79–81]. At this time, organ-specific aspects need further evaluations to explore potential benefits of MP in extra-renal organs.

Clearly, future studies will also need to explore protective mechanisms of pulsatile perfusion in more detail [82,83]. Protective mechanisms associated with pulsatile perfusion remain largely obscure. It can be speculated that pulsatile perfusion may not only perform provision of nutrition and the elimination of toxins, but as discussed above, may also sustain physiologic flow-mediated endothelial vasoprotective programs, which may have a significant impact on subsequent ischemia-reperfusion and early innate immune response events.

Conclusion

The analysis presented in this article supports the increasing interest in preserving organs by MP. Following the general assumption that marginal donor organs are more prone to injury, most previous clinical studies have tested the effects of MP in sub-optimal kidney grafts. Those retrospective studies showed improved outcomes and utilization rates when marginal kidneys had been preserved under conditions of MP. The only prospective large volume clinical study to date has shown significantly improved 1-year graft survival and significantly reduced DGF rates in a study population dominated by standard criteria donor organ recipients. While this important clinical study has initially not been powered for a subgroup analysis of ECD and DCD kidneys, more recent presentations by the same group had extended the inclusion of those subgroups and demonstrated beneficial effects of MP for DCD and very recently also for ECD kidneys

Table 1. Clinical studies comparing machine (MP) and cold storage (CS) perfusion.

Author, year & study	Patient volume (MP vs. CS)	Study design	Perfusion modus	Device	DGF in % (MP vs. CS)	Acute rejection rate in % (MP vs. CS)	Graft survival by 1 year in % (MP vs. CS)
Moers *et al.* 2009 [69]	336 vs. 336	Prospective	Pulsatile	LifePort	20.8 vs. 26.5 *P* = 0.05	13.1 vs. 13.7 *P* = NS	94 vs. 90 *P* = 0.04
Kwiatkowski *et al.* 2007 [84]	227 vs. 188	Retrospective	Pulsatile	Waters MOX machine	32.9 vs. 32.3 *P* = NS	48.9 vs.46.3 *P* = NS	87.7 vs. 85.0 by 15 months *P* = NS 68.2 vs. 54.2 by 5 years *P* = 0.02
Kwiatkowski *et al.* 2009 [85]	37 vs.37	Prospective	Pulsatile	Waters MOX machine	32.4 vs. 50 *P* = not mentioned	similar, details not reported	68.2 vs. 43.0 by 10 years *P* = 0.08
Gage *et al.* 1997 [86]	25 vs. 25	Retrospective	Pulsatile	Waters MOX machine	12 vs. 24 *P* = not mentioned	Not reported	100 vs. 100 *P* = NS
van der Vliet *et al.* 2001 [87]	38 vs. 38	Prospective	Pulsatile	Gambro pulsatile perfusion machine	20 vs. 66.7 *P* = not specified	Not reported	76.3 vs.84.2 *P* = NS
Shah *et al.* 2008 [88]	40 vs. 40	Retrospective	Pulsatile	Waters MOX machine	5 vs. 35 *P* < 0.01	Not reported	95 vs. 88 *P* = NS
Polyak *et al.* 2000 [89]	402 vs. 248	Retrospective	Pulsatile	Waters MOX machine	9 vs. 24 for conventional criteria kidneys *P* = 0.02 14 vs. 37 for ECD kidneys *P* = 0.02	Not reported	96 vs. 89 for conventional criteria kidneys *P* = 0.02 88 vs. 79 for ECD kidneys *P* = 0.02
Merion *et al.* 1990 [90]	51 vs. 51	Prospective	Pulsatile	Waters MOX machine	41 vs. 31 *P* = NS	9.8 vs. 3.9 *P* = NS	Not reported
Kozaki *et al.* 2000 [58]	16 vs. 16	Prospective	Continuous	LPS-II	62.5 vs. 81.3 *P* = NS	Not reported	Not reported
Halloran *et al.* 1987 [64]	91 vs. 90	Prospective	Pulsatile	Waters MOX machine	31 vs. 44 *P* = significant, not further specified	22.0 vs. 17.8 *P* = NS	75 vs. 70 *P* = NS
Alijani *et al.* 1985 [91]	29 vs. 29	Prospective	Not reported	Waters MOX machine	Post-transplant dialysis: 17 vs. 63 *P* < 0.01	Not reported	*P* = NS not further specified

NS = nonsignificant.

(R. Ploeg, personal communication). While those most recent data have not been published one could argue that the allocation policy of the European Senior program emphasizing on brief ischemic times may have 'prevented' more significant beneficial effects of MP on transplant outcome of ECD kidneys in this trial.

Moving forward, one wants to leave the comparative analysis of machine and CS preservation with a clinical recommendation on the superiority of one preservation period over the other. However, the current analysis of machine- versus pulsatile perfusion has left us with as many open questions as novel and important clinical data. Thus, to expand the availability and to optimize the quality of organs during pressing times of ever-increasing demands for transplantation, we feel that the clinical utilization of pulsatile perfusion should be currently focusing on marginal donor organs. This approach will also increase the safety of utilizing marginal kidneys as MP will allow the measurement of predictive flow parameters. Costs have recently increased when utilizing MP and previous studies on economics and organ preservation may need to be revisited prior to expanding the utilization of MP to all organs. At the same time, we need to focus our research efforts in this field to better understand potential mechanisms involved in postulated protective effects of MP. Mechanisms of injury and repair, as a consequence of ischemia remain only partly understood and aspects of improved organ quality and transplant outcome need further exploration. The targeted protection of the vascular endothelium may play an important role during organ preservation and endothelial activation/dysfunction may be critical for the initiation and progression of immune responses. Understanding these mechanisms of vasoprotection and its pharmacologic modulation should allow us to develop new interventional strategies. Benefits of pulsatile perfusion for extra-renal organs are also of clinical interest and will require further research. A more frequent utilization of pulsatile perfusion will also require a detailed documentation of mechanical injuries such as vascular damage associated with the attachment of vessels to the pumping device, which have so far only been reported anecdotally.

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