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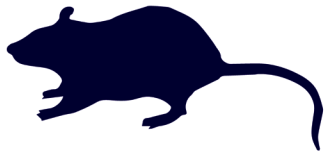
General introduction



General introduction

In Europe, more than 10000 patients with end-stage renal disease were waiting for a donor kidney in 2012. In the same year, 4812 patients received a donor kidney. From these kidneys, 3432 were derived from a deceased donor and 1380 from a living donor[1]. Since 2007, the waiting list has declined slightly. This effect can be mainly attributed to an increasing number of living kidney donors. On the other hand, the number of deceased donors has been stable for the last 20 years[1]. Thus, a significant increase in donor kidneys is required to provide all patients on the waiting list with a kidney. Expanding the donor pool is therefore of great importance for the transplant community. Public education and expansion of the inclusion criteria are primary means to reduce the number of patients waiting for a kidney.

There are three different donor types: living- (LD), deceased brain death- (DBD) and deceased circulatory death donors (DCD). Alternative nomenclature for DCD donation is deceased cardiac death or non-heart beating donors. The outcome of LD kidney transplants is superior to DBD or DCD transplants. This can be explained by the absence of primary warm ischemia and a shorter period of cold storage (CS). Brain dead donation also features minimal primary warm ischemia, but average CS is 15-20 hours. In addition, brain death per se causes hemodynamic instability, hormonal changes and systemic inflammation, which negatively affect the quality of potential donor organs. DCD donor organs perform the worst as these organs are subjected to a combination of warm ischemia prior to donation and subsequent CS. The duration of warm ischemia unavoidably increases renal ischemia/reperfusion (I/R) injury. The detrimental features of the deceased donor types result in different short- and long-term outcome after transplantation.

Death censored graft survival of LD, DBD and DCD kidney transplantation in the University Medical Center Groningen between 1993 and 2008 is shown in Figure 1. The major differences in survival between the donor types can be observed early after transplantation and are explained by a distinct higher incidence of primary non-function (PNF) in DCD compared to DBD and LD donor kidneys (9%, 5% and, 1% respectively). Excluding PNF grafts, overall graft survival of DCD-, DBD- and LD transplantation is 86%, 86% and 93%, respectively. LD death censored graft survival is superior to the deceased donor types, but graft survival of DCD transplants is not inferior to DBD transplantation. However, short-term outcome of DCD transplantation is evidently compromised compared to DBD transplantation, as the incidence of PNF (9% vs. 5%, respectively) and delayed graft function (DGF; 82% vs. 30%, respectively) are increased.

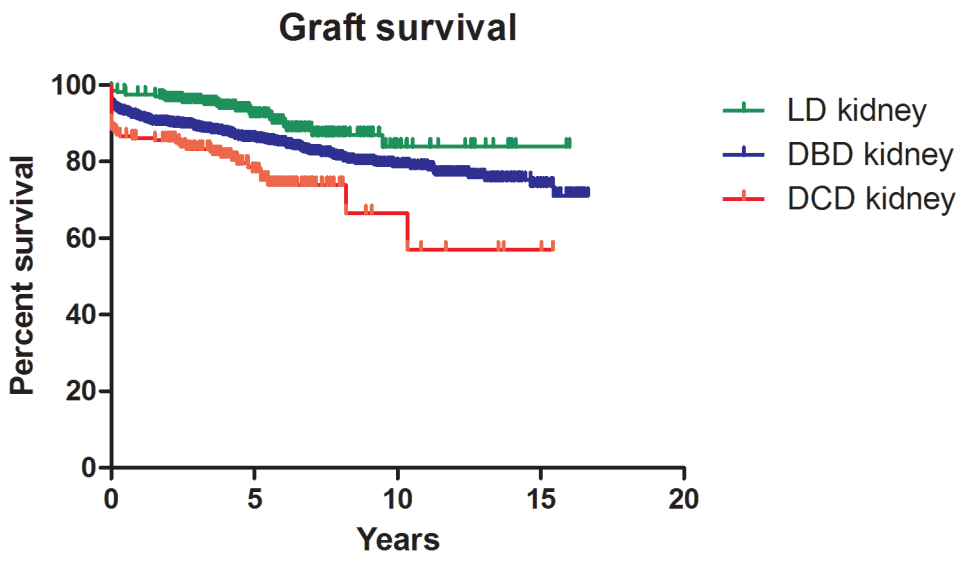


Figure 1 - Death censored graft survival between 1993 and 2008 in the University Medical Center Groningen. Graft survival of living donor kidneys is superior compared to deceased brain- and circulatory death donor kidneys. Differences in graft survival are mainly observed directly after transplantation.
LD - living donor kidney; DBD - deceased brain death donor kidney; DCD - deceased circulatory death donor kidney.

Although deceased donation negatively affects the outcome of renal transplantation compared to LD, the ten year graft survival is still over 85%, which indicates that transplantation of DBD and DCD donor organs are a great opportunity for patients with end-stage chronic kidney disease. Besides, it has been shown to be a cost-effective treatment[2].

To increase the number of donors several countries started to use DCD donors and expanded criteria donors (ECD). DCD donors are classified into five categories (Table 1).

Table 1 - Modified Maastricht classification for DCD donors[3]

Category	Classification	Status
I	Dead on arrival	Unexpected
II	Unsuccessful resuscitation	Unexpected
III	Awaiting cardiac arrest	Expected
IV	Cardiac arrest after brain death	Expected
V	Cardiac arrest in a hospital patient	Unexpected

ECD refers to older donors (≥60 year) or donors who are aged 50 to 59 years and have two of the following features: hypertension, terminal serum creatinine (>1.5 mg/dl) or death from a cerebrovascular accident[4]. In the future, the age criteria of ECD donors will be met by more potential donors because of the aging population. The shift from DBD- to ECD donors explains that the use of ECD donors did not result in a clear reduction of the waiting list for donor kidneys. Besides, part of the potential DBD donors are used as category III DCD donors, which means that the total number of donor kidneys does not increase.

To date, mainly expected, category III donors are used for DCD transplantation. The number of category III donors is low compared to the number of unexpected donors. However, an increasing number of transplantation clinics, in particular in Spain and Russia, also employ unexpected DCD donors[5–7]. Potentially, the pool of unexpected DCD donors is enormous and the use of unexpected DCD donor kidneys might significantly reduce the waiting list.

To utilize this potential, outcome of more marginal and unexpected DCD kidney transplants has to approach current outcome of deceased donor kidney transplantation. This can eventually result in a reduced waiting list for donor kidneys. As shown in figure 1, graft survival of DCD transplants is inferior compared to LD- and DBD transplants due to a higher incidence of PNF. Thus, particularly short-term function is compromised by more severe I/R injury. The use of more marginal DCD donors increases the risk of PNF, DGF and eventually overall graft survival. Thus, new performance enhancing treatments to reduce the effect of I/R injury are required to improve the function of these marginal kidney grafts.

I/R injury is a key player in DCD transplantation, but also in DBD transplantation as these donors also sustain I/R due to the hemodynamic instability[8,9]. After donation, the donor organ is cooled to 4°C to slow down metabolism, but also to turn down inflammatory and injury related processes. Recirculation of the donor organ results in oxygenation, supply of nutrients and warming of the donor kidney. Renal metabolism and function are re-established, but also detrimental pathways are induced[9]. The major part of I/R injury, however, develops during the reperfusion phase of transplantation[9].

This explains why many researchers focus on diminishing I/R injury to improve short-term outcome following transplantation of deceased donor kidneys. In this field of research several medications and preservation strategies are being tested. A major breakthrough in 2009 was the protective effect of machine perfusion on graft survival of deceased donor kidney transplants compared to cold storage[10]. However, the base for clinical studies originate in experimental models, in which we are currently aiming at improvement of preservation techniques and donor- or recipient treatment with cytoprotective medication[11–13].

The primary aim of this thesis is to show the performance enhancing- and renoprotective capacities of three different treatments regimens. One preservation technique and two potential cytoprotective treatments are therefore being investigated. However, improved understanding of the effects of brain death on donor kidneys is essential for development of new strategies to improve outcome of DBD kidney transplantation. Therefore, we investigated the direct effect of brain death on renal function, metabolism and inflammation in **chapter 2**. In this experiment, ureters of brain death donor pigs were cannulated, enabling us to continuously measure the glomerular filtration rate during brain death. This is the first study showing the immediate effect of brain death on renal function.

Furthermore, we focused on reduction of I/R injury and thereby improvement of short-term function following renal transplantation. Here, the three strategies will be shortly introduced.

α-melanocyte stimulating hormone

α-melanocyte stimulating hormone (α-MSH) is a pleiotropic neuropeptide produced by the pituitary gland. It is mainly known for its function in pigmentation, but it also plays a role in energy metabolism and it has anti-inflammatory capacities[14,15]. In models of acute kidney injury, α-MSH improved renal function[15-18]. Based on these anti-inflammatory and renoprotective capacities, we hypothesized that α-MSH improves outcome of DBD kidney transplantation.

In **chapter 3**, we test the hypothesis that α-MSH of recipients improves short-term graft function and reduces inflammation after transplantation of DBD donor kidneys.

Normothermic recirculation

Normothermic recirculation (NR) means recirculation for a limited time with warm oxygenized blood quickly after declaration of circulatory death. After NR, organs are retrieved and cold storage (CS) starts. NR is typically implemented by an extracorporeal membrane oxygenator, connected to a closed circuit in the femoral vessel of the DCD donor[19]. Thus, NR is an early organ preservation strategy for DCD donors. Several hospitals worldwide already have operational clinical NR protocols for potential DCD kidney- and liver donors[19–22]. However, experimental studies showing the protective effects of NR are limited[23].

In **chapter 4**, we test the hypothesis that normothermic recirculation protects renal transplants against warm ischemia in a rodent transplantation model.

Erythropoietin mediated cytoprotection

Erythropoietin (EPO) is primarily known as a regulator of erythropoiesis[24]. However, it became infamous because of its role in professional cycling as a doping agent[25]. Recently, it was discovered that the working mechanism of EPO is not that simple. EPO is pleiotropic and has also an endogenous protective function[26]. Perhaps, cycling athletes were not only champions because of a better capacity to deliver oxygen, but also because of the ability to recover faster after heavy exertion. Next to its questionable role in sports, it has been shown that EPO can be used as a performance enhancing agent in the kidney[27-29]. In models of acute renal injury, EPO improves renal function, reduces inflammation and reduces structural damage[27,28,30].

As shown in figure 2, stimulation of erythropoiesis and cytoprotection are regulated by binding of EPO to different receptor complexes[29,31]. This means that systemic EPO treatment activates several pathways. This causes a major drawback of EPO mediated cytoprotection, as it also increases risk of cardiovascular adverse events[32,33]. Therefore, we tested ARA290, a non-erythropoietic EPO derivative, derived from the binding site to the protective receptor complex[34-36]. This may result in protection of renal transplants without increasing the risk of cardiovascular adverse events.

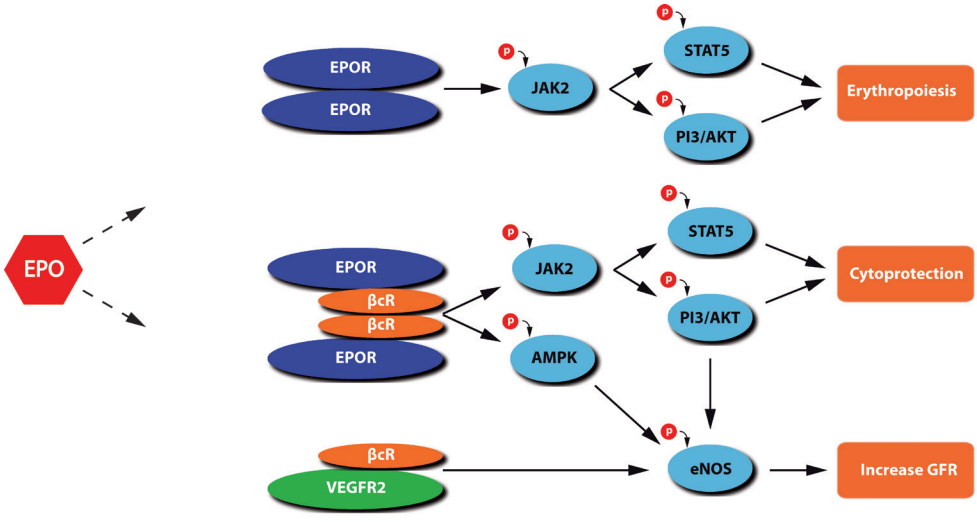


Figure 2 - Proposed pathways of erythropoietin. EPO is able to activate either the classical $EPOR_2$ complex, the $EPOR_2$-βCR_2 complex or an interaction between the βCR-VEGFR2. Regulation of erythropoiesis and cytoprotection is mediated by similar downstream pathways activating anti-inflammatory, anti-apoptotic and pro-survival pathways. PI3/AKT and AMPK, activated by the $EPOR_2$-βCR_2 complex and βCR-VEGFR2 interaction, are responsible for increased eNOS phosphorylation by EPO. The direct stimulative effect on renal function is presumably the result of enhanced eNOS activity.

In **chapter 5**, the protective capacities of brain death donor treatment with ARA290 are tested. Subsequently, we test the hypothesis that ARA290 protects against renal I/R injury in rats and pigs in **chapter 6 and 7**. In **chapter 8**, the role of the EPO receptor in the development of renal I/R injury and its consequent role in ARA290 mediated renoprotection are investigated. In **chapter 9**, recent clinical trials concerning high dose EPO treatment after renal transplantation to improve short-term function are reviewed. Furthermore, the benefits of non-erythropoietic EPO derivatives in renal transplantation are discussed. The endogenous role of EPO mediated cytoprotection is investigated in **chapter 10**. In this chapter, the role of a functional EPO gene polymorphism in deceased donor kidney transplantation is also demonstrated.

Discussion

The results of this thesis and the future implications are discussed in **chapter 11**.

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