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Novel preservation methods to increase the quality of older kidneys

Aukje Brat, Robert A. Pol, and Henri G.D. Leuvenink

Purpose of review

The purpose of this review was to summarize the novel developments in preservation of older kidneys.

Recent findings

The importance of older donors as a source of renal grafts is increasing, with a concomitant increase of posttransplant failure. Preservation of kidneys retrieved from older donors through hypothermic machine perfusion reduces delayed graft function rate and increases long-term graft survival. Assessment of renal function and selection through biomarkers or perfusion criteria to predict posttransplant function are limited. Normothermic perfusion offers the benefit of reperfusion under ideal circumstances, thereby reducing ischemic injury while having the opportunity to test graft viability and preselect kidneys on graft-specific characteristics. Both preservation methods enable active treatment of the isolated graft prior to transplantation, with stem cells or pharmaceuticals.

Summary

Older kidneys are more prone to acute kidney injury during ischemic periods combined with an impaired ability to fully recover after transplantation. Novel preservation and resuscitation methods provide the opportunity to select transplantable kidneys better founded or even repair re-existing damage reducing the risk of impaired graft function and improving survival.

Keywords

ECD, kidney, preservation, transplantation

INTRODUCTION

Renal transplantation, the most efficient treatment for end stage renal failure, is limited by the shortage of donor organs [1]. Ideally, only standard criteria donors (SCD), i.e., donation after brain death (DBD), aged 50 years or younger without comorbidities, should be used as a source of grafts for transplantation in order to limit the risk of primary non-function (PNF) or delayed graft function (DGF). The number of patients waiting for an organ is, however, far exceeding the number of ideal donors available for organ donation resulting in a high mortality rate on the waiting list. Therefore, many centers have expanded their acceptance strategy in order to reduce the risk of death on the waiting list [2].

Apart from transplantation of kidneys from living donors, organs from older donors or so called expanded criteria donors (ECD) are being used [3]. ECD donors are aged over 60, or over 50 years with at least two of the following conditions: a history of hypertension, a cerebrovascular cause of death or a terminal serum creatinine greater than or equal to

1.5 mg/dl [4]. In the last decade, the percentage of donors aged 50 years or older in the Eurotransplant region has increased from 49.6% to 58.6%, with a 6.4% increase of donors aged over 59 [5]. Next to brain-dead ECD donors, also kidney grafts from donation after circulatory death (DCD) donors which simultaneously exhibit ECD characteristics are being used for transplantation [6–8]. Recently, the kidney donor profile index has been implemented in the USA, which assesses the likelihood of graft failure after transplantation based on 10 donor factors including age, known at the time of organ offer [9,10]. It aims to give a better estimate of graft quality than the SCD/ECD dichotomy.

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KEY POINTS

- Because of age-specific injuries and an impaired ability to recover fully from ischemic damage, current preservation methods are deemed insufficient for ECD kidneys.
- Oxygenated hypothermic machine perfusion improves graft function and survival in ECD kidneys, although the value of biomarkers, perfusion characteristics and viability testing is limited.
- Normothermic regional perfusion gives the advantage of reducing ischemic injury in ECD/DCD donors with the ability to resuscitate the organs after a period of warm ischemia. Graft function assessment is feasible, but could be influenced by potential injurious blood-borne factors from other organs or body structures.
- Normothermic machine perfusion resuscitates and improves graft function and survival. It also gives the opportunity of assessment of the isolated kidney, through perfusion characteristics, urine production and biomarkers.
- Both hypothermic and normothermic machine perfusion exert the ability of treatment of an isolated kidney, without influences of or possible side-effects in other organs.

Usage of older donors introduces new risk factors and uncertainties to the outcome after transplantation. First, elderly donors are likely to have age-specific injuries such as interstitial fibrosis, tubular atrophy and glomerulosclerosis, affecting short and long-term graft survival possibly due to increased DGF rates and higher rates of acute rejection [11[¶]]. Second, older donors are more prone to ischemia reperfusion injury (IRI) and have, as a consequence of natural loss of nephron mass, an impaired ability to recover fully after transplantation [12]. Third, ECD/DCD grafts are also subjected to a period of warm ischemia as a result of poor and even absent organ perfusion prior to procurement, which has proven to be a significant predictor of acute kidney injury (AKI) [13,14].

Although enlargement of the donor pool by extending the acceptance criteria potentially increases the chance of a patient to be transplanted earlier, with the advantage of a shorter period on dialysis resulting in a better patient survival, ECD kidney grafts have an increased risk of developing DGF or even PNF resulting in a decreased long-term graft survival [6]. Therefore, transplantation of kidneys retrieved from ECD donors is a balance between shorter waiting time leading to reduced costs and morbidity because of dialysis versus the risk of DGF and lower graft and/or patient survival [2,15,16].

Strategies to increase organ quality are, therefore, needed to reduce posttransplant complications. The period of preservation provides an ideal window of opportunity for therapeutic interventions of a single organ. With the increased use of ECD kidneys there is a growing interest in the transplant community for improvement of the current preservation methods. The aim of this review was to give an overview of the different preservation techniques with emphasis on their indication, application and outcomes, especially in older and ECD kidneys.

Static cold storage

After procurement and during transportation organs are placed on ice, reducing but not completely arresting cell metabolism. Reduction in temperature, combined with deprivation of nutrients and oxygen, however causes depletion of energy and an increase in cellular stress leading to AKI. For SCD kidneys static cold storage is an effective and save preservation method. For kidneys procured from ECD and ECD/DCD donors which are more prone to ischemia-reperfusion injury, the standard cold storage method is, however, considered to be insufficient.

Hypothermic machine perfusion

Currently, outside the USA, only a small number of kidneys is being preserved by hypothermic machine perfusion (HMP). In the USA, some centers pump most of their kidneys while others only pump DCD and ECD kidneys. A recent randomized controlled trial comparing HMP versus cold storage showed a significant reduction in DGF rate after HMP compared with cold storage for all donor types (DBD, DCD and ECD) (Fig. 1) [17^{¶¶}]. Especially for ECD kidneys, machine preservation not only resulted in a decrease in DGF and PNF but also in better graft survival [18,19].

The exact mechanism for the beneficial effect of HMP is still unknown, but it is believed that by continuous perfusion of specialized preservation solutions, a reduction in damage to the endothelial lineage and better parenchymal integrity can be achieved [20]. Especially ECD kidney grafts, which are more susceptible to IRI, may potentially benefit most from HMP [17^{¶¶},21,22].

The optimal duration and timing of HMP remains a subject of debate. A recent article on hypothermic reconditioning of kidney grafts to improve graft function showed that 4 h of HMP, after a period of cold storage, was only slightly better compared with 1 h of postischemic HMP, further

FIGURE 1. HMP in ECD donation. Schematic presentation of injurious periods during preservation in older kidneys reflected by color intensity by cold storage (a) versus hypothermic machine perfusion (b). CS, cold storage; ECD, expanded criteria donors; HMP, hypothermic machine perfusion. Source: Original figure.

questioning whether continuous hypothermic machine perfusion is required to optimally condition the kidney graft for transplantation [23,24²²]. Reoxygenation of an organ during preservation was demonstrated in livers and kidneys to reduce injury and resulted in improved short-term graft outcome [25,26]. Currently, a large international randomized controlled trial is investigating the efficacy of oxygenated short-term perfusion following cold storage, particularly in ECD kidneys [27].

Although HMP may improve kidney quality, still a reasonable amount of ECD kidneys fail to function optimally after transplantation [19,28]. Being able to select these kidneys prior to transplantation could prevent morbidity of patients having to dialyze after transplantation. Unfortunately, the specificity and sensitivity of biomarkers in the perfusate or flow characteristics such as renal resistance are poor and clinically of minor relevance [19,29,30,31²³]. Most promising are measurements of different iso-enzymes of glutathione-S-transferase (GST) [32]. Although the α -GST subtype especially has a strong association with proximal tubular injury after transplantation (31), the π -GST subtype has the strongest predictive value for the risk of DGF [31²⁴,33]. An important element to keep in mind is the limited time frame between procurement and transplantation. Biomarker measurement may need several hours, but also the release of biomarkers into the perfusate before levels are detectable can take up precious time. Prolonging cold ischemia time for biomarker assessment could lead to additional risks for the graft and is therefore unfavorable. Nuclear magnetic resonance techniques with the ability to produce a broad spectrum analysis in a limited amount of time have been proposed as a possible solution although clinical benefit has yet to be proven [34].

Normothermic regional perfusion

In addition to the preexisting damage in the donor due to, for example, age, hypertension or brain

injury, warm ischemia is detrimental to the quality of the graft [13,14]. A recent development to reduce warm ischemia time resulting from poor and even absent perfusion is the application of normothermic regional perfusion (NRP) in which the abdominal organs are perfused *in situ* with perfusion solutions based on autologous blood [35]. This provides not only the opportunity to diminish the period of warm ischemia in ECD/DCD donors but also possibilities to resuscitate and evaluate graft quality before procurement and transplantation.

The potential of NRP was described by Reznik *et al.* who observed peristalsis of intestine and ureters in all cases and in over 60% of the cases urinary output during NRP in spite of a mean warm ischemia time of more than 1 h [36,37]. Next to being a predictor of quality of perfusion and kidney function, NRP could give the opportunity to measure urinary biomarkers associated with AKI and in theory the possible improvement over time. For example, high levels of neutrophil gelatinase-associated lipocalin (NGAL) measured in the urine of a deceased donor are associated with prolonged DGF [38]. Comparing measurements before and during NRP could give an insight into the development of AKI and predict DGF after transplantation.

Applying NRP in a donor is technically challenging and requires arterial and venous access usually obtained via the femoral vessels. Ideally, the cannulation takes place before withdrawal of treatment in DCD-III category donors. One should, however, not influence the agonal phase and several countries are bound by legal and ethical issues making it impossible for this to become common practice [39]. British laws, for example, prohibit cannulation and heparinization before cardiac arrest and a no-touch period of 5 min (Fig. 2). In spite of this caveat, experience from Cambridge has shown that even with these restrictions NRP is feasible and provides promising results [35,40²⁵]. Next to improvement in kidney function, it could potentially lead to an increased utility rate of abdominal organs of these donors.

FIGURE 2. NRP in ECD/DCD-III donation. Schematic presentation of injurious periods during donation and preservation in older DCD-III donors by in-situ perfusion as current practice (a) versus normothermic regional perfusion (b) as a strategy to improve kidney quality. CS, cold storage; ECD, expanded criteria donors; HMP, hypothermic machine perfusion; NRP, normothermic regional perfusion. Source: Original figure.

It should be noted that perfusion at (sub)normothermic temperatures holds the risk of intestinal contamination and infection [41]. In the series from Butler *et al.* positive cultures were obtained even though the extracorporeal circuit was primed with a variety of antimicrobial agents [40²²]. These drugs should, however, not be given without considering possible nephrotoxic effects when administered at a high dose. For prophylactic use, a high dose of cefazolin is recommended without risk of nephrotoxicity [41].

Ex-vivo normothermic machine perfusion

An alternative strategy to reduce ischemic injury and assess organ function prior to transplantation is ex-vivo normothermic machine perfusion. With this technique, kidneys are procured and preserved in a routine fashion and after a period of cold storage resuscitated through normothermic machine perfusion (NMP). Main benefits of this technique are the reduction of cold ischemia time and the opportunity to test renal function without the influence of blood with potential injurious blood-borne factors from other organs or body structures as is the case in NRP.

The duration and timing of NMP are currently subject of debate and research (Fig. 3). A limited period of end ischemic NMP seems to effectively resuscitate kidneys, leading to a significant decline in DGF and making the full period of normothermic perfusion between donation and transplantation redundant [42]. This would mean that many logistical and financial problems could be overcome by simply applying cold storage followed by NMP after arrival in the transplant center. Also intermediate NMP has been described in a case report, with a second cold ischemic period to prepare the kidney for transplantation [43²]. The authors hypothesized that NMP prior to cold storage could protect against additional cold ischemic injury. This could be an alternative method of ischemic conditioning, which has been shown to result in early recovery of renal function after transplantation [44].

An important feature of NMP is that reperfusion takes place under ideal circumstances, with blood-based perfusion solutions depleted from leucocytes preventing immunological reactions and with optimized flow and pressure [42]. Pressure-controlled NMP seems to give significantly better results when focusing on renal hemodynamics, kidney function

FIGURE 3. Timing of NMP during preservation. Schematic presentation of end ischemic resuscitation (a) versus intermediate ischemic conditioning (b). CS, cold storage; HMP, hypothermic machine perfusion; NMP, normothermic machine perfusion. Source: Original figure.

and acid–base homeostasis, possibly because of a better self-regulation of flow in the kidney [45].

After the first transplantation of a NMP kidney described by Hosgood *et al.* [46], a clinical trial was conducted with ECD kidneys that underwent end ischemic NMP for 1 h after a period of cold storage [42]. Despite small numbers, the clinical potential was shown as illustrated by the reduced DGF rate of 5.6% in the NMP group compared with 36.2% in the control group ($P=0.014$), with no increase in rejection. As mentioned before, NMP provides the opportunity to evaluate kidney function before transplantation. Measurements of intrarenal resistance and urinary output could give an estimation of kidney function. Currently however, no clear guidelines or cut-off points have been formulated to discard or transplant a kidney. Even though intrarenal resistance and urinary output seem to correlate to donor age, no obvious decline in kidney function or graft survival has been shown.

A potential further improvement could be to apply oxygenated HMP instead of cold storage before resuscitation of the kidney by NMP. Cold ischemia-induced damage could potentially be avoided by means of oxygenated HMP resulting in better preservation of the endothelial lineage, improved parenchymal integrity as well as partially restored levels of ATP. At present, no direct evidence for this assumption is available.

Another advantage of ex-vivo normothermic machine perfusion over applying regional perfusion is the possibility of treating and optimizing an isolated organ. New research is being done on influencing protein expression by enhancing or silencing genes involved in deteriorating kidney function and rejection. Especially genes and proteins involved in immunological and coagulation pathways which are associated with IRI and rejection are of potential interest to improve graft function and survival [47].

In addition to gene therapy, another possibility is to add pharmaceuticals to the perfusion circuit. Endothelial cells, which are vulnerable cells in the pathogenesis of IRI, are relatively easy to reach during perfusion given the direct contact with blood or other perfusion fluids. For example, treatment with antithrombin nanoparticles has been described to protect renal function after induction of AKI by clearance of microvascular thrombosis and inhibition of thrombin-mediated inflammation [48,49]. Administration of these drugs in a machine perfusion setting gives an isolated target without potential side-effects in other organs.

In addition to the acute injury inflicted during the donation and transplantation process, kidney grafts are prone to chronic kidney failure with fibrosis and chronic rejection. Older kidneys have a

stronger allogenic effect, provoking a higher rejection rate [11[■]]. Innovative research has been done using stem cells to modulate the immune response in the recipient by creating an immunogenic tolerance toward the graft [50]. In addition to silencing of the immune response, stem cells have the ability to stimulate regeneration. This feature could not only contribute to a decrease in short-term AKI but also to a reduction of fibrosis occurring in the graft on the long term. The exact mode of action, however, is still unclear. Potentially, stem cells may home to injured tissue and differentiate to form new structures or the endocrine/paracrine release of growth factors elicits a regenerative response from residual cells. Although in rodents promising results have been shown for application of stem cells in transplantation, more research is needed to show their value in human kidney transplantation [51].

CONCLUSION

For years kidneys have been preserved using conventional cold storage. Because the shortage of available grafts forced the transplant community to use older grafts that are more prone to injury, these preservation methods are deemed insufficient. Novel preservation methods are developed and implemented in clinical practice. In addition to the promising results in reducing DGF, PNF and rejection rates, the possibility of evaluating the quality of the graft before transplantation is interesting. This could create a larger donor pool with more usable grafts and the opportunity of selection on graft-specific criteria instead of the current risk assessment based on donor characteristics.

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Conflicts of interest

There are no conflicts of interest.

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- of outstanding interest

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