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# Donor pretreatment in clinical kidney transplantation: a critical appraisal

D.K. de Vries, L.G.M. Wijermars, M.E.J. Reinders, J.H.N. Lindeman, A.F.M. Schaapherder

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## Abstract

Kidney transplantation represents one of the medical achievements of the 20th century. Its continued success, however, is limited by the increasing shortage of donor grafts. As a result more kidney grafts from marginal donors are being considered for transplantation, with concomitantly more initial graft injury and limited organ and patient survival. This has led to an increased need for interventions aiming to optimize and preserve graft quality. Interventions within the donor may protect against ischemia/reperfusion injury and therefore donor pretreatment is a promising strategy to increase graft function and survival. During the last decade, diverse donor pretreatment interventions have been explored in animal studies. Moreover, the first human trials concerning donor pretreatment in kidney transplantation have provided encouraging results. Unfortunately, it remains difficult to determine how and where to intervene in the multifactorial and complex processes that affect the donor kidney. Moreover, ethical matters play a critical role in donor interventions, and pretreatment should principally not have any potentially unfavorable effects on other organs to be transplanted or on the living donor. This review provides an overview of promising therapeutical strategies for donor pretreatment in kidney transplantation and discusses the clinical trials that have been conducted thus far.

## Introduction

Kidney transplantation is the preferred treatment of patients with end-stage renal disease.[1-3] The previous decade has been characterized by a steady increase in the number of kidney transplantations. This increase largely reflects improved medical therapy for renal failure. Besides the fact that more patients are being considered eligible for kidney transplantation, improved patient survival rate after transplantation led to the emergence of patients requiring re-transplantation, because of progressive loss of graft function in the long run.[4-6] The augmented demands for kidney transplantation resulted in organ shortage and as a consequence, waiting lists for kidney transplantation are ever increasing. As of June 2012 more than 99,000 American citizens were on the waiting list for a kidney transplantation.[7-9] Kidney grafts can be derived from living or deceased donors. For the deceased donors, more kidneys are recovered by donation after brain death than from donation after cardiac death. The current organ shortage, however, necessitates expansion of the donor pool by the increased use of marginal donor kidneys. Since these marginal donor kidneys have worse long term outcome, it has been proposed that these may benefit most from pre-transplantation interventions that preserve or even improve graft quality.[10]

Before and during the process of transplantation the graft is exposed to various noxious events, including donor brain death, cold preservation and ischemia/reperfusion (I/R) injury; potentially all contributing to the functional deterioration of the graft. The importance of these harmful mechanisms is illustrated by the superior results of living donor transplantation. Despite generally more accepted HLA mismatches, living donor transplantation is associated with minimal delayed graft function and improved long term outcomes. This observation suggests that non-HLA-specific factors such as donor health and duration of the ischemic period before transplantation have substantial impact on short and long term graft function.[11-13]

Consequently, interventions in the donor, aimed at minimizing pre-transplantation graft injury, may potentially have large effects in preventing acute and long term graft dysfunction. This review will focus on the prevention of harmful processes that initiate graft damage in the donor. Various intervention strategies for donor pretreatment that have been tested in clinical kidney transplantation or in animal experiments involving kidney transplantation will be discussed.



### Processes in brain death

To date, the majority of deceased kidney grafts are derived from donation after brain death. Brain dead donor kidneys unfortunately have a worse graft and patient survival rate

as compared to living donor kidneys. Brain death leads to dysregulation of the autonomic nerve system, inducing many pathophysiological processes in the human body. Brain death is usually provoked by a period of increased intracranial pressure exceeding the mean arterial pressure and thereby blocking brain perfusion. The physiological responses to this increased pressure and brain damage can have effects on multiple organ systems. The most prevalent derangements are cardiovascular. With increasing intracranial pressure, a compensatory arterial hypertension is induced, sometimes with bradycardia. The catecholamine storm sets in next, with sympathetic stimulation, vasoconstriction, raised systemic vascular resistance, and tachycardia. After the catecholamine storm, there is a loss of sympathetic tone and peripheral vasodilatation. Next, brain death results in severe hemodynamic instability and the resulting hypotension, if untreated, leads to hypoperfusion of all organs. This phase is well-known for the damage it can inflict in organs to be transplanted. Other common clinical problems associated with donor brain death may include diabetes insipidus, disseminated intravascular coagulation, arrhythmias and pulmonary edema. Injury to the hypothalamus and the pituitary gland causes disturbances of hormonal homeostasis and thermoregulation.

On the microvascular level, brain death is associated with the induction of adhesion molecule expression and endothelial cell activation.[14] The hemodynamic, neurogenic, hormonal and microvascular disturbances lead to a generalized inflammatory response in the donor. This is characterized by the release of cytokines into the circulation which can trigger an inflammatory response in all organs [15,16] with tissue infiltration by granulocytes,[16] monocytes and lymphocytes.[17] All these physiological derangements should be limited as far as possible to maintain optimal graft condition before donation. Donor management is the primary approach to do so.

### Donor management

After the diagnosis of brain death, there is a change from curative patient care to optimizing organ function for subsequent transplantation. This donor management is the active care of the donor from the time of diagnosis of brain death until procurement of organs and involves correction of the widespread physiological changes that occur during brain death. Early recognition of the potential organ donor and aggressive correction of the non-physiological state, even before consent to organ donation, are crucial to optimize post-transplantation graft function.

In order to standardize management, donor goals have been developed. These aim to maintain physiology close to normal values and were based on measurements performed routinely in patients in the intensive care unit. They include objectives to maintain body temperature, ensure adequate oxygenation, circulating volume, cardiovascular stability,

and adequate urine output. Indeed, in a prospective study, the application of a standardized donor management protocol increased the number of retrieved and transplanted organs per donor substantially.[18]

One part of donor management is providing cardiovascular support. This support principally includes stabilization of hemodynamics in the donor. Treatment of hypertension associated with the catecholamine storm may significantly increase graft availability for transplantation.[19] In the consecutive hypotensive period, the first priority is to maintain an adequate intravascular volume. Fluid therapy should, however, be carefully titrated. Recent studies recommend restrictive fluid management, since this restriction increases the number of transplantable lungs without influencing kidney graft function or survival after transplantation.[20, 21]

Hormone replacement can aid by correcting the loss of pituitary function after brain death, as a method of stabilizing the donor. Posterior pituitary function is lost very commonly, leading to diabetes insipidus with associated fluid and electrolyte changes. Anterior pituitary function may be preserved or only partially affected. Most hormone replacement therapies use a combination of methylprednisolone, vasopressin and thyroid hormone. The fraction of donors that received replacement therapy with these three hormones had an increase in the number of procured organs by 22,5%, as demonstrated by a retrospective study.[22]

Aggressive donor management increases the number of organs available for transplantation and has minimized loss of potential donors due to cardiovascular collapse in the process of brain death. Most studies, however, deny a major effect on graft quality and survival of donor management. Grafts may therefore benefit from additional interventions that more specifically prevent organ damage before procurement. This donor pretreatment aiming to further maximize organ quality is an evolving field that constitutes the next step in optimizing kidney graft survival.

## Donor pretreatment

Donor pretreatment is the active treatment of the donor in order to improve organ quality before and after transplantation. It distinguishes itself from donor management by the fact that donor management concentrates on stabilizing the donor to normal physiological ranges, while pretreatment aims to inhibit potentially harmful processes. Many therapies used as donor pretreatment have been investigated for their ability to reduce or prevent renal I/R injury in animal experiments. Few interventions have been studied in clinical trials, which are summarized in Table 1. Here we discuss the most frequently applied and most promising approaches to donor pretreatment.

**Table 1**: Human clinical trials of donor pretreatment and result on outcome in kidney transplantation.

| Intervention | Design | n | Main result | Reference |
|---|---|---|---|---|
| Dopamine | Single blind RCT | 264 brain dead donors | Dopamine pretreatment decreased the incidence of dialysis post-transplantation. No change in acute rejection or patient or graft survival after 3 years. | Schnuelle et al., 2009 [42] |
| Steroids | Double blind RCT | 306 brain dead donors | Donor pretreatment with corticosteroids did not reduce the incidence or duration of DGF. | Kainz et al., 2010 [60] |
| PUVA | Non-randomized | 59 deceased donors | PUVA pretreated grafts had a significantly lower number of rejection episodes, other outcome parameters were not different. | Oesterwitz et al., 1987 [70] |
| Hyperoxia | Double blind RCT | 60 living donors | Donor oxygen pre-treatment the day before transplantation improved kidney function at 10 days after transplantation. | Montazeri et al., 2011 [79] |

RCT: randomized controlled trial
DGF: delayed graft function
PUVA: psoralen plus ultraviolet A

### *Ischemic preconditioning*

Over the past decades several studies were performed, exposing an organ to brief periods of ischemia to protect against subsequent periods of ischemia and reperfusion. This phenomenon of ischemic preconditioning has been first described in 1986 in the heart.[23] Many animal studies, mainly in rats, have reported beneficial effects of donor renal ischemic preconditioning before kidney transplantation since then.[24-27] Our present understanding of the molecular mechanisms causing these effects is still largely incomplete. Experimental studies have shown that the protective effects of renal ischemic preconditioning are mediated by adenosine[28], nitric oxide [29, 30] and subsequent activation of signal networks involving protein kinases and transcription factors. More complex mechanisms have been proposed recently as well, including cellular actions of regulatory T cells and endothelial progenitor cells.[31, 32] It is generally acknowledged that the mechanism of ischemic preconditioning may differ between species and organs and it still remains controversial whether ischemic preconditioning is beneficial in large animals as well. Studies on kidney transplantation in dogs and renal I/R injury in pigs in fact both failed to confirm beneficial effects of renal ischemic preconditioning. Although the first description of ischemic preconditioning dates from almost thirty years ago, the technique has not yet been successfully translated into the clinical setting.[33, 34]

More recent studies demonstrated that remote ischemic preconditioning confers protection to I/R injury by preceding ischemia and reperfusion of another organ or tissue. In animal experiments, the donor kidney can be protected after transplantation by remote ischemic preconditioning of the hindlimb.[35] Remote ischemic preconditioning suggests the involvement of humoral mediators and consequently protection is both dialyzable, transferable, and receptor-mediated.[36] Remote ischemic preconditioning has the advantage that it is more easily applicable in clinical transplantation than ischemic preconditioning of the graft itself. At present, clinical studies in which remote ischemic preconditioning of the lower limb is explored to improve outcome of kidney transplantation have been initiated and patients are being recruited (www.clinicaltrials.gov).

### *Catecholamines*

Before transplantation, the graft is exposed to harmful periods of warm and cold ischemia. Dopamine is capable of protecting endothelial cells from damage during cold preservation by inducing protective enzymes, such as heme oxygenase-1 (HO-1).[37,38] Dopamine could therefore be a good donor pretreatment candidate, rendering the kidney graft more resistant to I/R injury. In a rat allogeneic kidney transplantation model it was shown that donor dopamine pretreatment diminished histological damage, monocyte infiltration and cytokine expression in the kidney graft. Moreover, both short- and long-term graft function significantly improved.[39,40] Other catecholamines, like dobutamine and norepinefrin, did not influence post transplantation kidney function.[41]

This preclinical experimental evidence resulted in a clinical trial of donor dopamine pretreatment.[42] Almost 300 brain dead donors were randomized to receive low dose dopamine pretreatment or placebo. Donors had to be stable on low dose noradrenalin and dopamine was continuously infused at a standard rate. The main outcome measure, need for dialysis during the first week after transplantation, was significantly reduced in recipients of a dopamine pretreated graft. Dopamine pretreatment however, did not affect graft or patient survival. Donors in the dopamine administered group showed a significant but clinically not relevant increase in systolic blood pressure. In addition, effects of dopamine pretreatment were more pronounced with increasing cold ischemia time, supporting the hypothesis that the beneficial effects of dopamine are mediated by its protective effects on the endothelium. Dopamine pretreatment may therefore even have the largest effects in marginal donors.[42]

### *Heme oxygenase-1*

The ischemic period before transplantation can induce oxidative stress, which on its turn induces the release of the cytoprotective enzyme HO-1. Both HO-1 and carbon monoxide (CO),

a product of HO-1 metabolism, are potential candidates for donor pretreatment. Induced expression of HO-1 in rat kidney donors led to decreased cell infiltration, downregulation of inflammatory genes and diminished histological signs of chronic rejection, resulting in increased graft function and survival after transplantation.[43]

The effects of HO-1 may be mediated by its downstream CO production, since induction of CO in the donor improved graft function similarly to HO-1, and both HO-1 and CO were able to diminish donor immunogenicity.[44-47] Cellular effects however, may also be involved in the protective effect of HO-1. HO-1 induction in the donor decreased early post transplant alloreactivity, donor-derived dendritic cells, and T-cell reactivity in the recipient.[48] Moreover, donor pre-treatment with HO-1 has been shown to improve microcirculation after transplantation.[49] Both HO-1 and CO appear promising opportunities for donor pretreatment. Whether their application is feasible and beneficial in clinical practice still remains uncertain.

*Anti-complement therapy*

The inflammatory storm in the process of brain death may induce complement activation in the donor, thereby causing damage in the kidney graft before transplantation.[50] Inhibition of complement activation in the donor could therefore be beneficial for the kidney graft. The complement system is part of the innate immune system and it can be activated through the classical, alternative and lectin pathway. Soluble complement receptor 1 (sCR1) acts as an inhibitor of the common part of all three complement pathways. Pretreatment of donor rats with sCR1 around induction of brain death prevented the increase in circulating C3d and significantly improved renal function immediately after transplantation.[51] These first encouraging results should be confirmed in further animal experiments applying other complement inhibitors, before translation to clinical application can be made.

*Erythropoietin*

Erythropoietin (EPO) was originally identified for its role in erythropoiesis, but is now known for its anti-apoptotic and cytoprotective effects as well. These protective effects are mediated by different receptors and mechanisms than the ones regulating the hematopoietic effects and may defend the kidney from I/R injury, potentially even when administered to the donor. This is particularly interesting considering the conceivably unfavorable side effects of systemic EPO treatment to the recipient.

Two very recent animal studies addressed donor pretreatment with EPO.[52, 53] In a rat model, brain dead donors were pretreated with EPO or carbamylated EPO (cEPO), which lacks the hematopoietic effects of EPO. Although kidneys were not actually transplanted, short term graft function was analyzed in an isolated perfused kidney set-up. Both EPO and

cEPO diminished the influx of polymorphonuclear cells into the kidney and grafts showed a normalization of creatinine clearance in this isolated perfused kidney model.[53] In a study with larger animals, involving porcine kidney transplantation, cardiac dead donors were pretreated with EPO. In as little as 4 hours after reperfusion, renal injury and inflammation decreased and renal function improved in the EPO pretreated group.[52]

*Immunosuppressive and anti-inflammatory agents*

The period of donor brain death is well known for causing a systemic, nonspecific inflammatory reaction, and organs from brain dead donors are influenced by this inflammatory state.[17, 54-57] Moreover, the ongoing inflammatory reaction after transplantation is responsible for reperfusion induced tissue damage.[58] When translating findings of animal experiments into human therapies, the general immunosuppressive effects of corticosteroids could be suitable to suppress the inflammatory response in brain dead donors. Methylprednisolone is frequently administered in donor management as part of hormone replacement therapy, but may also be administrated at a higher dose than normally given for replacement therapy, to decrease the inflammatory response. Limited recent data from a large randomized, blind, placebo-controlled trial on donor steroid pretreatment are available. The results show that expression of pro-inflammatory genes in brain dead donors normalized after steroid pretreatment. Although the incidence and duration of delayed graft function did not change with steroid pretreatment, the follow-up period was fairly short and information on longer term graft function is not available as yet.[59,60] Nevertheless, reducing the pro-inflammatory storm after brain death remains a promising approach, as illustrated by the improved kidney graft survival rate in rats after donor pretreatment with JNK signal transduction inhibition.[61]

Preconditioning of rat donors with calcineurin inhibitors cyclosporine A or tacrolimus decreased structural damage and resulted in improved graft function after kidney transplantation.[62] Pretreatment with tacrolimus combined with rapamycin even improved outcome synergistically.[63] Not all studies could confirm these results, potentially explained by gross differences in experimental set-up and dosing of immunosuppressives between studies.[64]

The basic mechanism behind immunosuppressive donor pretreatment remains unknown. The responsible mechanism appears not to be, as expected, an additional inhibition of the alloimmune response. An effect on renal I/R injury is more likely, since the protective effects have been observed in syngeneic kidney transplant models and appear related to acute renal stress.[65]

*Other pretreatment therapies*

Photosensitizer + UVA (PUVA) treatment was applied in a series of studies nearly 30 years ago. Donor rats were pretreated with a photosensitizer and during hypothermic storage the kidney was irradiated with UVA. PUVA pretreatment improved graft survival in rats, with a dose-response relationship of the irradiation period.[66] The positive effect is attributed to decreased graft immunogenicity.[67-69] To validate these results, a clinical study was performed in 1986 showing that PUVA pretreated grafts had a significantly lower number of rejection episodes, although all other outcome parameters were not different.[70] Altogether, PUVA pretreatment showed some successes but since then results have never been validated or reproduced by others. Multiple rodent studies report on donor pretreatment with various substances with antioxidant capacities. Among others N-acetylcysteine (NAC), melatonin, danshen, and taurine were applied in animal kidney donors. Most studies showed improvements in biochemical parameters or secondary endpoints only, although some also demonstrated increased survival rates.[71-74] Nevertheless, unconfirmed results of these single studies on various antioxidants will probably not have a great impact on clinical donor pretreatment in the next few years, unless confirmed or applied in clinical studies.

Miscellaneous studies using diverse donor pretreatment strategies have been published the last few years with varying results. Vagus nerve stimulation was applied as donor pretreatment in brain dead donors for its potential anti-inflammatory effects. Vagus nerve stimulation decreased the expression of pro-inflammatory genes, decreased TNF-α production, diminished monocyte infiltration and more importantly, improved post-transplantation graft function.[75] Another study showed that donor statin pretreatment increased graft function and reduced renal inflammation in a rat kidney transplantation model.[76] Others showed that ICAM-1 inhibition in rat kidney donors improved graft survival, although effects were even larger when ICAM-1 was inhibited in the recipient or during preservation.[77] Disappointing results in animal studies have also been reported; glutamine donor pretreatment did not affect the post-transplantation renal function in rats.[78]

Finally, in humans a remarkable randomised clinical trial showed that hyperoxic donor pretreatment resulted in improved urine production and creatinine clearance after transplantation.[79] Previous animal experiments involving renal I/R without transplantation showed identical results.[80,81] It may be speculated that hyperoxia induces oxidative stress in the donor, which enhances endogenous antioxidant mechanisms of the kidney. Another explanation is that hyperoxia leads to an improved oxygen reserve capacity of the kidney that protects the energy metabolism during the ischemic period. Finally, there have been reports on successes of hyperthermic donor preconditioning in rodent experiments. Two studies of the same group described beneficial effects of donor hyperthermia on kidney

function and graft survival after transplantation. Hyperthermia induced renal expression of heat shock proteins was held responsible for the beneficial effects.[82, 83]

## Future perspectives

Although the principle of donor pretreatment is not new, the clinical trend to use more marginal donors for transplantation only recently necessitated the search for new ways to optimize donor organ quality. Much preclinical research on donor pretreatment has been done, with promising results. The first human trials have recently shown protective effects of donor pretreatment with dopamine and corticosteroids, and these are likely to be of great influence in the years to come. Despite all the promising results from preclinical studies, clinical trials studying donor pretreatment are scarce, especially studies aiming specifically at the effects in marginal donor grafts. It has been suggested that the great amount of groups of interests involved in transplantation, the difficult ethical debate concerning informed consent of deceased donors and the effect of pretreatment on other organs considered for transplantation hampers the translation into the clinical setting.[9,84] In the near future we expect results from some ongoing clinical trials, studying the effects of glucose, ischemic preconditioning, HO-1 induction and dopamine. Donor pretreatment targeting the immune system or oxidative stress responses remain interesting topics, but still have to prove themselves in the clinical setting.

In this review we focused on kidney transplantation. Research on transplantation of other organs will also provide new targets for pretreatment of kidney donors. Animal experiments with 17β-Estradiol as donor pretreatment, for example, showed improved outcome after transplantation of several different organs.[85] Donor pretreatment with metformin improved acute and chronic rejection in cardiac transplantation in mice.[86] Ultimately, it is expected that a combination of agents will be used as tailored donor pretreatment, and timing may turn out to be crucial in the success of donor pretreatment.

## Ethical considerations

In trying to translate donor pretreatment strategies to the clinical setting, ethical issues may be raised, particularly involving deceased donors. Most issues are related to the fact that manipulations will not benefit the donor directly in any way. Living donors provide the simplest situation, where the donor is fully aware of the donation procedure and can provide detailed consent, with prolonged time for consideration and reflection. It is obvious that donor pretreatment may never harm the living donor. The situation is more difficult for deceased donors. The act of joining an organ donor register or carrying a donor card is considered as consent to organ donation, but it is not clear whether such consent would extend to invasive pretreatment strategies, and when they could be started. There is a

need to establish whether pretreatment could be seen as no different from other medical interventions, such as the administration of heparin to donors. One can argue that consent to organ donation suggests consent to all techniques required to allow optimal quality of grafts. Pretreatment may represent part of the normal process of organ donation in the near future, and therefore should not require further consent or lack of objection beyond that associated with organ donation itself.

The timing of pretreatment may be crucial, particularly with regard to agents that require a significant length of time prior to donation, to provide the beneficial effect. Thus, there may be requirements to commence administration of the agent before lack of objection is obtained. Although this might be an unusual principle, this type of approach has already been approved by ethical committees and has been used in clinical trials on organ donors.[87] Deceased donors are often multiple organ donors. Intervention on behalf of one organ may be harmful to other potentially transplantable organs or at least affect them differentially. Donor pretreatment should be carefully tested for the effects on other potential grafts. With progression of the clinical applications of pretreatment, potential new ethical issues will arise and boundaries have to be continuously redefined.

## Conclusion

Nowadays we are facing a scarcity of donor organs, hence it is absolutely necessary to search for therapeutical options to render more marginal donor organs suitable for transplantation and to improve graft quality. During and before the process of transplantation the graft is exposed to various noxious events, which will lead to functional deterioration. Prevention of injury already in the donor could facilitate transplantation of more marginal donor grafts and provide better outcomes. Donor management is the first step to prevent derangements in the donor and has been much improved by standardization. Further, more specific improvement of graft condition is the aim of donor pretreatment. Donor pretreatment by various strategies, including ischemic preconditioning, HO-1 induction, anti-inflammatory and anti-complement interventions, erythropoietin and catecholamines has been successful in animal experiments. Although many of these promising results in animals have yet to be confirmed in human kidney transplantation, the first pretreatment strategies have already shown encouraging beneficial effects in clinical studies.

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