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REVIEW

Hypothermic machine perfusion of kidneys retrieved from standard and high-risk donors

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Summary

Hypothermic machine perfusion (HMP) of kidneys is a long-established alternative to static cold storage and has been suggested to be a better preservation method. Today, as our deceased donor profile continues to change towards higher-risk kidneys of lower quality, we are confronted with the limits of cold storage. Interest in HMP as a preservation technique is on the rise. Furthermore, HMP also creates a window of opportunity during which to assess the viability and quality of the graft before transplantation. The technology might also provide a platform during which the graft could be actively repaired, making it particularly attractive for higher-risk kidneys. We review the current evidence on HMP in kidney transplantation and provide an outlook for the use of the technology in the years to come.

Introduction

Decades before deceased donor kidney transplantation reached clinical practice, the concept of machine perfusion was already coined. In 1935, Charles Lindberg described a device designed to maintain a sterile, pulsating circulation of oxygenated fluid through organs [1]. However, a true clinically applicable device was not developed until Belzer became interested in hypothermic machine perfusion (HMP) in the 1960s. His preclinical work, showing that cryoprecipitated plasma could be used to perfuse canine kidneys [2–4], led to the first successful human HMP kidney transplant in 1968 [5]. With technological progress came portable, although still rather large, machines [6], and HMP was commonly used, predominantly in the USA during the 1970s. However, two events led to the apparent demise of HMP. Firstly, simple and cheap static cold storage (CS) solutions became available [7,8]. Secondly, the definition of brain death radically changed the deceased donor profile. Kidneys were no longer donated after circulatory death (DCD) but were retrieved from brain-dead donors (DBD) [9,10]. These pristine DBD kidneys tolerated cold ischaemia in the available preservation solutions relatively well. It appeared that the use of rather cumbersome

HMP units was no longer necessary. Nevertheless, Belzer continued his work on HMP and developed a synthetic HMP solution replacing his previous human albumin-containing solution. This solution could preserve canine kidneys for up to 5 days [11,12] and it still is the standard HMP solution today. Furthermore, it is the concept of this solution and its particular components that, with a few modifications, gave rise to the University of Wisconsin solution, the gold standard for abdominal organ preservation [13].

Technological advances have made it possible to construct user-friendly, portable machine perfusion devices that are now commercially available (Fig. 1).

Over the past decade, our deceased donor profile has significantly changed again and continues to change. The use of (older) DCD and expanded criteria donor (ECD) kidneys is increasing exponentially and with it so is the use of HMP (Fig. 2). These higher-risk kidneys are particularly susceptible to preservation-induced injury, delayed graft function (DGF), and primary nonfunction (PNF) and can experience reduced long-term graft survival. CS of higher-risk kidneys has reached its limits, and HMP is thought to preserve them better. Furthermore, HMP creates a window of opportunity during which to assess the viability and quality of the graft before transplantation. The technology might also provide a platform during which the 'grafts-to-be' might be actively repaired.

We review the current evidence regarding HMP in kidney transplantation and provide an outlook for the use of the technology in the years to come.

Does HMP provide better preservation?

Even though HMP has been used for over 40 years, there are relatively few well-conducted randomized controlled trials (RCT) comparing HMP to CS.

Data from the 20th century

A meta-analysis of all 16 studies [14–32] prospectively comparing HMP with CS between 1971 and 2001 showed that HMP is associated with a relative risk of DGF of 0.80 (0.67–0.96) compared with CS [33,34]. No effect of HMP on 1-year graft survival was detected; however, all studies were severely underpowered with respect to the likely impact on graft survival [33]. Furthermore, the evidence spreads out over decades during which CS solutions, donor profile, outcome measures, etc. have changed considerably. The report concludes that a definite study establishing the effect of HMP on DGF and long-term graft survival, together with an economic evaluation, would be of great value. To provide these data, a few RCTs were set up in Europe and these are reviewed below.

Recent randomized controlled trials of HMP versus CS

HMP in deceased donor kidneys

In 2009, the report of an international RCT in Eurotransplant comparing HMP to CS of kidneys from all deceased donor types (standard criteria donors (SCD), ECD, controlled DCD) was published [35]. This MP-Trial analysed 336 kidney pairs. DGF occurred in 20.8% of HMP kidneys versus 26.5% in CS kidneys. Logistic regression showed that HMP reduced the risk of DGF (adjusted odds ratio (AOR) 0.57 (0.36–0.88)). If DGF developed, its duration was 3 days shorter after HMP (10 days vs. 13 days, $P = 0.04$). Functional DGF [36] developed in 22.9% HMP kidneys and in 30.1% of CS kidneys ($P = 0.03$). PNF occurred in 2.1% of HMP kidneys and in 4.8% of CS kidneys ($P = 0.08$). Graft survival was also improved by HMP (94% vs. 90%, $P = 0.04$) (Fig. 3). Cox regression analysis showed that HMP reduced the risk of graft failure in the first year after transplantation (adjusted hazard ratio

Figure 1 Currently available hypothermic machine perfusion devices for clinical kidney preservation. Panel (a): RM3 (Waters Medical systems, Birmingham, AL, USA), www.wtrs.com; panel (b): LifePort Kidney Transporter (Organ Recovery Systems, Itasca, IL, USA), www.organ-recovery.com; panel (c): Kidney Assist (Organ Assist, Groningen, the Netherlands); www.organ-assist.nl.

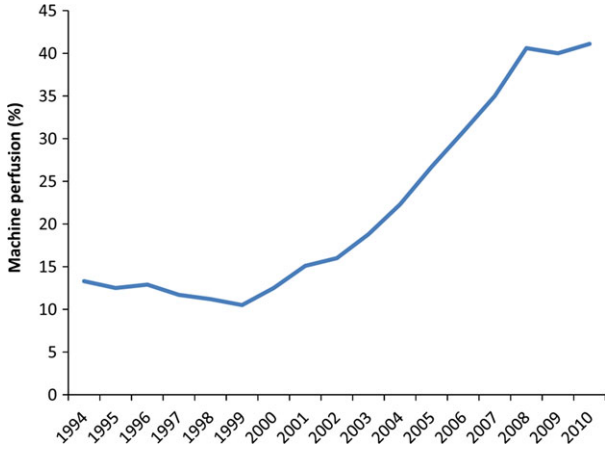

Figure 2 Percentage of deceased donor kidneys preserved by hypothermic machine perfusion in the United States of America each year. Graph constructed using OPTN data [117].

(AHR) 0.52 (0.29–0.93)). The 3-year follow-up data of this trial confirmed the improved graft survival of HMP kidneys (91% vs. 87%; AHR 0.60 (0.37–0.97)) [37] (Fig. 3).

HMP of expanded criteria donor kidneys

The beneficial effect of HMP might be more pronounced in ECD kidneys [38,39]. Multivariate regression of the 91 randomized ECD kidney pairs of the MP-Trial showed that HMP reduced the risk of DGF compared with CS (AOR 0.46 (0.21–0.99)). PNF was also lower in HMP compared with CS kidneys (3% vs. 12%, $P = 0.04$) [40]. One-year graft survival was higher after HMP (92.3% vs. 80.2%, $P = 0.02$) with an AHR of 0.35 (0.15–0.86). The 3-year graft survival advantage after HMP was maintained for ECD kidneys (86% vs. 76%, AHR 0.38 (0.18–0.80) [37].The presence or absence of DGF seems to have an impressive effect on graft survival, especially in CS ECD kidneys. Although there was a difference of nearly 10% for 1-year HMP kidney graft survival if DGF occurred compared with kidneys with immediate function, this difference was not significant (94% vs. 85%, $P = 0.16$). However, in CS kidneys that developed DGF, graft survival was significantly worse compared to when the graft functioned immediately (41% vs. 97%, $P < 0.0001$) (Fig. 3). If only recipients of grafts that developed DGF were analysed, there was a significant difference in 1-year graft survival between HMP and CS kidneys (85% vs. 41%, $P = 0.003$).

Additional data will become available from an ongoing RCT comparing HMP with CS of ECD kidneys in France (NCT01170910).

HMP of kidneys donated after circulatory death

Previous studies have suggested that HMP of DCD kidneys results in better early function and improved graft survival

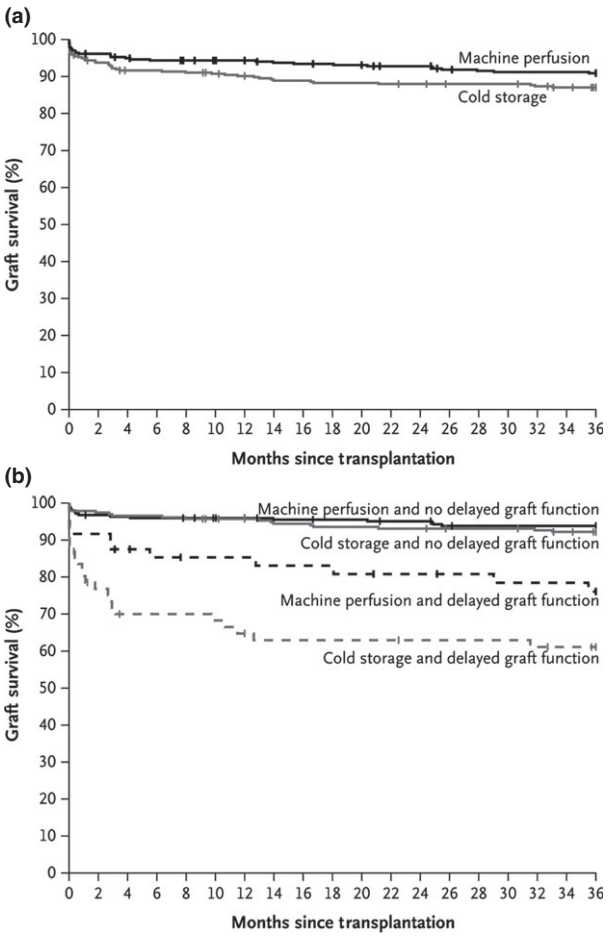

Figure 3 Three-year graft survival of deceased donor kidneys included in the Machine Preservation Trial. Panel (a) shows graft survival in 672 kidney recipients, with a hazard ratio for graft failure in the machine perfusion group of 0.60 (95% confidence interval, 0.37–0.97; $P = 0.04$). Panel (b) shows the post hoc analysis of a subgroup of 588 recipients of kidneys donated after brain death, with data split according to whether delayed graft function developed in the recipient. Delayed graft function was defined as the need for dialysis in the first week after transplantation. From [37] Copyright © 2012 Massachusetts Medical Society. Reprinted with permission.

compared with CS. However, other studies do not support this conclusion [14,41–44]. A comprehensive meta-analysis failed to show a significant risk reduction of DGF in HMP DCD kidneys [33,34]. Although the MP-Trial included DCD donors, 87.5% of inclusions were DBD kidneys. In a separate randomized extension of the MP-Trial, data from 82 Maastricht III DCD kidney pairs were analysed [45]. HMP reduced the risk of DGF (AOR 0.43 (0.20–0.89)). Of HMP DCD kidneys, 53.7% developed DGF compared with 69.5% of CS DCD kidneys ($P = 0.007$). Functional DGF was even more reduced by HMP (19.5% vs. 51.2%; $P < 0.0001$). PNF occurred in only two cases in each study arm. HMP did not result in an increased 1-year or 3-year

graft survival (93.9% for HMP kidneys vs. 95.1% for CS kidneys). Although equal outcome might seem contradictory, this finding is in line with an increasing number of reports showing similar medium-term graft survival for DCD and DBD kidneys despite higher rates of DGF in DCD kidneys [46–48].

A parallel-run RCT, analysing outcome of 45 Maastricht III DCD kidney pairs in the United Kingdom, showed no difference in DGF between HMP and CS (58% vs. 56%; $P = 0.99$; AOR 1.14 (0.38—3.49)) [49]. One kidney in the HMP group suffered from PNF. One-year graft survival was similar in both groups (93.3% in the HMP group and 97.8% in the CS group, $P = 0.3$).

The contradicting results between the two trials, currently the largest RCTs performed and the best available evidence, might be related to the setting in which HMP is used. In the MP-Trial, kidneys were placed on the HMP device at the donor centre, immediately after retrieval, whereas in the United Kingdom trials, those kidneys removed away from the transplant centre were cold stored during transfer after which HMP was started. As such, it could be that HMP needs to be used in a continuous setting to achieve a benefit.

An ongoing RCT in the United Kingdom is duplicating the set-up of the MP-Trial with continuous HMP being compared with CS (ISRCTN 50082383).

Meta-analyses

A number of meta-analyses have recently been published reviewing the evidence of HMP and CS in kidney transplantation. Two meta-analysis analysed data from all donor types and reported a decreased risk for DGF with HMP (AORs of 0.83 (0.72–0.96) [50] and 0.81 (0.71–0.98) [51]). Two meta-analyses analysed data from DCD kidneys and found a protective effect of HMP on DGF (AORs of 0.56 (0.36–0.86) [52] and 0.64 (0.43–0.95) [53]). There is one meta-analysis that combined the data of HMP in ECD kidneys which also showed a protective effect of HMP on DGF (AOR 0.59 (0.54–0.66) [54]). Overall, even with the meta-analysis of prospective data, numbers have not been large enough to identify a benefit of HMP on outcomes that are relatively rare, such as PNF and 1-year graft loss. In studies that have individually identified a difference, this has largely been in ECDs.

Is machine perfusion cost-effective?

Meta-analysis assessing the cost-effectiveness of HMP could not draw any conclusions because of a lack of properly powered studies. Published economic evidence at the time was of poor quality and not based on randomized studies [33,55]. An economic evaluation of the MP-Trial data combined the 1-year results based on the empirical data from the study with a Markov model with a 10-year time horizon [56]. Short-term evaluation showed that HMP results in lower average costs in the first year post-transplant when compared to CS and across all deceased donor types but mostly apparent in the ECD group, related to the increased 1-year graft survival of pumped ECD kidneys. The costs of dialysis and readmission were mainly responsible. The long-term analyses showed a similar benefit; the Markov model revealed cost savings of $ 86 750 per life-year gained in favour of HMP. The corresponding incremental cost–utility ratio was minus $ 496 223 per quality-adjusted life-year gained.

Does timing or duration of HMP matter?

It is well known that renal vascular resistance (RR) falls during HMP, whilst perfusate flow increases [57,58]. This has prompted the question of whether or not there is a required minimum duration of perfusion for any potential benefit of HMP to be evident. Recently published experimental studies have found that between 1 and 4 h of HMP before reperfusion with blood could reduce RR and improve creatinine clearance compared with kidneys preserved by CS alone [59,60]. Then again, other studies have shown that despite reaching mean perfusate flows after two hours of perfusion, RR values continue to improve at 6 h [57]. As it is frequently necessary to transport organs many miles between donor and recipient, a beneficial effect of a short period of end-ischaemic HMP sounds attractive and avoids the logistics of transporting machines. Nevertheless, there is some suggestion that when following long periods of CS, relatively short periods of HMP (<4 h) have reduced or no benefit compared to continuous HMP throughout the preservation period [61]. Clinical trials that have shown significant benefits with HMP have used continuous HMP with the kidney being pumped for many hours, on average the majority of the total cold ischaemic time (median 11–15 h) [35,40,45,62]. Recent large registry analyses (over 90 000 kidneys) have shown that in the case of SCD kidneys, HMP reduces the risk of DGF compared with CS regardless of a very short or very long cold ischaemic time [63]. In the same study, the risk of DGF was reduced for ECD kidneys only if the total cold ischaemic time was >6 h, and in the case of DCD only if 6–24 h [63]. These discrepancies may be a function of the numbers available for power but if not, have important consequences. Furthermore, as total cold ischaemic time is a well-established predictor of DGF, a balance between minimizing cold ischaemic time and any potential benefits of HMP is required [64]. It seems that just a few hours of HMP, as long as the total cold ischaemic time is not extended, can have a positive impact on early graft function compared with CS

alone. However, there is currently no good evidence to suggest that HMP allows lengthening cold ischaemic times. Currently, an RCT comparing end-ischaemic HMP with CS in DBD kidneys is ongoing (ISRCTN 35082773). However, to the best of our knowledge, there are no clinical trials comparing continuous with end-ischaemic HMP.

Does machine perfusion predict graft viability and quality?

Kidney graft viability and quality cannot be assessed when kidneys are cold stored on melting ice. On the contrary, HMP allows the study of perfusion characteristics such as RR and perfusate biomarkers.

The value of renal vascular resistance

Retrospective evidence suggests that RR and flow rate during HMP correlate with kidney graft function [33]. However, in most of these studies, a selection bias was introduced because kidneys were systematically discarded based on arbitrarily defined parameter thresholds. Today, 'poor' perfusion dynamics are still frequently used to discard kidneys even though their true prognostic value on graft outcome had never been studied until recently. Indeed, more than 15% of HMP kidneys are discarded annually in the USA, partly based on elevated RR [65]. In the MP-Trial, the preservation method was not revealed at the time of organ offer and in case of HMP clinicians had no knowledge of the RR value. The decision to accept a given kidney was based solely on traditional donor data, making it possible to elucidate the real prognostic value of RR on PNF, DGF and 1-year graft survival [58]. Analysis showed that RR at the end of HMP was an independent risk factor for the development of DGF, independent of donor type (AOR 38.1 (1.56–934)). However, the predictive power of RR was low (AUC of the ROC curve 0.58). This means that, despite the association of RR and DGF, RR has a limited value in the prediction of DGF for a specific donor–recipient pair. RR was also a risk factor for 1-year graft failure but again with low predictive power (AHR 12.3 (1.11–136.9)). A retrospective study on RR in DCD kidneys showed that RR at the start of HMP is a risk factor for both DGF (AOR 2.34 (1.11–4.96)) and PNF (AOR 2.04 (1.36–3.06)) but again the predictive power was only moderate at best (0.61). Available evidence indicates that kidneys should not be discarded based on RR criteria alone.

The value of perfusate injury biomarkers

Retrospective data – systematically reviewed in [66] – also suggest that biomarker concentrations in the perfusate of HMP kidneys correlate with graft outcome. The groups of Newcastle and Maastricht have previously determined the perfusate concentration of total glutathione-S-transferase (GST), heart-type fatty acid-binding protein (H-FABP), and alanine aminopeptidase and found higher concentrations in perfusate of discarded kidneys [67–69]. Furthermore, lactate dehydrogenase (LDH) and GST concentrations correlated with warm ischaemia time, and GST and H-FABP were higher in Maastricht II compared with Maastricht III DCD kidneys. However, these data are confronted with the same methodological design and selection bias as the RR data, and none of the previous studies investigated whether perfusate biomarkers were independently associated with graft outcome. In prospectively collected perfusate of the MP-Trial, GST, *N*-acetyl-β-D-glucosaminidase and H-FABP were found to be independent predictors of DGF, but not of PNF or graft survival [70]. The predictive power of these three biomarkers was also moderate at best (0.67, 0.64 and 0.64, respectively). A retrospective study in DCD kidneys showed that LDH and IL-18 perfusate concentrations were associated with PNF (AOR 1.001 (1.000–1.002) for both markers) but that the predictive power was poor (0.62 and 0.59). Thus, similar to RR, perfusate biomarkers alone should not lead to kidney discard.

As there are multiple donors, preservation and recipient factors influencing graft outcome, it is not surprising that RR and individual perfusate biomarkers are not sufficient on their own to be used as sole predictors of outcome. However, given the fact that they do independently correlate with DGF (RR, biomarkers) and with graft survival (RR), they are valuable in assisting clinicians in decision-making. Perhaps that implementation of these markers in existing quality scores for DGF and graft survival will increase their predictive power [71–73]. Emerging technologies such as proteomics and metabolomics open new doors to investigate viability and quality assessment during HMP.

Can HMP be used for active repair of the injured kidney?

In the past decade, it has been found that cerebral injury and brain death contribute significantly to the cascade of injury of donor organs. Cerebral injury will result in a significant up-regulation of the innate immune system with a profound and progressive systemic inflammatory response leading to damage in the kidney graft-to-be. To reduce or even repair injury, optimization of donor management is a must, possibly also requiring more attention towards targeted intervention prior to organ retrieval. However, optimal preservation will always remain of utmost importance to avoid additional injury and possibly repair injury that has already taken place.

The capabilities of HMP to repair damaged organs and its use as a method to deliver a variety of therapies have been tested in several ways. The particular strength of this method is the ability to provide local effects without systemic exposure or uptake of drugs, cells, etc.

Reconditioning

One potential use of HMP is in the reconditioning of organs prior to transplantation. Any mechanistic effect that HMP has on the kidney graft is not well understood, other than the drop in RR that is clearly evident [58]. As a downstream effect, there is some evidence from experimental kidney transplant studies that biomarkers of injury and of ischaemia reperfusion injury are reduced by HMP [74–76]. Kidneys preserved by HMP in these studies had improved tubular and renal cell function, and less protein excretion after reperfusion [74]. There is some evidence that HMP improves ATP recovery and reduces perforin expression and pro-apoptotic signals [77]. Potentially, the up-regulation of HIF-1α and altered expression of caspase proteins may play a protective role [78]. The preserved expression of flow-dependent genes might also be important [79].

An improved preservation solution

Currently, Belzer's HMP solution is used to perfuse kidneys. It is a gluconate-based perfusate that contains hydroxyl-ethyl starch and, contrary to the CS preservation solution, has a low potassium concentration to avoid vasoconstriction. The constitution of the perfusion solution is likely to play an important role in the outcome of the graft after transplantation. Keeping in mind the mechanisms of ischaemia reperfusion injury, it is possible that improved solutions or the addition of specific reagents targeting these mechanisms to the preservation solution could be developed. New solutions that resemble cell culture media (AQIX RS-I, Lifor) show potential [80,81]. They contain amino acids, metabolic substrates, vitamins, salts and organic buffers that make them ideal potential new solutions for HMP.

Drug delivery

Hypothermic machine perfusion has proven to be a useful way to deliver potentially protective therapies to the kidney during preservation without systemic administration. Administration in this way permits adequate penetration of vascular compartments without altering perfusion dynamics and allows assessment of the degree of uptake during perfusion [82]. So far, experience with this method of delivery has included heparin conjugates that adhere to vascular endothelium [82]. A water-soluble preparation of the drug propofol has also been added to HMP with improved early graft function [83]. These studies demonstrate the potential to administer therapies in this way; however, clinical results are yet to be reported. An ongoing clinical study aims to administer the inhibitor of complement, Mirococept, during HMP which would act locally and not affect systemic complement activation (ISRCTN49958194). There are several chemical compounds including hydrogen sulphide, carbon monoxide and nitrous oxide which have anti-apoptotic and vasodilatory effects when applied in experimental conditions [84]. These could be applied during HMP. In experimental models of ischaemia reperfusion injury, hydrogen sulphide administration during ischaemia results in reduced TNF-alpha and IL-2 levels and neutrophil invasion with improved microvascular circulation and subsequently improved renal function [85–87].

Gene therapy

The transfection of genes targeted at inhibiting harmful pathways or stimulating protective ones is also a potential adjunct to HMP. Delivering gene therapy during perfusion would mean that the vector (usually a virus) does not have to be administered systemically to the patient. Several gene-therapy targets have been identified which may improve ischaemia reperfusion injury in transplanted kidneys [88]. Potential targets to reduce acute rejection include genes which up-regulate IL-4, IL-10, IL-13, Fas-ligand or blockades of costimulatory molecules [88]. Tolerogenic strategies and stimulation of regeneration are also potential avenues for gene therapy that are now being explored [88]. This technique is in the very early stages of investigation, and so far, there is limited evidence that renal cells can be transfected in the cold [89] and during normothermic perfusion [90].

Stem cells

Hypothermic machine perfusion may allow the direct administration of mesenchymal stem cells (MSC) to the kidney during preservation [91]. In a murine model of acute kidney failure, the administration of MSC improved recovery from ischaemia reperfusion injury and subsequent early kidney function [92]. In a ground-breaking clinical study of living-related kidney transplants, the administration of MSC at reperfusion, and 2 weeks later, resulted in lower rates of acute rejection, less infection and improved graft function at 1 year compared to IL-2 receptor antagonists [93]. Subsequent studies have assessed the potential role of donor-derived MSC in a regimen to reduce immune suppression, administered to the recipient [94]. Pretransplant administration of autologous MSC has also been tested in very small series, with some advantages over

post-transplant administration [95]. Further research is required to assess HMP as a potential method to deliver MSC directly to the organ, bypassing the difficulty of trafficking cells to desired site. So far, these studies have administered MSC under normothermic conditions only.

How can HMP be improved?

Effect of temperature

Traditionally, both static cold storage and machine perfusion of kidney grafts have been done at ice-cold temperatures (0–5 °C) to slow down metabolism as much as possible. Machine preservation at temperatures below normothermia, but above ice-cold temperatures has been investigated (subnormothermia), but the mechanism of action is not fully understood, and the results so far in kidney transplantation are experimental only. A recent animal study in a DCD model found that kidneys stored by machine perfusion at 20 °C had improved creatinine clearance compared with both CS and oxygenated HMP [96]. Given the higher metabolic demands at higher temperatures, it seems that provision for oxygenation and higher perfusate flow needs to be made [96]. Another experimental model of kidney preservation used 'room temperature' perfusion with the preservation solution Lifor. This type of preservation was associated with improved perfusate flow and reduced renal resistance during perfusion [81]. Preservation at temperatures just below normal (30 °C) with the preservation fluid AQIX RS-I has been tested for short periods (2 h) [97]. The same fluid was also tested as a potential subnormothermic flush before CS in experimental studies [80]. More work has been carried out with liver models of subnormothermic preservation at temperatures as diverse as 7–21 °C; however, the information available is still limited in its scope and applicability to clinical practice [98]. Maintaining a kidney graft at temperatures closer to normothermia comes with ongoing metabolism at a faster pace that must be supported with oxygen, nutrients and clearance of metabolites, making it a much more complex technique compared with HMP [99]. The use of normothermic machine perfusion has also been extensively investigated over the past decade and has recently been introduced in the clinics [99–105]. However, an in-depth review on normothermic machine perfusion of kidneys is beyond the scope of this article.

The addition of oxygen

As can be made up from the construction of the Lindbergh apparatus that included a gas mixture of carbon dioxide, nitrogen and oxygen, the latter was considered to be a vital part of kidney preservation from the start of preservation technology [1]. Although the rational of using oxygen to sustain metabolic cell processes makes perfect sense, it is quite paradoxical that the majority of clinically applied preservation methods nowadays do not use oxygen. However, with the availability of high-tech perfusion platforms, the deteriorating quality of donor kidneys and the need to 'repair' these higher-risk kidneys prior to transplantation, oxygenation is finding its way into transplant research. Increasing evidence suggests that kidneys preserved by HMP will consume oxygen and that the level of oxygen consumption is correlated with postpreservation glomerular filtration rate [106]. In that sense, adding oxygen to the preservation solution would be beneficial, especially when it comes to restoring cellular levels of ATP after the kidney has been exposed to ischaemia [107]. On the other hand, the presence of oxygen could potentially increase the production of radical oxygen species and thereby cause increased injury [108]. The delicate balance between active oxygenation and antioxidant properties of the preservation solution will no doubt need to be taken into account.

Oxygen can be delivered in a number of ways: by simple retrograde persufflation of oxygen directly through the renal vein (without necessarily using machine perfusion); during machine perfusion where oxygen can be dissolved into the perfusate; or by adding artificial oxygen carriers (e.g. acellular solution based of perfluorocarbons by Brasile *et al.* [109–111], Hemo2Life [112]). The most recent studies have used newer technology in which a membrane oxygenator complements a more typical HMP device. There are animal auto-transplant studies published which have found indications for some benefit of oxygen provision, particularly if following a prolonged warm ischaemic time [113]. The major benefit seen so far is in early kidney function; the study was unfortunately too small to comment on graft survival. A similar study, however, did not find such a stark difference in renal function when following a DBD model, with no first warm ischaemic time [114]. Other recent studies have compared oxygenated HMP against CS, rather than HMP, again showing some improvement in early graft function [115,116]. The use of oxygen during kidney preservation has recently been reviewed by Hosgood *et al.* [107].

As there is no clear clinical evidence to support widespread use of HMP with supplemental oxygenation, clinical trials with this new technology are required. Ongoing trials of oxygenated HMP are investigating the benefit of end-ischaemic oxygenated HMP versus CS in ECD kidneys (ISRCTN63852508) and continuous oxygenated HMP versus HMP in DCD kidneys (ISRCTN32967929).

Conclusions

Hypothermic machine perfusion is a long-established technique to preserve kidney grafts before transplantation.

There is now good evidence showing a reduction in DGF and survival benefit in ECD when kidneys are preserved by HMP compared with CS. Numbers have not been large enough to identify a clear benefit of HMP on outcomes that are relatively rare, such as PNF and 1-year graft loss, despite meta-analysis of available data. Furthermore, more consistent good data are needed focusing on the benefit of HMP on graft function, including 6 and 12 months glomerular filtration rate. It appears that there is a minimum time the kidney needs to be pumped to benefit from HMP, but we do not know the exact number of hours required. Other remaining questions are whether pumping should be continuous or whether a short period of oxygenated HMP before transplantation (end-ischaemic HMP) is enough, and evidence has to be gained that HMP can be used to prolong cold ischaemia. Increasing evidence suggests a benefit of oxygenated HMP; however, this remains to be confirmed in clinical trials. It is also still unclear whether (sub)normothermic temperatures could improve outcome.

Hypothermic machine perfusion provides additional insight on viability and quality of the kidney; however, none of these data should be used as stand-alone tools to decide whether to accept or discard kidneys. Contrary to CS, HMP offers the opportunity to repair the kidney by reconditioning, adding several drugs acting against ischaemia reperfusion injury, or stems cells, and this is an important challenge to be explored in preclinical and clinical trials.

To date, we see a number of emerging novel techniques allowing *in vivo* and *ex vivo* normothermic perfusion of organs showing good initial outcome after transplantation. In the coming years, we will have to unravel which type of donor kidney will benefit most from what kind of combination of CS, hypothermic and/or normothermic reperfusion technique – with or without active repair – to result in both optimal kidney function and longer graft survival.

Authorship

IJ, JO'C, JP and RJP: writing and editing the manuscript.

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