Preclinical evaluation of a new organ preservation solution
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Chapter 5

Machine perfusion preservation of the liver: a worthwhile clinical activity?
Machine perfusion preservation of the liver: a worthwhile clinical activity?

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Abstract

Purpose of review
Although the first successful machine perfusion (MP) procedures of the liver have been performed almost four decades ago, it never gained general acceptance, mainly due to technical and logistical constraints. Interest in liver MP has recently been renewed, because of its potential to resuscitate marginal organs. This review describes experimental and clinical liver MP studies as well as current developments.

Recent findings
Liver MP is increasingly reconsidered since experimental studies have shown that oxygenated MP provides a complete wash-out and is able to restore parenchymal energy status, a phenomenon of particular importance in preservation of livers from compromised donors. Also, perfusion of the hepatic artery can prevent ischemic-type biliary lesions. Short term as well as continuous MP prior to or after cold storage (CS) preservation, has proven beneficial when compared to CS alone.

Summary
The benefits of MP for both heart-beating and non-heart-beating liver grafts seem promising in view of expanding the donor pool. To date, MP preservation is clinically used only for kidney grafts since liver MP systems have not yet become commercially available. Clinical application appears feasible in the near future since new, easy to use MP systems and new MP solutions are currently under development.
Introduction

Following the publication of a landmark paper in 1967 by Belzer et al.1, kidney preservation by hypothermic machine perfusion (MP) has become an established clinical activity. To date, however, preservation of the liver by MP has not followed although in 1968, the first successful clinical application of liver MP was described2. After the introduction of simple cold storage (CS), the clinical focus averted from MP since CS was easier to use, was cheaper and did not involve the logistical drawbacks of MP. Also, CS of liver grafts using the University of Wisconsin solution allowed for significantly longer preservation periods compared to the previously used preservation solution, Euro-Collins3. Nevertheless, the potential of liver MP to extend preservation periods compared to CS was well recognized. Currently, since MP has continued to be used for kidney preservation, liver perfusion preservation has regained interest in the light of improving the quality of marginal donor organs. In this review we focus on experimental studies as well as on recent clinical developments in order to answer the question whether liver MP remains a worthwhile clinical activity.

Current status

To date, the gold standard for preservation of regular cadaveric organs is cold flush followed by static, CS using an appropriate organ preservation solution. However, as suggested by St. Peter et al.4, the potential for static CS preservation has presently reached its limit. In order to expand the donor pool, the procurement of organs from marginal donors, such as from non-heart-beating donors (NHBD) has become a necessity. Clinical studies comparing graft and patient survival of heart-beating and NHBD livers show clear differences in favour of heart-beating donor (HBD) grafts5-7. Obviously, ischemically damaged organs need a more effective method of preservation and of equal importance, require a means for viability assessment prior to transplantation. In contrast to CS, MP holds the potential of viability assessment8 and allows for pharmacological intervention. In addition, MP potentially reverses the effects of ischemic injury that occur during warm ischemia, whereas CS leads to sequential injury with the risk of rendering grafts unviable for transplantation9. Results of studies concerning MP preservation of kidney grafts showed a benefit of MP, in particular for NHBD kidneys10. The same advantage is expected for perfusion preservation of ischemically damaged livers11-13.

In organs subjected to warm ischemia such as in NHBD livers, it is essential to restore cellular energy levels lost during warm ischemia. Continuous perfusion via the portal vein supplies metabolic substrates, removes waste products and allows for pharmacological interventions. Also, the delivery of oxygen to the organ during the preservation period has shown encouraging results. The ultimate goal of liver preservation by MP is to improve the quality of donor livers available for transplantation in order
to expand the donor pool. In anticipation of clinical application, several landmark experimental studies have been performed of which an overview is provided together with an insight in the more recent developments.

**Single or dual perfusion**

Machine perfusion of kidneys is relatively straightforward, in contrast to MP of liver grafts which is more challenging because of the dual continuous and pulsatile blood supply. Approximately 80% of the vascularization of the liver originates from the portal vein (PV), the remaining 20% finding its origin in the hepatic artery (HA). Although responsible for a relatively small part of total blood supply, the HA is accountable for the largest part of the oxygen supply to the liver and as it also supplies blood to the biliary tract, is not to be neglected\(^\text{14}\). Nevertheless, various studies have shown that perfusion of the PV alone resulted in viable livers upon reperfusion. Seventy-two hour in situ perfusion of porcine livers via the PV, improved preservation of energy metabolism compared to simple CS for 48 hr\(^\text{15}\). The solutions used were UW supplemented with an oxygen carrier and UW alone, respectively. Kim et al.\(^\text{16}\) compared the mitochondrial function of rat livers after 48 hr CS or MP via the PV. Tissue ATP was significantly depleted after 48 hr CS, whereas MP resulted in stimulation of ATP production in liver tissue. It was concluded that liver energy-generating capabilities are significantly better preserved by MP than by CS. To investigate the efficacy of different routes of perfusion, a study was performed in rats evaluating perfusion via the PV, hepatic veins (by retrograde perfusion) or HA. Twenty-four and 48 hr of MP was compared with 24 and 48 hr CS, confirming the superiority of MP over CS for liver preservation. Retrograde perfusion via the hepatic veins provided results similar to PV perfusion whereas perfusion via the HA proved less beneficial\(^\text{17}\). The first successful clinical results of MP of the liver were however, achieved perfusing both the PV and the HA\(^\text{18}\). Belzer, who was the first to successfully pump canine kidneys in a pulsatile fashion, later focussed his experimental work on MP of the liver. In MP studies of the porcine liver using cryo-precipitated plasma, the concept of dual perfusion was applied using continuous portal flow in combination with pulsatile arterial flow. Eight to ten hours of MP resulted in seven-day survival of four out of five animals. When perfusion was extended to 24 hr, only two of twelve animals survived beyond 12 hours\(^\text{18}\). Two decades later, Belzer’s group published the best results so far in MP of the liver, reporting successful continuous MP of canine livers for 72 hr via the PV only, using the UW-Gluconate (UW-G) solution\(^\text{19}\). Seven of eight dogs survived for 7 or more days following orthotopic liver transplantation. More recent studies using large-animal models described liver perfusion through both the PV and HA. In a porcine study comparing preservation by MP and CS\(^\text{20}\), livers were preserved for 24 hr followed by a 4 hr phase of normothermic reperfusion to assess hepatocellular function and tissue viability. Perfusion was achieved using a prototype Liver Transporter (LTR, Organ Recovery Systems, Des Plaines, IL, USA)
with constant perfusion pressures of 10-12 mmHg through the PV and 30 mmHg through the HA, resulting in perfusion flow rates of 0.3 ml/min per g liver in the PV and 0.1 ml/min per g liver in the HA. Compared to CS, MP preserved livers showed improved hepatocellular function and overall less parenchymal damage as indicated by improved oxygen consumption and lower ALT levels, respectively. The improved results compared to CS preservation were attributed to better sinusoidal endothelial cell function and homogenous sinusoidal perfusion. Using the same prototype machine for MP, Monbaliu et al. performed a porcine transplant study\textsuperscript{21}. Four hours of CS (\(n=6\)) was compared to 4 hr MP (\(n=8\)) and post-transplant recipient and graft survival, as well as liver damage and hepatocellular function were assessed. Three day survival was 5 of 6 in the CS group and 2 of 8 in the MP group. Although hepatocellular damage appeared to be reduced by MP, TNF-α production and the degree of endothelial cell dysfunction after reperfusion were more markedly present in the MP group. For that reason, the authors concluded that transplantation after liver MP is feasible, but seems less advantageous over CS as compared to MP of kidney grafts. Deprivation of oxygen during MP might have played a major role in these poor results. Another liver transplant study was performed by Guarrera et al.\textsuperscript{22} in 6 miniature swines, comparing CS (\(n=3\)) to MP (\(n=3\)) perfusing both the PV and HA in a flow controlled system. Post-transplant results of liver function were comparable between groups, with all animals surviving for 5 days. The same report describes a study of perfusing human discarded donor livers. Ten non-transplantable human livers were obtained and subjected to centrifugal MP of both the PV and HA, according to a flow-controlled system with target flow (0.7 ml/g/min) adjusted for graft weight. PV and HA pressures ranged from 3 to 5 and 12 to 18 mm Hg, respectively. From this study it was concluded that a safe and reliable method of centrifugal liver MP was developed. Using the same technique, MP was used in a human liver transplant setting for the first time. Three donor livers were transplanted after MP during 4-5.5 hr and all grafts functioned immediately. Biopsies taken after reperfusion showed well preserved histology. Although this report does not provide for long-term survival data, all three recipients survived with a mean survival of 7 weeks\textsuperscript{22,23}. MP supplemented with oxygenation was applied by Van der Plaats et al.\textsuperscript{24} in their development of a new MP system. Porcine livers were preserved for 24 hr by CS using UW or 24 hr of MP using UW-G in the Groningen Hypothermic Liver Perfusion pump. Continuous perfusion of the PV at 4 mmHg was applied simultaneously with pulsatile perfusion of the HA (30/20 mmHg at 60 BPM). Perfusion pressures used were based on results from a previous study\textsuperscript{25} from the same group performed in rats, concluding that perfusion at 25% of normothermic liver circulation, showed complete perfusion of the liver without induction of endothelial injury. Flow was approximately 350 and 80 ml/min, respectively, but decreased in the PV, possibly due to edema formation. The arterial oxygen tension was thereby maintained at 100 kPa. In contrast to cold-stored
livers, MP preserved livers showed complete and uniform perfusion. For further development of the concept of hypothermic dual perfusion of the liver, the system will have to be subjected to feasibility testing in a transplant setting.

**Continuous or pulsatile perfusion**

Techniques of liver perfusion have evolved over the years with the most successful results achieved in a transplantation study by Pienaar et al.\textsuperscript{19}, who perfused canine livers in a pulsatile fashion through the PV only. Using an isolated perfusion model, Yamamoto et al.\textsuperscript{15} succeeded in 72 hr MP of porcine livers by continuous perfusion of the PV. Most recent attempts of simulating normal liver physiology in MP systems led to the application of pulsatile flow for the HA and continuous or non-pulsatile flow for the PV\textsuperscript{20,21,24,26}. Monbaliu et al. performed a porcine liver transplantation study\textsuperscript{21} using pulsatile and non-pulsatile perfusion but results were poor, probably due to the lack of oxygen. Therefore, further studies are necessary to show whether the liver will indeed benefit from a combination of pulsatile and non-pulsatile flow in an oxygenated environment, based on the rationale of mimicking normal liver physiology.

**Effects of oxygenation**

The exposure of liver grafts to ischemia and the shortage of energy substrates during static cold storage, is detrimental to the liver since hepatic metabolism, although reduced, is still active at hypothermia\textsuperscript{27}. Therefore, oxygenated MP was developed as a means of improving the quality of liver preservation\textsuperscript{28}. Oxygenated preservation enables grafts to restore tissue homeostasis\textsuperscript{29} and allows for maintenance of functional integrity of hepatocytes during ischemia. Mitchell et al.\textsuperscript{30} have demonstrated that brief periods of temporary, substrate-free hypothermic oxygenation during long-term storage of rat livers significantly improved post-ischemic recovery of metabolism. Furthermore, it has long been recognized that continuous hypothermic perfusion of the liver, under optimal conditions of oxygenation, perfusate flow, and substrate supply, can yield excellent preservation of the donor liver\textsuperscript{19}. Poor results in MP preservation studies using porcine livers were probably caused by the lack of oxygen\textsuperscript{21,22}.

**Combined CS and MP**

The combination of CS and MP for liver preservation was already shown to be effective in a canine transplant study almost two decades ago\textsuperscript{31}. Six hours of CS followed by 42 hr of MP was considered an alternative preservation method with the aim of facilitating organ sharing between transplant units. Recently, enhanced shear stress induced by long-term continuous hypothermic perfusion has been reported to cause vascular injury and was therefore considered to be a serious drawback of MP\textsuperscript{32}. For that reason, the effect of shorter periods of MP as an adjunct to CS
was evaluated. Minor et al. performed a rat liver preservation study comparing 18 hr CS to 18 hr MP and 2 hr MP followed by 16 hr CS of liver grafts subjected to 30 min of warm ischemia. From the results of enzyme release, excretory function and energy recovery upon reperfusion, it was concluded that MP during 18 hr significantly improved hepatic integrity compared to CS. Only 2 hours of oxygenated perfusion preceding 16 hr of CS seemed to be sufficient for recovery of parenchymal liver function and reduction of vascular activation to an extent comparable with long-term MP. It was also suggested that combining short-term oxygenated MP upon harvest with a subsequent period of CS, is an alternative method of preservation of pre-damaged grafts as it unites the advantages of both techniques. Another study from the same group, demonstrated that reversal of the deleterious effects of warm ischemia is possible even after a period of CS. In rat livers subjected to 30 min of warm ischemia, a subsequent period of 18 hr CS was compared to 16 hr CS followed by 2 hr oxygenated MP. Pre-damaged livers recovered after short-term, hypothermic oxygenated perfusion, even without energetic support or nutritive stimulation. These results were confirmed by Dutkowski et al. who showed that a liver metabolically depleted during cold storage or warm ischemia, could be energy recharged by short-term MP. In a HBD liver model, oxygenated perfusion after CS resulted in increased cellular energy and upon normothermic reperfusion, less oxidative stress and decreased cell death compared to CS alone. Liver transplantation after short-term MP of NHBD grafts resulted in a significant extension of animal survival. Dutkowski et al. investigated the effects of 30 min normothermic perfusion prior to cold preservation of ischemically damaged rat livers. Oxygenated Krebs-Henseleit buffer with or without the antioxidant biliverdin was used as perfusate. It was concluded that normothermic, pre-CS perfusion improved the viability of grafts from NHBDs. The addition of biliverdin further increased the energy status of grafts.

**Normothermic perfusion preservation**

Hyptothermic preservation is based on the principle of reduction of the metabolic rate and hence, decrease of oxygen demand. Preservation at near-body temperatures requires an adequate supply of oxygen to the tissues which can only be delivered by continuous perfusion with the addition of an oxygen carrier to the perfusate. Several advantages of warm perfusion of liver and kidney have been described. The deleterious effects of hypothermia (primarily aggravation of endothelial cell injury and prolongation of ischemia) are avoided by subnormothermic ([15-35°C]) or normothermic oxygenated preservation. Normothermic, sanguinous perfusion can mimic the physiological state of the organ and is therefore assumed to be the least damaging method of preservation. Normothermic perfusion can potentially prolong preservation time when compared to CS and enables real-time viability assessment prior to transplantation. Experimental studies have shown advantages of this method over CS in liver transplantation.
maintaining viability of both HBD\textsuperscript{40} and NHBD liver grafts\textsuperscript{41}. One hour of warm ischemia followed by 24 hr CS in UW solution resulted in a completely non-viable organ. In contrast, 24 hr of normothermic perfusion preservation produced a functioning liver, despite comparable ischemic injury. Using the same model for the preservation of porcine livers by isolated perfusion\textsuperscript{42}, it was demonstrated that livers subjected to 60 min of warm ischemia had superior bile production and metabolic activity after 24 hr of continued normothermic perfusion with oxygenated blood compared to a 4 hr CS period in UW followed by 20 hr of normothermic perfusion preservation. Even a short period of cold ischemia proved to be deleterious to the function of ischemically damaged (NHBD) livers. These results were comparable to a transplant study addressing the preservation of warm, ischemically damaged livers described by Schön et al.\textsuperscript{43}. After a period of 60 min warm ischemia, livers were preserved by 4 hr of CS or normothermic perfusion. Unlike the normothermically preserved livers, the cold preserved livers all suffered from primary non-function. Normothermic perfusion of warm ischemically damaged livers throughout the preservation period using autologous blood, not only replenishes cellular substrates but also ameliorates ischemic injury and provides for a clear assessment of liver function. This technique (combined with use of viability markers) could therefore permit the use of severely compromised organs. The only normothermic perfusion system under development currently for heart preservation, the Organ Care System (Transmedics Inc, Andover, MA, USA), is reported to be modified for future application of normothermic perfusion of the liver. Aside from the potential advantages of normothermic perfusion preservation over CS, this demanding technique will evidently lead to high costs and, due to its complexity will place a significant burden on the workload of transplant personnel. For these reasons, it is uncertain whether this technique will really take off in clinical organ preservation.

**Prevention of post-transplant biliary complications**

The results of graft and patient survival and the complication rate in terms of ischemic type biliary lesions (ITBL) vary according to the type of donor (heart-beating, non-heart-beating), the method of procurement, the preservation method and the sequence of revascularization\textsuperscript{44}. First and foremost, a successful organ procurement and preservation procedure has to provide for a complete wash-out of the graft and, simultaneously, an effective preservation of the biliary tree. It is well-known that ITBL lead to considerable morbidity following orthotopic liver transplantation\textsuperscript{45}. From experimental studies it was suggested that a proper initial wash-out procedure during organ procurement should be performed using high-perfusion pressure perfusion\textsuperscript{46} and back-table arterial pressure perfusion\textsuperscript{47}. Protection against biliary strictures was assumed to be dependent on the aortic flush solution's viscosity, rather than the cold ischemic time\textsuperscript{48}. A clinical study comparing preservation using HTK or UW
showed no difference between groups in terms of ITBL (18.8% vs. 16.0%, respectively)\textsuperscript{45}. It was suggested that a low viscosity by itself does not guarantee reliable perfusion of the small arteries of a liver graft and that pressure perfusion might be beneficial even when using a low viscosity solution such as HTK.

**Recent developments**

After an interval of almost four decades, clinical application of the technique of liver preservation by perfusion now appears to be in sight as both new MP systems and perfusion solutions are currently under development.

**Machine perfusion systems**

Due to high costs, logistical and technical complexity as well as the need for trained personnel to operate an MP system, MP up to now, never gained widespread acceptance. However, it has been suggested that if a MP system could be applied to the donor liver graft only for a limited time during the preparation of the recipient, the procedure would be easier to manage in the clinical setting\textsuperscript{35}. Also, if a readily transportable MP system could be applied without the high costs and the complicated technology associated with MP systems used previously, widespread acceptance of liver preservation by perfusion would even stand a better chance. To our knowledge, three systems are currently under development, all of which are transportable MP units. Two systems have already been mentioned: the prototype Liver Transporter (Organ Recovery Systems)\textsuperscript{20,21} and the Groningen Hypothermic Liver Perfusion pump\textsuperscript{24}. The third machine perfusion system currently under development is a disposable MP device named the Airdrive (Doorzand Medical Innovations, Amsterdam, The Netherlands). To date, it is the only system that enables oxygenated perfusion preservation of both kidney and liver grafts\textsuperscript{26}. Whereas the kidney is perfused in a pulsatile fashion, perfusion of the liver is established by non-pulsatile flow through the PV and simultaneous pulsatile flow through the HA. The Airdrive is now assessed in a final series of preclinical testing.

**Machine perfusion solutions**

Hypothermic machine perfusion of the liver was accomplished in the 1960s using perfusates based on autologous blood\textsuperscript{2} or cryo-precipitated plasma\textsuperscript{49,50}. Subsequently, acellular perfusion solutions were used such as saline\textsuperscript{51} and lactated Ringer’s solution\textsuperscript{52}. In the past decade, the most effective perfusion solution for liver preservation by MP has been UW-G\textsuperscript{19}. Recently, liver perfusion studies have been described using (modified) HTK\textsuperscript{33,34}, oxygenated Krebs-Henseleit solution\textsuperscript{37}, modified UW\textsuperscript{12,13,32,35} and Celsior-Hydroxyethyl starch\textsuperscript{17}. Most recently, studies reported the use of Vasosol (Waters Medical Systems, Rochester, Minn, USA)\textsuperscript{22} and Polysol
(Doorzand Medical Innovations, Amsterdam, The Netherlands)\textsuperscript{53-55}. Vasosol is a UW-G based solution with additional compounds in order to enhance the vasodilatory and antioxidant capacity\textsuperscript{56}. The Polysol perfusion preservation solution was originally developed for application in the Airdrive system and was shown to improve liver preservation quality in rat and porcine MP preservation studies of HBD\textsuperscript{53,54} as well as NHBD liver grafts\textsuperscript{55}. Since Polysol is a colloid based solution with low viscosity, it appears to meet the suggested requirements\textsuperscript{57} for optimal perfusion preservation as the low viscosity allows for an effective wash-out and the colloid ensures prevention of edema formation and ITBL\textsuperscript{48}.

**Conclusion**

Machine perfusion preservation of liver grafts is increasingly reconsidered since experimental studies have shown a benefit of oxygenated MP in liver preservation, particularly in livers retrieved from marginal donors. Both hypothermic and normothermic perfusion preservation provide a complete and uniform wash-out and are able to restore the energy status of liver grafts. The current development of easier to use MP systems for liver preservation and new perfusion solutions exemplify the regained interest in liver preservation by MP. After an intermission of approximately 40 years, clinical application of this promising technique now appears to be in sight also for the preservation of livers.
References