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MACHINE PRESERVATION OF THE LIVER (C MILLER AND C. QUINTINI, SECTION EDITORS)

Kidney Machine Preservation: State of the Art

Laura Ioana Mazilescu[1,2] · Markus Selzner[1]

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Abstract

Purpose of the Review Machine perfusion has become an important asset in solid organ transplantation to improve the constant organ shortage. This review summarizes advances in machine perfusion of kidney grafts over the last 3 years.

Recent Findings Because of the severe organ shortage, more and more marginal grafts are being accepted for transplantation. In an attempt to decrease preservation injury and better assess grafts before transplantation, research studies have focused their attention on ex vivo machine perfusion. Hypothermic machine perfusion has been used in a clinical setting for years and has proven to be superior to cold storage. Recently, novel technologies, such as normothermic ex vivo machine perfusion, controlled oxygenated rewarming, and normothermic in situ perfusion, have triggered interest to decrease preservation injury and improve the outcome of marginal grafts. Keeping grafts metabolically active allows for a better assessment, reconditioning, and organ repair. Preclinical results suggest that normothermic perfusion is superior to hypothermic perfusion and static cold storage. Normothermic ex vivo perfusion has been translated into clinical trials, with encouraging first results. Currently, there is no consensus regarding a protocol for warm perfusion. Normothermic regional perfusion is used to recirculate blood in situ to restore the changes after warm ischemic injury. First results are promising, but further assessments are needed to explore the potential of this novel approach.

Summary Ex vivo machine perfusion is a superior preservation method compared with cold storage. Optimal perfusion solution, temperature, and machine technology are still controversial. Graft assessment and repair are the central research focuses at the moment.

Keywords (Ex vivo) machine perfusion · Marginal grafts · Kidney transplantation · Graft assessment · Graft repair · Ischemia reperfusion injury

Introduction

Ex situ machine preservation of solid organs is a concept older than 100 years, which has experienced a renaissance due to the organ shortage for transplantation. In 1935, Alexis Carell and Charles Lindbergh demonstrated the viability of normothermic ex situ perfusion of abdominal organs [1]. In the 1960s, Belzer developed the first clinical hypothermic machine perfusion set-up for kidney preservation [2]. Due to the high costs and difficult logistics, these techniques were replaced by static cold storage (SCS) [3]. When using grafts from standard criteria donors, static cold storage has proven to be a reliable preservation method. However, marginal grafts have been increasingly used in the past decade in an attempt to increase the donor pool [4]. A major problem in using grafts from extended criteria donors (ECD) and donation after circulatory death (DCD) is their susceptibility to cold ischemic injury, which results in a higher rate of postoperative delayed graft function (DGF), primary non-function (PNF), and graft loss [4–6]. Ex vivo machine perfusion is a promising option to minimize ischemic injury and assess the graft function and viability.

Brasile and Stubenitsky developed in the early 1990s a subnormothermic perfusion system called exsanguinous metabolic support (EMS) and showed that EMS can repair marginal grafts [7, 8]. Several years ago, multiple groups reported

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✉ Markus Selzner
markus.selzner@uhn.ca

[1] Multi Organ Transplant Program, Department of Surgery, Toronto General Hospital, University Health Network, Toronto, Ontario, Canada

[2] Division of Nephrology, The Hospital for Sick Children, Toronto, Ontario, Canada

that adding oxygen to the hypothermic perfusion could result in better graft function and several clinical trials were performed to investigate the benefits of oxygenated perfusion [9, 10]. Results from these studies are still pending. In the early 2000s, Nicholson's group studied brief normothermic warm perfusion following cold storage for graft assessment and reconditioning [11, 12]. The same group also determined the feasibility of short periods of warm perfusion after hypothermic machine perfusion (HMP), however without improvement in renal function compared with HMP [13]. In 2015, controlled oxygenated rewarming (COR) for kidney grafts was introduced as a preservation method which allows a gentle recovery of the mitochondria [14].

Perfusion parameters depend on the temperature at which the perfusion is performed. For HMP, a pressure of 25/30 mmHg has proven superior outcomes with better preservation of renal proximal tubules and better functional recovery compared with a higher pressure of 60/40 mmHg [9]. For the warm perfusion, a physiologic arterial pressure of 65 mmHg has shown best results [15]. In order to maintain a normal urine production, osmolarity and oncotic pressure should also be keep at physiologic ranges [16]. Depending on the device, the arterial flow can be pulsatile or continuous.

This review summarizes the latest developments in machine-based kidney preservation technologies over the last 3 years.

Hypothermic Machine Perfusion

Preclinical Animal Studies

Molecular Mechanisms

Several animal studies have proven that hypothermic machine perfusion is superior to static cold storage (SCS) [9, 17]. Still, at this point, the mechanisms that are involved in this process remain unclear. Therefore, efforts have been made in the last years to better understand which factors are responsible for the protective effects.

Nath and colleagues [18] used 1H-NMR spectrometry to investigate the metabolic profile of kidneys preserved for 24 h with either cold storage or HMP. Several central metabolites such as lactate, aspartate, glutamate, fumarate, and acetate were higher in the perfusate and tissues preserved with HMP, suggesting de novo production of these metabolites during HMP.

Using isotope-labeled glucose, the same group [19] investigated metabolism changes during HMP in the perfusate and kidney tissue. Results showed not only accumulation of lactate and alanine as glycolytic endpoints metabolites, but also enrichment of glutamate as non-glycolytic metabolite, suggesting the presence of aerobic TCA cycle activity.

He et al. [20] found evidence of activation of the Ark-Erk pathway along with decreased activity of proapoptotic proteins (Bad, captase-3, cytochrome c) and increased activity of anti-apoptotic proteins (Bcl-2, Hsp27) during HMP. Also, HMP was associated with less accumulation of toxic metabolites in the perfusate, improved electrolytes homoeostasis, and reduced oxidative stress.

Yang et al. [21] investigated in a rabbit model the anti-apoptotic effect of A20, a potent anti-inflammatory protein, and found that HMP increases the expression of A20, which in turn inhibits inflammation and decreases the apoptosis and necroptosis of renal cells. This mechanism might be responsible for the HMP protection of the kidney grafts from ischemic reperfusion injury.

Ex Vivo Graft Treatment

Graft treatment during HMP is another attempt to minimize ischemic injury and better preserve the kidney grafts.

Gregorini et al. [22] determined that adding mesenchymal stromal cells (MSC) or MSC-derived extracellular vesicles to the perfusate during HMP resulted in less ischemic damage and an upregulation of genes responsible for improving cell energy metabolism and ion membrane transport.

Bhattacharjee and colleagues [23] investigated whether exposure to carbon monoxide before reperfusion can decrease the ischemia reperfusion injury of DCD kidney grafts. Organs treated with carbon monoxide showed less tubular necrosis, reduced apoptosis and captase 3 activity, and decreased kidney injury molecule 1 (KIM-1) and neutrophil gelatinase–associated lipocalin (NGAL) levels in the urine produced during 10 h of simulated reperfusion.

Recently, Opatrny and colleagues [24] compared perfusion via hydrostatic pressure with HMP while the kidney was still in the animal's body. They concluded that machine perfusion ensures better flow, more rapid cooling, and better washout.

Hamaoui et al. [25] supplemented the perfusate during HMP with a cytotopic membrane-associated anticoagulant (thrombalexin) in an attempt to minimize microvascular coagulation. During reperfusion, grafts treated with thrombalexin showed enhanced macrovascular and microvascular perfusion; also, cortical rapid sampling microdialysis revealed lower tissue lactate levels suggesting better tissue perfusion.

Another group added corline heparin conjugate, which can reduce inflammation and inhibit activation of thrombosis, to the perfusate and observed a better creatinine clearance, higher urine output, and lower lactate levels in the treated group compared with the control group during simulated reperfusion. Moreover, in the treated group, histology showed less tubular injury and lower urine NGAL, all suggesting protection from preservation injury [26].

Oxygenated Hypothermic Machine Perfusion and End-Ischemic Machine Perfusion

During cold storage and perfusion, about 5% of the kidney metabolism is maintained; therefore, supplying oxygen could possibly induce the restauration of ATP levels, which is essential for the organ' s metabolic activity.

In a porcine model of autotransplantation, Darius and colleagues compared the results of oxygenated HMP with non-oxygenated HMP and SCS [27•]. During the first 3 postoperative days, the oxygenated HMP group showed significantly lower serum creatinine levels compared with the non-oxygenated group and SCS. End-ischemic oxygenated HMP did not show any significant differences in daily serum creatinine levels compared with SCS alone. Moreover, end-ischemic normothermic perfusion after SCS or non-oxygenated HMP also did not result in a lower daily serum creatinine.

In a rodent model, Kron et al. [17] compared the benefits of different 1-h end-ischemic perfusion models; the grafts were exposed to 30 min of warm ischemia, followed by 4 h of cold storage and then 1 h of either warm or cold perfusion with or without oxygen. The grafts exposed to oxygenated end-ischemic cold perfusion showed significantly less oxidative stress, mirrored by less oxidized nuclear DNA and HMGB-1 release, and also less endothelial activation and tubulus injury, as well as lower levels of danger-associated patterns (DAMPs) than perfusion at normothermia.

Clinical Studies

Several controlled clinical studies demonstrated that anoxic HMP decreases the incidence of DGF compared with cold storage for all donor types (standard criteria donor—SCD, extended criteria donor—ECD, DCD) [28]. Moreover, there is evidence that 1- and 3-year graft survival is improved by HMP compared with cold storage [29, 30].

A more recent study investigated whether end-ischemic machine perfusion (eHMP) also results in a decreased DGF rate and could potentially improve graft survival. Grafts from extended criteria donors were subjected to anoxic hypothermic machine perfusion after arrival at the transplant center. Serum creatinine levels were similar in both groups, but there was a tendency of improved graft survival in the eHMP groups. Also, eHMP resulted in a higher acceptance of marginal grafts than cold storage, since 10% of the contralateral cold stored kidneys were discarded. eHMP reduced PNF significantly and was an independent factor for prevention of DGF [31].

Two multicenter European trials investigated the benefits of end-ischemic oxygenated hypothermic perfusion over static cold storage (COPE-POMP; ISRCTN63852508) and, respectively, the benefits of oxygenated versus non-oxygenated hypothermic perfusion in DCD grafts (COPE-COMPARE) in the last years. Both trials have been completed and results are expected soon.

Sandal and colleagues [32•] found varying results regarding the benefits of HMP. This group analyzed DGF rates, patient, and graft survival in 78.207 patients that received kidney grafts from standard and extended criteria brain dead (NDD) and DCD donors that had been preserved with either anoxic HMP or SCS. DGF rate for grafts preserved with anoxic HMP was significantly lower in all groups, except the ECD-DCD group. In contrast, 1-year graft survival was improved by anoxic HMP only in the ECD-DCD group. No benefits of anoxic HMP versus SCS in regard to 1-year graft survival were observed in the other groups. Also, 2- to 5-year graft survival was similar in all groups.

Ex Vivo Graft Treatment

A high concentration of proinflammatory cytokines like TNF-alpha is a possible mediator of graft injury and might contribute to worse outcomes in kidney recipients. Diuwe and colleagues investigated the effects of adding TNF-alpha inhibitor etanercept to the perfusate during HMP. Recipients were followed for 12 to 46 months, and results showed no significant differences regarding occurrence of DGF, graft, and patient survival or serum creatinine levels [33].

At this moment, it remains uncertain to which extend HMP improves function of marginal organs and if the addition of oxygen to the machine perfusion benefits the kidney grafts. Moreover, parameters of function that can be measured during HMP are lacking and remain to be further investigated.

Controlled Oxygenated Rewarming

Recent data suggests that abrupt rewarming of a kidney graft after cold preservation leads to reperfusion damage and possibly inferior graft function. Controlled oxygenated gradual rewarming (COR) might prevent this type of graft injury.

Minor et al. [34] investigated in a DCD model, whether gradual rewarming up to 20° results in a better graft function than static cold storage. After 30 min of warm ischemia, grafts in the COR group were gradually rewarmed to 20 °C in 1 h and perfused at this temperature for further 30 min. During simulated reperfusion, grafts treated with COR cleared creatinine and urea significantly better and also expressed less inflammation and endothelial activation.

The same group compared gradual rewarming with normothermic perfusion. After 20 min of warm ischemia, followed by 20 h of static cold storage, grafts were subjected to either 2 h of normothermic perfusion, 2 h of gradual rewarming up to 35 °C, or no treatment. During simulated reperfusion, grafts subjected to COR demonstrated significantly better creatinine clearance and fractional excretion of sodium and glucose

compared with the other two groups. Also, grafts in the COR group showed less lipid peroxidation and tubular cell injury [35].

Minor et al. also tested whether the addition of washed erythrocytes as oxygen carriers during COR would result in a better kidney function. No improvement was observed during simulated reperfusion by adding erythrocytes in the COR phase [36].

In a follow-up study, the same group evaluated the benefits of COR in a porcine autotransplantation model. Grafts were subjected to 20 min of warm ischemia, 21 h of static cold storage, followed by either transplantation or COR for 90 min and then transplantation. Animals were followed for 7 days. COR resulted in significantly reduced serum creatinine and urea levels, suggesting less reperfusion injury. Also, the COR group showed significantly lower levels of lipoperoxides, suggesting that COR reduced the oxidative stress. Histology revealed less tubular cell damage in the COR group [37•].

At this point, it remains unclear whether COR protects grafts from reperfusion injury and if it is a reliable preservation method. Additional studies are needed to further investigate the possible advantages of this method.

Normothermic Machine Perfusion

Preclinical Animal Studies

Hosgood group introduced normothermic machine perfusion (NMP) as a novel technique for graft reconditioning (1–2 h) after cold storage [13]. Kaths et al. developed a normothermic ex vivo kidney perfusion model (NEVKP) that allowed the warm perfusion of grafts for the whole preservation period and up to 16 h, eliminating the entire cold storage phase [15, 16, 38]. This method allowed also for the assessment and modification of grafts prior to transplantation [39]. In a series of experiments, Kaths and colleagues demonstrated that NEVKP is a superior storage strategy for marginal renal grafts which prevents the development of delayed graft function (DGF) in a porcine renal autotransplantation model [40•]. Moreover, they demonstrated that prolonged NEVKP provides better outcomes than short time NEVKP [38, 41]. The same group showed data which suggested that NEVKP reduces kidney injury in marginal renal grafts [42•].

Graft Assessment and Repair During Ex Vivo Normothermic Perfusion

Normothermic machine perfusion represents also a window for graft assessment and treatment.

Hosgood et al. [43] exposed kidney grafts to hydrogen during simulated reperfusion in an attempt to reduce ischemic injury, considering that hydrogen has antioxidant and anti-inflammatory effects. After 15 min of warm ischemia and 22 h of cold storage, ex vivo reperfusion was performed for 6 h. Two percent hydrogen was added to the gas that was administered in the treatment group (2% hydrogen, 25% oxygen, 5% carbon dioxide, 68% nitrogen). During reperfusion, no differences were found between the hydrogen group and the control group in terms of creatinine clearance, urine output, and renal flow.

Another attempt was made by adding a CytoSorb adsorber into the circuit. Cytokine removal using hemadsorption has been successfully used in the treatment of systemic inflammatory response syndrome. The CytoSorb adsorber can remove both pro- and anti-inflammatory cytokines, so its effects during ex vivo perfusion could be beneficial or not. Even though the renal blood flow was improved in the CytoSorb group, there was no difference regarding glomerular and tubular function of the grafts between groups [44].

Horn et al. [45] investigated whether pulsatile versus continuous flow results in better perfusion during ex vivo normothermic perfusion. They demonstrated that grafts that had been previously cold stored for 20 h showed an improved renal flow upon pulsed perfusion. This was not the case for undamaged grafts that were perfused directly after retrieval, without any cold storage. Also, creatinine clearance and fractional excretion of sodium were significantly better in the pulsatile perfused group.

Clinical Studies

Hosgood et al. [46] were the first group worldwide to use kidney NMP in a clinical trial. Since then, several other leading centers in the UK, the Netherlands, Canada, and USA have translated the NMP technology into clinical trials.

At present, it remains unclear, which parameters during machine perfusion allow the assessment of the grafts and predict a better outcome. Also, several attempts have been made to modify and repair renal grafts during warm machine perfusion.

One multicenter UK trial is investigating the benefits of 1 h of ex vivo normothermic perfusion compared with cold storage in DCD kidneys. The trial is expected to be completed in 2020 (ISRCTN15821205) [47].

Graft Assessment During NMP

Hosgood and colleagues used kidneys deemed unsuitable for transplantation to correlate perfusion parameters during normothermic perfusion with urine markers of kidney injury after reperfusion. They assessed renal blood flow, oxygen consumption, and urine output during 1 h of normothermic ex vivo perfusion and correlated it with three parameters of kidney injury after transplantation measured in a urine sample

collected at the end of the perfusion—NGAL, KIM-1 and endothelin-1 (ET-1). The group found a correlation between the perfusion parameters and urinary ET-1 and NGAL levels. In addition, the terminal renal function in the donor was predictive for post transplantation urine ET-1 and NGAL levels. No correlation was found regarding KIM-1. This suggests that urinary biomarkers levels during perfusion could offer additional information regarding organ quality [48].

In a recent trial, Hosgood and colleagues developed a scoring system for assessing kidneys during NMP. The score uses the macroscopic appearance, the mean renal blood flow, and the total urine output. Kidneys with a score of 3 or less are considered to be of sufficient quality for transplantation. Based on this score, Hosgood et al. successfully transplanted five kidneys that had been considered unsuitable for transplantation. One of five grafts had delayed graft function which required dialysis and then had suboptimal graft function. However, this was also the kidney with the highest score and highest levels of NGAL, suggesting poorer quality of this graft. The other four grafts functioned immediately [49•].

Graft Treatment During NMP

Organ treatment and repair before transplantation is a challenge that remains unsolved at the moment. Several attempts have been made to identify therapies that would result in a better function of the kidney grafts.

Tietjen and colleagues investigated whether polymeric nanoparticles delivered during normothermic perfusion can accumulate within a target cell type. They could prove that targeted nanoparticles delivered to human kidney grafts during NMP can accumulate in the vasculature of the renal cortex, which implicates that they have the potential to be used for targeted delivery of therapeutic drugs [50].

Mesenchymal stromal cells, which have regenerative properties, have been administered to kidney transplant patients, but they could not reach the kidneys because they were trapped in the lungs. In order to avoid the complications of intravenous delivery, administration of MSC during NMP could be a solution [51]. Brasile et al. added MSC to the perfusate during warm exsanguinous metabolic perfusion of discarded human kidney grafts and results showed that MSC did not integrate into the renal tissue, since 95% of the MSC remained in the perfusate after 24 h of perfusion. Kidney grafts treated with MSC produced less inflammatory cytokines, showed an increased synthesis of ATP and growth factors and increased mitosis [52].

In porcine models, NEVKP was found to be a safe method for the preservation of marginal grafts with better graft survival compared with cold storage. First clinical studies have been started and results will determine the reliability of this preservation method. The optimal length of perfusion, best markers for the assessment of graft function, and possible treatments during perfusion remain to be further investigated. Also, there is a need for the development of portable devices for NEVKP that have little technical requirements and could be easily transported.

Normothermic Regional Perfusion

The newest approach in organ preservation is normothermic regional perfusion (NRP). During NRP, the abdominal organs are reperfused in situ with the donor's own blood, using extracorporeal membrane oxygenation (ECMO). This method was

Table 1 Clinical outcomes from single-center series of kidney transplantations with marginal grafts that had been previously subjected to either NRP or ex vivo normothermic perfusion

Author	*N*	DGF (%)	PNF (%)	1-year graft survival (%)	Perfusion time (min)	DCD category	Data collection time; site
In situ normothermic regional perfusion							
Reznik et al [55]	44	52.3%	0	95.5%	145.5 ± 6.1	uDCD	2009–2011; Russia
Rojas-Pena et al [56]	48	31%[a]	3.5%[a]	NR	86 ± 5	cDCD	2000–2013; USA
Demiselle et al [57]	19	53%	5%	94.4%	60–240	uDCD	2011–2013; France
Hessheimer et al [58]	43	56%	0	91%	NR	uDCD	2007–2013; France
Oniscu et al [59]	32[b]	40%	0	NR	120	cDCD	Unknown–2014; UK
Minambres et al [60]	37	27%	5%	91.8%	109	cDCD	2014–2016; Spain
Ex vivo normothermic machine perfusion							
Hosgood et al [49]	5	20%	0	NR	60	cDCD	2012–2014; UK
Nicholson et al [61]	18	5.6%	0	NR	63 ± 13	ECD	2010–2012; UK

DCD donation after circulatory determination of death, *cDCD* controlled/expected donation after circulatory determination of death, *DGF* delayed graft function, *min* minimum, *NR* not reported, *PNF* primary non-function, *uDCD* uncontrolled/unexpected donation after circulatory determination of death

[a] Rates of DGF and PNF are for 29 transplanted kidneys

[b] Including four double kidney transplantation

first used in Spain in 1989 for uncontrolled DCD donors [53]. Although there is only a limited number of cases that have used NRP, studies report minimal PNF and an excellent 1-year survival [54•] (Table 1).

Minambres and colleagues reported recently about the Spanish experience with abdominal regional perfusion. Thirty-seven kidney grafts from DCD donors underwent at least 60 min of NRP and were then successfully transplanted. There were two cases of PNF due to arterial thrombosis (5%) and ten cases of delayed graft function (27%), and 1-year death-censored kidney survival was 91.8% [60] [Table 1].

A recent animal study investigated kidney function in DCD grafts that were subjected to 2, 4, or 6 h of NRP compared with a control no-NRP group. NRP grafts compared with no-NRP grafts showed lower resistance during HMP, and also grafts perfused for 4 h or more showed better early renal function recovery and also late outcomes (GFR). In regard to several injury markers, such as aspartate aminotransferase, neutrophil gelatinase–associated lipocalin, and kidney injury molecule-1, 4-h NRP appears to be the optimal perfusion time [62]. The ideal duration and perfusion parameters of NRP remain to be established in future studies.

Conclusion

Anoxic hypothermic machine perfusion remains the most widely used kidney machine preservation method worldwide, which results in less DGF for all donor types (SCS, ECD, DCD). However, there is increasing evidence that NEVKP could offer better graft preservation than HMP. Keeping the kidney grafts metabolically active allows for assessment and treatment, which remains the main focus in research at this moment.

Several attempts have been made to modify the existing perfusates; however, no ideal composition has been established yet. Several clinical trials are using normothermic ex vivo warm perfusion in human kidneys with positive first results, but there is still no consensus regarding optimal perfusate composition and duration of perfusion. There is data suggesting that graft assessment with NEVKP allows the use of very marginal grafts that would have otherwise been discarded. NRP is a new promising alternative for enlarging the donor pool by extending the use of DCD grafts. Future studies need to investigate the optimal protocol for NRP perfusion.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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