Title of review article:

Ex-vivo Normothermic Perfusion in Renal Transplantation: Past, Present and Future.

Author (s):

Cissy Yong, Sarah A. Hosgood, Michael L. Nicholson

Authors' Affiliations:

Department of Surgery, University of Cambridge, Cambridge CB2 0QQ UK.

Author of correspondence:

Name: Professor Michael L. Nicholson

Address: Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ

Telephone number: +44 (0)1223 245 151

Email address: mln31@cam.ac.uk

Abstract (200 words max):

Purpose of review:

Marginal donor kidneys make up a substantial proportion of transplanted kidneys. Nonetheless, they are more susceptible to injury during procurement and preservation. Normothermic perfusion is an alternative method of organ preservation that can be used to improve the quality/resuscitate, assess and potentially repair the organ. This review provides an up to date summary of the role of ex-vivo normothermic perfusion (EVNP) in renal transplantation and what we can expect in the future.

Recent findings:

EVNP has been demonstrated to be a feasible and safe method of organ preservation in series of extended criteria donor kidneys (ECD). Furthermore, EVNP can be used as a quality assessment tool for kidneys pre-transplant. In the future, EVNP could be used to manipulate the organ using gene and stem ameliorating renal function after injury.

Summary:

In the last 5 years EVNP has been translated from an experimental laboratory technique into clinical practice with promising results. EVNP has demonstrated itself to be multi-faceted in its application in renal transplantation, offering an improved preservation technique, particularly for marginal donor kidneys and real-time quality assessment of the kidney pre-transplant. The implications of these applications are crucial in maximizing the use of donor organs available in the perpetual climate of organ shortage.

Keywords: marginal donor kidneys, ex-vivo normothermic perfusion, extended donor criteria, donation after cardiac death

Introduction

Ex-vivo normothermic perfusion (EVNP) in kidney transplantation is a promising area of research that has seen translation from experimental models into early clinical practice. The potential applications of EVNP are multi-fold; from optimising organ preservation techniques, particularly apt in the climate of an increasingly used pool of marginal donor organs, to real-time quality assessment of donor organs. It also provides an ideal platform for targeted therapies such as gene and stem cell therapies. This review provides an update on EVNP within renal transplantation and the progression to date. It will also focus on what can be expected for the future of EVNP in kidney transplantation drawing upon research from other transplantation fields.

History of EVNP

Although experimental normothermic perfusion techniques were first introduced in the early twentieth century by the French surgeon and scientist Alexis Carrel (1-5), isolated organ perfusion at normothermic temperatures *ex-vivo* gained momentum only in the last few decades. This has been fuelled by technology, such as extracorporeal membrane oxygenators, cardiopulmonary bypass systems (6-7) and the shortage of organ donors. A great deal more emphasis has been placed on using marginal donor, such as donations after cardiac death (DCDs) and extended criteria donors (ECDs) (8-10).

The principle of normothermic perfusion is to maintain an organ in a near to normal physiological state by providing the medium for aerobic metabolism to take place. This reduces the deleterious effects associated with hypothermic preservation (11-12).

In the 1980s, several groups demonstrated an extended preservation time of up to 6 days for canine kidneys with the adjunctive use of EVNP and hypothermic techniques (13-15). The aim of this research was to extend the organ preservation period to afford more time for cross-matching and immunological techniques. However, this particular facet is no longer necessary in clinical transplantation, as cross-matching technology has advanced and the average cold ischaemic time in the UK for kidney transplant is only 16 hours (16).

More recent research has also focussed on using EVNP as a method of resuscitating marginal donor kidneys. Experimental studies by Brasile *et al* demonstrated the successful resuscitation of canine kidneys subjected to warm and cold ischaemic injury (17). The findings were corroborated in a porcine model by our group, with the demonstration of reversal of some detrimental effects of cold ischaemic injury and improved renal blood flow on reperfusion, after only 2 hours of renal normothermic perfusion (18).

EVNP up-to-date

The use of marginal donor kidneys has significantly reduced the donor kidney deficit, and globally, an increasing proportion of transplant kidneys are from DCDs and ECDs (8,19). However, the use of marginal donor kidneys is not without risk, and has been associated with higher rates of primary non-function, delayed graft function (DGF), acute rejection and a greater susceptibility to preservation injury (20-24).

Based on previous experimental work, in 2011 our group (25) reported the first clinical case of an ECD donor kidney transplanted into a recipient after a short period

of EVNP (35minutes) following nearly 11 hours of cold ischaemia. The recipient, a 55-year-old dialysis dependent female had initial slow graft function post-transplant but remained dialysis free, and at 4 years follow-up her kidney function has remained excellent. Furthermore, in 2013, we demonstrated the safety and feasibility of EVNP in a series of 17 ECD kidneys subjected to 60 minutes of EVNP prior to transplantation (26). The outcomes were compared to a matched cohort of 47 ECD kidneys, which had undergone cold storage alone. Although there was a reduced incidence of DGF in the EVNP group compared to the control group; 1/17 recipients (6%) vs 17/47 (36%), respectively, no difference in graft or patient survival at 12 months was observed between the two groups.

In 2014, we reported a case of EVNP resuscitation of a pair of human kidneys that were originally declined for transplantation due to inadequate *in-situ* perfusion (27). After 60 minutes of EVNP, both kidneys appeared pink, evenly perfused and blood flow improved throughout perfusion with a significant amount of urine produced. Biopsies taken after EVNP showed some evidence of acute tubular injury, however nuclei appeared normal and there was no evidence of cortical necrosis in either kidney. The kidneys were not transplanted but this study demonstrated the potential ability of EVNP to resuscitate kidneys declined for reasons such as inadequate perfusion. EVNP also allows the assessment of the functional capacity of the kidney prior to transplantation.

While these studies demonstrate that EVNP in renal transplantation is both safe and feasible as a means for kidney preservation, and may have a role in resuscitating and assessing marginal donors kidneys, no long-term data is available yet to fully assess

the effects. Interestingly, rates of DGF in DCDs and ECDs are not associated with overall graft survival, unlike donation after brain death (DBDs) (26, 28-29), and it will be important to study other long-term data such as graft function and post-transplant related complications in future studies.

A randomised controlled trial conducted by our group is underway to assess EVNP and will recruit 400 patients undergoing a DCD kidney transplant (Maastricht Categories III & IV). Patients will be randomly allocated to static cold storage alone or in conjunction with 60 minutes of EVNP. Outcomes will include DGF, short and long-term graft function (12 months), graft and patient survival and episodes of acute rejection. The trial will enable a better understanding of the efficacy of EVNP in renal transplantation.

Pre transplant assessment using EVNP has major implications for re-evaluating discarded organs, and also kidneys from marginal donors. We have formulated a scoring system, which uses several markers of quality assessment: macroscopic appearance, perfusion parameters of renal blood flow and urine output of the kidney during 60 minutes of EVNP (30). The scores ranged from 1 (least injured, highest quality) to 5 (most injured and lowest-quality). This scoring system was applied to a series of 36 marginal kidneys that underwent EVNP prior to transplantation. Recipients with an EVNP assessment score of 3 had a significantly higher rate of DGF (38%) compared to those scoring 1 (6%) and 2 (0%). Post-transplantion kidneys with a higher score also had significantly higher serum creatinine levels and a lower eGFR at 12 months. This study demonstrates a promising innovative technique

for assessment of marginal kidneys pre-transplant and assessment of their suitability for transplantation.

Future of EVNP

Ex-vivo normothermic perfusion research in heart (31), lung (32) and liver transplantation (33-34) has been more expansive than in renal transplantation. As such, we can draw upon this research to facilitate expansion of EVNP in renal transplantation.

An appealing quality of EVNP that has attracted a growing amount of research in transplant communities, is the principle of administering therapeutic agents solely to the organ pre-transplantation, allowing direct manipulation. This also avoids unwanted systemic effects from administering these agents to the patient and obviates problems with targeting particular organ cells. The applications of such could include use of stem cell and gene therapy to target complex transplantation issues such as rejection and fibrosis.

Using human kidneys, Brasile *et al* demonstrated effective transfection of a recombinant adenovirus with an encoded GFP reporter protein delivered during EVNP (35). Localisation of the reporter gene to the intimal layer of blood vessels demonstrated the ability to target a specified delivery site. Furthermore, using gene-silencing techniques our group demonstrated a reduction in expression of caspase-3 (36). Caspase-3 is a pro-apoptotic protein that is upregulated in ischaemic-reperfusion injury, and after addition of caspase-3 specific small interfering RNA into the cold preservation solution for 24hours, the porcine kidneys showed decreased

caspase-3 expression level. Treated kidneys demonstrated better renal oxygenation and acid-balance homeostasis in comparison to the control group. Although combining EVNP with this technique may be more efficacious, it is likely that prolonged periods of EVNP will be required for effective manipulation of the kidney before transplantation. Nonetheless, the research to date paves the way for investigating gene therapy in EVNP.

Recent experimental work by McConnell *et al* (37) used a novel silicon microparticle and viral nanoparticle hybrid model, as a means of a delivery platform. They were able to demonstrate successful uptake and gene delivery in endothelial cells during EVNP in a porcine lung transplantation model. The inherent mechanisms for endosomal escape and nuclear localisation make the use of viral-associated nanoparticles an effective gene-delivery platform, whilst the microparticle enabled a high quantity of nanoparticles (up to 1,000) to be attached, thereby exponentially increasing the impact of the therapy per microparticle uptake into cells. Although much more research is required to determine mechanistic processes of uptake and downstream processes of gene expression, this holds an exciting prospect for effective targeting of cells within a heterogeneous cellular environment. In collaboration with Yale University, preliminary work is underway by our group to assess the uptake of endothelium targeted nanoparticles using EVNP in declined human kidneys.

Another growing area of interest in transplantation is the use of mesenchymal stem cells (MSCs). Although stem cells are notable for their differentiation properties, which modulate tissue repair and regeneration (38), their immunomodulatory and paracrine properties, such as anti-apoptotic and anti-fibrotic effects make them

attractive in the transplant setting. Experimental studies using different models of kidney injury with MSCs have demonstrated amelioration in renal function following ischaemia-reperfusion injury, reduced tubular injury and prolonged survival (39-42). Currently, a phase I clinical trial is underway assessing the use of multipotent adult progenitor cells (MAPCs) in liver transplantation (43). Recipients will be administered MAPCs day 1 and 3 post-transplantation and it is postulated that these MAPCs may reduce the dose of immunosuppressive therapy required.

To our knowledge only one study has combined the use of MSCs and ex-vivo perfusion. Lee *et al* (44) demonstrated attenuation of E. Coli endotoxin induced - acute lung injury after addition of allogenic human MSCs during EVNP of a human lung. Specifically, alveolar epithelial fluid transport mechanisms were restored with reduction in pulmonary oedema injury. Early research using MSCs is promising and warrants further work to delineate their role and function in renal transplantation. EVNP provides an ideal route for such therapies because not only does it allow direct targeting of the organ of interest, bypassing undesirable systemic effects, but also the organ is in a functioning state whilst receiving the therapy. This allows the effects to be closely monitored and the mechanisms and therapeutic actions of these agents to be studied in isolation.

Conclusion

To date, *ex-vivo* normothermic kidney perfusion has been translated from experimental models through to clinical practice. EVNP has exciting and promising prospects for the future of clinical renal transplantation. It is a safe and feasible alternative organ preservation technique, which can be used in conjunction with

hypothermic preservation techniques. It is particularly apt for marginal donor kidneys providing an innovative way of assessing quality of organs pre-transplant and improve their quality. EVNP can also be used to ensure that we maximise the use of all available donor organs and reduce the number of organ that are discarded. Stem cell and gene therapies have demonstrated the ability to facilitate organ repair and immunomodulation. The administration of such therapies directly to the kidney using EVNP is a novel and exciting application in renal transplantation.

Key Points

1. EVNP is a safe and feasible alternative method of organ preservation in renal transplantation that may be more beneficial in marginal donor kidneys.
2. EVNP has the potential to resuscitate kidneys, particularly from marginal donors and provides an innovate means of quality assessment of the organ pre-transplant.
3. The future of EVNP research includes gene and stem cell therapy, which have demonstrated organ repair and immunomodulation.

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Conflicts of interest

None

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