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Review

ADVANCES IN NORMOTHERMIC MACHINE PERFUSION OF THE KIDNEY: EVIDENCE FOR CLINICAL PRACTICE AND UNDERLYING MECHANISTIC ACTIONS

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Summary

Normothermic machine perfusion (NMP) has been introduced into transplantation to improve the outcomes of kidneys from marginal donors. This review focuses on the application of NMP detailing the clinical evidence and examining the underlying mechanistic actions. It then explores the evidence for more prolonged periods of NMP and modifications to the protocols. There are only a handful of clinical studies reporting the outcomes of NMP on early renal allograft function. All of the studies used an end period of NMP approach in conjunction with hypothermic preservation techniques. All validated the safety and feasibility of NMP and several reported improved early graft function. Although NMP restores oxidative phosphorylation to support active metabolism and replenish cellular adenosine triphosphate (ATP), inflammatory and immune processes are also activated. This is in response to the period of ischaemia prior to NMP but may also be exacerbated by the perfusion conditions. Removing the inflammatory mediators during NMP, altering conditions or extending the NMP time to minimise ischaemic injury may be beneficial for future application. NMP is a promising new technology in kidney transplantation. Ongoing clinical studies and further development of NMP will determine the benefit of this technology in the coming years.

Key words: kidney, preservation, normothermic machine perfusion

Abbreviations

AST: aspartate aminotransferase; ATP: adenosine triphosphate; COR: controlled oxygenated rewarming; CXCL: chemokine (C-X-C motif) ligand; DAMPs: damage-associated molecular patterns; DBD: donation after brain death; DCD: donation after circulatory death; DGF: delayed graft function; ECD: extended criteria donor; eGFR: estimated glomerular filtration rate; ICAM-1: intercellular adhesion molecule 1; L1β: interleukin 1β; NADPH: nicotinamide adenine dinucleotide phosphate; NEVKP: normothermic *ex-vivo* kidney perfusion; NFkB: nuclear factor kappa B; NLRP3: NLR family pyrin domain containing 3; NMP: normothermic machine perfusion; OXPHOS: oxidative phosphorylation; PPAR: peroxisome proliferator-activated receptors; RCT: randomised controlled trial; ROS: reactive oxygen species; SCS: static cold storage; TLR: Toll-like receptors; TNFα: tumour necrosis factor α

INTRODUCTION

Kidneys from donation after circulatory death (DCD) and extended criteria donors (ECD) are routinely used in deceased donor kidney transplantation [1-3]. However,

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they are susceptible to delayed graft function (DGF) and more likely to be rejected for transplantation due to donor factors or concerns about their quality [1-6]. Furthermore, limited access to theatre due to the growing burden on health services can extend the hypothermic preservation period beyond an acceptable duration, increasing the likelihood of discard [7].

The anoxic environment during hypothermic preservation results in the gradual depletion of adenosine triphosphate (ATP). Maintaining an organ below 10°C slows this process, but over time this leads to the build-up of toxic substances resulting in irreversible cellular injury [8]. Kidneys from older donors are less likely to recover from significant ischaemic injury [3]. Furthermore, DCD kidneys incur additional warm ischaemic injury which results in a higher likelihood of DGF or the possibility of irreversible damage resulting in primary non-function [3,4,9].

Preserving organs at a normothermic temperature as an alternative or adjunct to hypothermic preservation may help to improve graft outcomes and increase organ utilisation [10]. *Ex-vivo* normothermic machine perfusion (NMP) involves the circulation of an oxygenated perfusate through the kidney at a near-normal or normal body temperature and normal mean arterial pressure [10]. In most cases banked red cells are added as an oxygen carrier and various nutrients, amino acids/electrolytes and supplements added to support cellular metabolism and replenish ATP [10]. The evidence for NMP in clinical practice is limited to a small number of case series and several case reports [11-25]. One randomised controlled trial (RCT) has recently been completed (results not yet published) [26]. All of the studies have used a short period of NMP at the recipient centre following hypothermic preservation.

NMP can also be used to assess viability and as a platform for the administration of therapies directly to the kidney to aid regeneration and repair. These topics have been reviewed in several recent publications [27-33].

This review focuses on the application of NMP in kidney transplantation, detailing the clinical evidence and examining the underlying mechanistic actions. It then explores the experimental evidence for more prolonged periods of NMP and modifications to the protocol and highlights the future clinical trials.

CLINICAL APPLICATION OF NORMOTHERMIC MACHINE PERFUSION

The first clinical application of NMP was reported in 2011 using an adapted paediatric cardiac bypass system [11]. The kidney was perfused for 35 minutes after a period of static cold storage (SCS) using a red cell and Ringer's based solution. The recipient of the ECD kidney had slow graft function but remained dialysis independent. A series of 1 h NMP in 18 ECD kidneys using the same protocol was reported in 2013 [12]. The results were extremely encouraging with rates of DGF, defined as the requirement for dialysis within the first 7 days post-transplant, of 5.6% compared to 36.2% in a historical control group of 47 matched SCS kidneys. Graft and patient survival were similar at 12 months [12].

In 2019, Minor et al published the first case of controlled rewarming (COR) and NMP using a cell-free solution in a kidney from an ECD [15]. After 12.5h of SCS the kidney was gradually rewarmed from 8°C to 35°C over a period of 90 minutes followed by 30 minutes of NMP at 35°C before transplantation. The kidney was perfused with STEEN solution diluted with Ringer's solution and oxygenated via a membrane oxygenator with 100% oxygen on the Kidney Assist device (XVIVO). The kidney had immediate graft function. The group have since reported the outcome of 6 COR ECD kidneys and compared them to 6 SCS matched controls [16]. Creatinine clearance on day 7 post-transplant was 66 ml/min in the COR kidneys compared to 27 ml/min in the cold stored kidneys and the DGF rate was 0 *vs* 33% in the control kidneys. The estimated glomerular filtration rate (eGFR) at 3 months was significantly higher in the COR kidneys (70 *vs* 45 ml/min; p = 0.023) [16].

In 2021, the Rotterdam group published the results of a series of 11 NMP kidneys from ECDs over the age of 65 years [13]. After hypothermic machine perfusion (HMP), kidneys were perfused for 2 h using a red-cell based solution at 37°C on the Kidney Assist device (XVIVO) with 100% oxygen. The rate of DGF was not significantly different in NMP kidneys compared to in a historical control group of 53 mostly HMP kidneys (36 *vs* 53%; p = 0.320) [13]. There was no difference in eGFR at 3 months or in graft and patient survival at 12 months. There were also no significant differences when pairs of kidneys from the same donor (n = 8) were allocated to NMP or HMP [13].

In 2022, the Toronto group published a series of 13 NMP kidneys from donation after brain death (DBD) (n = 6) and DCD (n = 7) donors [14]. These were compared to a series of HMP kidneys from both DCD and DBD donors. Kidneys were perfused using an adapted paediatric cardiac bypass system with STEEN solution and 400 ml of red cells at 37°C. The median perfusion time was 171 minutes (range 44-272 minutes). There were no complications or recipient infections and all of the perfusion cultures were negative. The rate of DGF was similar between groups: 30.8% in the NMP kidneys and 38.5% in the control. Serum creatinine and eGFR up to 12 months post-transplant were similar between the groups [14].

A UK multicentre RCT comparing 1 h NMP (n = 143) with SCS (n = 147) in DCD kidneys has just been completed and the results are due to be published later this year [26]. Each of the reported studies uses different protocols and technologies, therefore it is difficult to establish the optimal conditions for and duration of NMP. There is a growing

body of research examining the molecular effects of NMP which may guide future protocols.

UNDERLYING MECHANISMS

The overarching goal of NMP is to replenish ATP and restore cellular homeostasis [33]. NMP conditions are designed to support metabolism and provide an environment that is protective [34]. A common feature of NMP is the use of red cells, either banked units or fresh blood that has been filtered to remove the white cells and platelets [10]. This is to minimise inflammation and neutrophil infiltration, prevent platelet adhesion and the formation of thrombi to promote perfusion and recovery [34].

A number of recent studies have used molecular techniques such as RNA sequencing or proteomic approaches to examine the mechanistic effects of NMP. In a series of cortical biopsies from non-transplanted human kidneys undergoing 1 or 2 h NMP after SCS, Ferdinand et al. [35] found a significant upregulation of oxidative phosphorylation (OXPHOS) genes after NMP. OXPHOS is a key pathway required to generate ATP, with potential benefits for cell viability and restoration of cellular homeostasis. However, the upregulation of OXPHOS was accompanied by a significant upregulation of immune and inflammatory processes. Tumour necrosis factor α (TNFa) signalling via nuclear factor kappa B (NFkB) had the largest increase but interleukin 1β (IL1β) and the neutrophil-recruiting chemokine (C-X-C motif) ligand 8 (CXCL8) and CXCL2 were also significantly upregulated. String analysis of the top 50 upregulated genes showed clusters of four main nodes: IL8 and neutrophil recruiting chemokines, inflammasome associated genes, NFkB signalling and transcriptional regulation. To examine the impact of the inflammatory and immune response the same group examined the gene expression in a series of 33 NMP kidneys that were transplanted [35]. Kidneys with a lower expression of OXPHOS pathways and enhanced upregulation of inflammatory cytokines had more prolonged DGF compared to those with immediate graft function or just 1 day of DGF [35].

Inflammation is a defensive immune response to facilitate tissue repair [36]. When oxygen and circulation are re-introduced after a period of ischaemia, membrane bound receptors such as Toll-like receptors (TLRs), c-type lectin receptors and cytoplasmic nod-like receptors are stimulated and identify damage-associated molecular patterns (DAMPs) released by injured cells [36]. This activates the innate immune system stimulating an acute inflammatory response with the secretion of cytokines and chemokines. During NMP, levels of inflammatory cytokines in the circulating perfusate were found to increase throughout perfusion and may further drive the inflammatory response [35,37].

The re-introduction of oxygen also causes the production of reactive oxygen species (ROS), which cause damage to tissue membranes, DNA, lipids and proteins via apoptotic pathways [36]. One of the most important sources of ROS in ischaemia reperfusion injury after transplantation is from the respiratory chain at the mitochondrial site [38,39]. Depletion of ATP and reduction of the electron carrier pool during ischaemia causes succinate to accumulate. At reperfusion succinate serves as an electron store and is rapidly re-oxidized by reverse electron transport at complex I [39]. This reverse action is accompanied by the generation of superoxide anion radicals. Another source of ROS is nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that is present in a variety of cells including endothelial cells [36]. ROS are also a key signalling molecule for inflammation [36].

In a similar model and protocol to the previously described study using 1 h NMP after SCS, Hameed et al also found significant upregulation of IL1β, CXCL2 and TNFα in a small series of non-transplanted human kidneys [40]. However, pathways promoting cell survival and proliferation were also upregulated suggesting a beneficial effect. Compared to the SCS paired kidney, renal function and blood flow were improved during a reperfusion phase with whole blood. Kidneys also had reduced levels of apoptosis, complement activation and oxidative stress compared to SCS kidneys [40].

It is likely that the inflammatory response during NMP is driven in part by the ischaemic injury acquired during SCS. Nonetheless, the perfusion conditions may also have an underlying effect. During storage the deformability and surface charge of banked red cells decreases and the fragility and aggregability increases [41]. The heat generated by the perfusion pump, contact with artificial surfaces, gravity assisted drainage and shear stress are among the factors that can cause haemolysis and the release of free heme into the circulating perfusate during NMP [42]. Free heme is highly toxic and can induce oxidative stress and inflammation [42]. Hosgood et al. found high levels of free heme in banked red cells before NMP [43]. Older units of red cells had higher levels of free heme compared to younger units and levels increased significantly over 1 h of perfusion. Although, high levels of free heme had no association with renal perfusion parameters, there was a significant upregulation of genes associated with apoptosis, inflammatory cytokines, chemokines and oxidative stress after NMP [43]. No direct correlation could be made; however, free heme may have been a contributing factor and could have a greater impact if longer durations of NMP were performed.

It has also recently been observed that during SCS fibrinogen accumulates within the tubular epithelium [44]. When NMP commences, this triggers the intravascular release of fibrinogen which causes red cell aggregation and microvascular plugging. This reduces blood flow and stimulates inflammation within the kidney during NMP. DiRito et al demonstrated that these microvasculature obstructions could be cleared with the addition of tissue plasminogen activator and plasminogen [44]. This reduced

intrarenal resistance, levels of IL-6 and intercellular adhesion molecule 1 (ICAM-1) and tubular cell injury.

REDUCING INFLAMMATION AND OXIDATIVE DAMAGE

Removing inflammatory mediators from the circulation during NMP may help to reduce the inflammatory response. Haemoadsorber filters remove molecules within a range of 10-50 kDa and have been used to treat sepsis, severe respiratory syndrome and the inflammatory response induced by COVID-19 [45-47]. In a porcine NMP model the haemoadsorber reduced inflammation and improved renal blood flow [37]. When incorporated into the NMP circuit in a study using non-transplanted human kidneys, the haemoadsorber significantly removed a range of cytokines from the circulating perfusate [35]. Transcriptional analyses demonstrated a 50% reduction in the number of upregulated genes after 4h NMP compared to the paired kidney perfused on a circuit without the haemoadsorber filter [35]. There was a significant downregulation of NLR family pyrin domain containing 3 (NLRP3) inflammasome activating genes such as IL1β, NLRP3, CASP1 and neutrophil recruiting chemokines. The haemoadsorber filter also increased OXPHOS and fatty acid metabolism pathways, demonstrating improved energy metabolism. Lower levels of inflammation and enhanced metabolism suggest improved function post-transplant [35]. However, the benefits of removing circulating cytokines from the perfusate have not yet to be tested in clinical practice.

The abrupt change in temperature from hypothermia to normothermia can trigger dysfunction in respiratory control and cause cellular injury [38]. As described in the clinical study, COR over 90 minutes of perfusion after SCS preserves better metabolic efficiency compared to abrupt rewarming [15,16]. Several studies by Minor et al describe benefits from COR including improved mitochondrial efficiency, improved oxygen consumption and tubular reabsorption, better glomerular function and lower levels of aspartate aminotransferase (AST) after reperfusion [48,49].

LONGER PERIODS OF NORMOTHERMIC MACHINE PERFUSION

It may be beneficial to maintain kidneys under NMP conditions for longer periods to minimise the effects of ischaemic injury. Weissenbacher et al. demonstrated the capacity to preserve human kidneys for up to 24 h or 48 h with a red cell-based solution [50,51]. A unique feature of the system to maintain a physiological environment was the recirculation of urine into the perfusate during NMP. Replacing high volumes of urine with a crystalloid solution can result in elevated levels of sodium and abnormal acid-base balance. Urine recirculation enabled kidneys to be perfused for longer with normal acid-base homeostasis. Proteomic profiling by mass spectrometry revealed that urine recirculation was associated with a reduction in DAMPs and an increase in proteins involved in anion transport to maintain pH [52]. Without urine recirculation, DAMPs, proteins involved in succinate metabolism and vasoconstriction were upregulated. Furthermore, kidneys could not be perfused for more than 7-8 h [50].

Selzner et al. have published a series of experimental studies using a porcine autotransplant model to examine the effects of more prolonged periods of normothermic *ex-vivo* kidney perfusion (NEVKP) [53-59]. Kidneys with 30 minutes warm ischaemia followed by 8 or 16 h NEVKP had improved graft function post-transplant compared to SCS or 1 h NMP [57,58]. McEvoy et al found that proteins involved in key metabolic pathways including fatty acid β-oxidation, the tricarboxylic acid cycle and oxidative phosphorylation were upregulated during 8 h of NEVKP [59]. They identified members of the peroxisome proliferator-activated receptors (PPAR) family including PPARA, PPARD and RXRA as upstream regulators. Urbanellis et al performed transcriptional profiling using microarrays in a similar model on samples taken on day 3 post-transplant [60]. NEVKP kidneys were compared to kidneys that were transplanted immediately with no preservation interval and kidneys that were cold stored. NEVKP kidneys had comparable levels of renal function on post-operative day 3 to kidneys without preservation. Fatty acid metabolism used in the generation of ATP by proximal tubular epithelial cells, genes involved in the tricarboxylic acid cycle, oxidative phosphorylation, amino acid synthesis and ATPase binding were all significantly upregulated. Pathways relating to small molecule transport, interferon signalling and chemokine signalling, binding and chemotaxis were enriched in NEVKP indicating recovery from ischaemic injury [60]. Furthermore, they noted that uromodulin expression was higher after NEVKP. Uromodulin is produced by tubular epithelial cells and is abundant in normal kidneys. It protects against ischaemia reperfusion injury to regulate the innate immune response in the kidney and is proposed as a biomarker of tubular health [60]. The experimental evidence suggests that performing NMP for longer periods is favourable. Weissenbacher et al. found that after 6 h of NMP kidneys with urine recirculation were metabolically more active in the subsequent 18 h period of NMP, suggesting a better recovery of metabolism [52]. Nonetheless, it is challenging to carry out long periods of NMP in clinical practice. Portable systems and significant resources are required to transport kidneys using NMP between donor and recipient centres.

Alternatively, the COR technique may rival the necessity for prolonged NMP. Von Horn et al recently investigated the effect of COR compared to continuous NMP for 8 h or 8 h SCS [48]. Porcine kidneys with 30 minutes warm ischaemia and 6 h SCS underwent COR for 2 h. Kidneys

were auto-transplanted and animals recovered for 7 days. Renal function was significantly improved in the COR and NMP kidneys compared to SCS kidneys and was equally as good as continuous NMP [48]. COR kidneys had less tenascin C expression in the tissue compared with SCS kidneys indicating reduced proinflammatory regulation [48].

ONGOING AND FUTURE CLINICAL TRIALS

There are a number of ongoing and new clinical trials registered in the clinical trials databases (Tab. I). Several of these trials are examining the effects of longer periods of NMP using newly developed NMP systems. The new OrganOX Metra K system is a portable device designed to maintain kidneys under NMP conditions for prolonged periods. An ongoing study using the OrganOX Metra K and the urine recirculation protocol is examining the effects of NMP for periods of up to 24 h. Other protocols include a comparison between NMP and HMP.

CONCLUSIONS

NMP is a promising new technology in kidney transplantation. At present the clinical evidence is sparse and limited to performing a short end ischaemic period of NMP after hypothermic preservation. There is insufficient evidence to draw any conclusions on the impact on graft function or graft survival. However, most importantly, the safety and feasibility of NMP has been established.

There are some limitations to performing a short end period of NMP. Although NMP has beneficial effects, with upregulation in the expression of genes that promote the generation of energy, there is also an induction of proinflammatory genes which may be harmful. Furthermore, by performing NMP for 1-2 h there may not be enough time for recovery or repair mechanisms to be instigated. The condition of these kidneys may be improved by removing inflammatory cytokines from the circulating perfusate using the haemoadsorber filter or using the COR protocol to better protect the mitochondrial reducing ROS production. Prolonged NMP may have greater potential to facilitate repair. In the coming years, ongoing clinical studies and the further development of NMP kidney systems will determine the benefit of NMP on early graft function.

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Conflict of interest statement

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Table I. Registered clinical trials assessing the effects of NMP in kidney transplantation.

	Title	Registration number	Trial	Status	Intervention
1	Investigation of the safety and feasibility of preservation of kidneys for up to 24 hours at normal body temperature prior to transplant (Oxford, UK)	ISRCTN13292277	Single group	Recruiting	n = 36 NMP up to 24 h
2	Normothermic machine perfusion: an additional value for kidney transplant outcomes? (Rotterdam, Netherlands)	NCT04882254	Randomised 1:1 NMP *vs* HMP	Recruiting	n = 80 NMP 2 h
3	Normothermic machine perfusion versus static cold storage in human kidney transplantation (Germany)	NCT05031052	Randomised 1:1 NMP *vs* SCS	Not recruiting	n = 194 End NMP 4 h DBD kidneys
4	Prolonged *ex-vivo* normothermic machine perfusion for kidney regeneration (Groningen, Netherlands)	NCT04693325	Single group	Recruiting	n =18 Up to 6 h NMP DCD kidneys
5	The feasibility and safety of normothermic *ex-vivo* kidney perfusion (Toronto, Canada)	NCT03136848	Single group	Unknown	n = 25 NMP 4-10 h
6	*Ex-vivo* normothermic perfusion in kidney transplantation (Eber Medical Technology)	NCT05175885	Non-randomised NMP *vs* SCS or HMP	Not yet recruiting	n = 100 NMP

DBD: donation after brain death; DCD: donation after circulatory death; HMP: hypothermic machine perfusion; NMP: normothermic machine perfusion; SCS: static cold storage

Author contributions

SAH: wrote and reviewed the manuscript; MLN: co-wrote and reviewed the manuscript.

Ethical consideration

Not applicable.

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