Brain death and organ donation

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CHAPTER 1

Introduction

Renal transplantation is the most effective therapy for end-stage renal disease. In 2015, 442 kidneys from deceased donors, the largest donor pool, were transplanted in the Netherlands[1]. At the end of this year, 554 patients were still awaiting a kidney transplant. The total amount of kidneys transplanted in the Eurotransplant region was 3206 in the year 2015 while 10400 patients were waiting for a kidney transplant. In the United states, 17.107 kidney transplants were performed while 100.791 patients were still on the waiting list[2,3]. These data indicate that the demand of donor organs outweighs the supply. Therefore, an increase in donor organs is necessary to meet the pressing demand.

Kidneys can be retrieved from living donors (LD) and deceased donors. Between the two, deceased donors form the majority since living donors are scarce[4]. Deceased donors can be classified into deceased brain-dead (DBD) and deceased circulatory death donors (DCD)[5]. Of the kidneys transplanted from deceased donors, most are retrieved from brain-dead donors. However, in many countries, the number of kidneys obtained from DCD donors are increasing and in some countries, like the Netherlands, these kidneys are already transplanted as frequently as DBD kidneys. This is because increased amounts of kidneys from DCD donors who die unexpectedly are being more frequently used for transplantation. Most DCD donor kidneys used for transplantation are from donors who die expectedly in the sense that cardiac arrest is anticipated. In the future, an increased amount of DCD donors could be realized as some countries are starting to use organs from unexpected DCD donors.

The outcome of kidneys retrieved from different donor types varies. The graft survival rates of kidneys from different donor types from the University Medical Center Groningen are depicted in Figure 1. Kidneys from living donors are superior to deceased donor kidneys which can be explained by shorter warm- and cold ischemia times. DBD kidneys do not suffer warm ischemia but are usually subjected to long periods of cold ischemia during transport. In addition to cold ischemia, DCD kidneys usually suffer prolonged periods of warm ischemia which results in the worst outcomes amongst the three donor types. This effect is typically observed soon after transplantation as the incidence of primary non-function (PNF) of DCD grafts is 9%, while DBD and LD grafts show PNF rates of 5 and 1%, respectively. However, overall graft survival of DCD-, DBD- and LD transplantation is 86%, 86% and 93%, respectively. Therefore, outcomes from LD kidneys is superior to deceased donors but overall outcome is not compromised between either two deceased donor types. However, short term outcome of DCD kidneys is inferior compared to DBD kidneys with regard to, like mentioned above, PNF, but also delayed graft function (DGF; 82% and 30%, respectively). Yet, despite that deceased donation influences graft survival negatively, transplanting deceased donor kidneys represent a good solution for patients with end stage renal disease as the ten-year graft survival rate exceeds 85%.

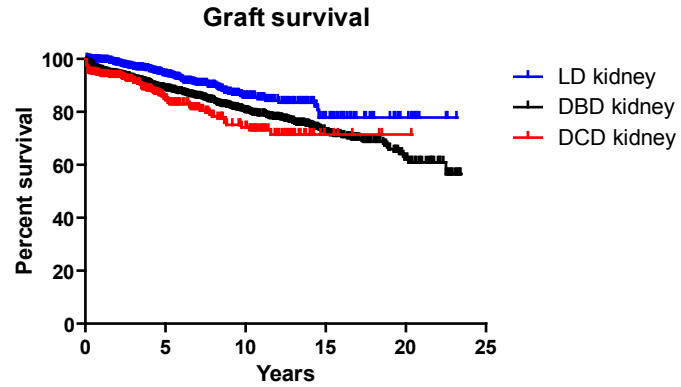

Figure 1. Renal graft survival in transplant recipients transplanted between 1993 and 2015 in the University Medical Center Groningen. Graft survival is superior when organs are retrieved from living donors (LD) compared to deceased donors. Within the deceased donor group, organs retrieved from brain-dead donors (DBD) show superior graft survival compared to cardiac-death donors (DCD).

There are different options to meet the increasing demand for donor organs. Many countries have resorted to the use of expanded criteria donors (ECD) to meet the increased demand of donor organs[6]. ECD donors are older donors (>60 years) or donors aged 50 to 59 years with two of the following: cerebrovascular accident as the cause of death, pre-existing hypertension, or terminal serum creatinine greater than 1.5 mg/dl. In the future, more ECD donors will be evident due to the aging population. Regarding DCD donors, an increased amount of DCD donors could be realized by increased use of organs from unexpected DCD. In Spain and Russia, kidneys from unexpected DCD donors are being increasingly used[7-9]. Unexpected DCD donors form a large group and may represent a potential option to decrease the demand for donor kidneys.

Transplanting kidneys from more marginal donors like ECD and DCD donors is likely to be associated with decreased rates of graft survival. Therefore, developing strategies to improve graft survival rates of transplanted kidneys could be advantageous. By doing so, these strategies could result in increased graft survival and thereby prevent patients from being relisted on the kidney waiting list. These strategies could also benefit more conventional donor types such as DBD donors as these kidneys suffer numerous insults in the donor. Furthermore, all transplants are subjected to ischemia-reperfusion (I-R) injury during the transplantation process and therefore even LD donor kidneys could benefit from such strategies. A major break-through was evident with the beneficial effect of machine perfusion on graft survival of deceased donor kidney transplants compared to cold storage[10].

As mentioned above, kidneys from brain-dead donors lead to inferior outcomes compared to kidneys from living donation[4]. This phenomenon is related to pathophysiological changes that take place in the brain-dead donor. Brain death (BD) leads to major hemodynamic derailments, systemic inflammation, and altered metabolism which potentially affects future donor organs[11-13]. Furthermore, brain dead donor organs suffer increased I-R injury [14]. Major hemodynamic derailments are due to the catecholamine storm which is characteristic for the onset of BD[13]. The catecholamine storm is believed to be the bodies final attempt to maintain cerebral perfusion against increasing intracranial pressure. The

large amounts of secreted catecholamines lead to severe vasoconstriction and possible ischemic damage to organs[15]. Furthermore, spinal cord ischemia will result in vascular collapse and consequently decreased blood flow and increased ischemia. Circulating cytokines are evident soon after the onset of BD with IL-6 being the most implicated cytokine in BD. Expression of adhesion molecules and infiltration of inflammatory cells in organs is also evident soon after the onset of BD[16]. The increased production of cytokines could be secreted by the dying cerebrum or by organs that suffer ischemic insults due to the hemodynamic instability. Altered metabolism is observed in the kidney and liver of brain-dead animals. Most notably a switch is apparent from aerobic to anaerobic metabolism. The altered metabolism could be the result of both hemodynamic changes and inflammatory mediators. Furthermore, brain death-related processes such as the catecholamine storm are affected by the speed at which intracranial pressure (ICP) increases[17]. A faster increase in ICP leads to higher levels of circulating catecholamines, which is particularly detrimental for cardiac and pulmonary graft function. Indeed, traumatic brain injury, the most common cause of BD preceded by a rapid increase in ICP, is a risk factor for mortality in heart recipients[18]. In contrast, a cerebrovascular cause of death, usually preceded by a slower increase in ICP, is a risk factor for renal and hepatic graft dysfunction[19,20]. However, this phenomenon is not believed to be associated with a slower increase in ICP. Rather, donor characteristics such as obesity, old age, and the presence of cardiovascular disease are regarded as the underlying cause.

Many studies have focused on attempting to counteract BD-related pathophysiological processes. Hemodynamic, inflammatory, and metabolic changes have all been counteracted with often good experimental results. Experimental research shows that blocking the catecholamine storm has a beneficial effect on lung function parameters and histology [21]. Treating brain-dead rats with methylprednisolone leads to improved renal graft survival and reduced rejection[22]. Metabolic changes have been tackled through administration of thyroid hormones which has differential effects on the liver and kidney [23,24].

Many beneficial experimental interventions in brain-dead donors have been studied clinically as well. Early studies involving the administration of methylprednisolone to brain-dead donors however did not show beneficial effects[25-27]. In a later study, a reduction in pro-inflammatory cytokines in the donor kidney prior to transplantation was evident[22]. No beneficial effects of methylprednisolone on kidney function after transplantation were observed. Improved renal function after transplantation has only been observed with the administration of dopamine to brain-dead donors[28-31].

Oxidative stress has been documented in brain-dead kidneys in both experimental and clinical studies[16,32]. Several studies show that BD is associated with oxidative damage of cellular lipid membranes[12,33]. Lipid peroxidation leads to membrane permeabilization and impairment of enzymatic processes and ion pumps which results in membrane dysfunction and cell toxicity[34-36]. BD-related lipid peroxidation is correlated with DGF in renal transplant recipients[32]. The levels of malondialdehyde (MDA), a product of lipid peroxidation, in the preservation solution of kidneys retrieved from brain-dead donors correlate well with DGF. Moreover, donor serum MDA levels correlate with acute rejection and immediate and long-term renal allograft function. In expanded criteria donors (ECD), MDA levels in machine perfusion solution also correlate with DGF[37].
Reactive oxygen species (ROS) are mainly formed in the mitochondrial electron transport chain (ETC, Fig.2) and are essential for cellular homeostasis, mitosis, differentiation, signaling and survival[38]. Superoxide can be generated from complexes I and III of the

mitochondrial electron transport chain (ETC), by xanthine and NADPH oxidase, the tricarboxylic acid (TCA) cycle enzymes aconitase and α-ketoglutarate dehydrogenase, by non-TCA cycle enzymes and by monoamine oxidases and cytochrome b5 reductase, located in the outer mitochondrial membrane[39]. Endogenous antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GpX) and catalase regulate the levels of ROS accurately[40]. However, certain pathological conditions increase radical production which can overwhelm antioxidant protection. Excessive ROS generation leads to damaged nucleic acids, proteins, and lipids. which damages enzymes in the ETC leading to mitochondrial dysfunction, decreased ATP production and increased generation of ROS.

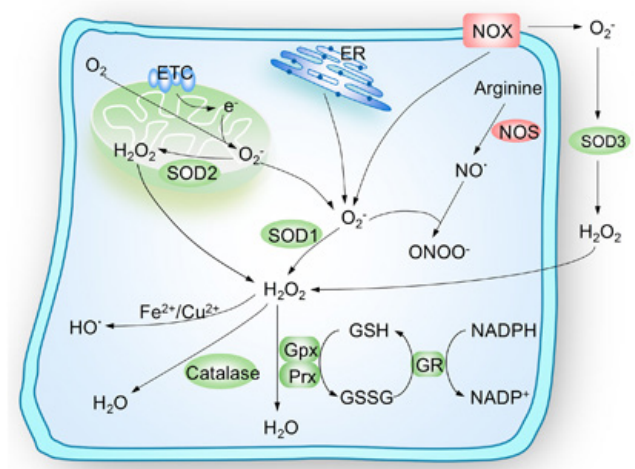

Figure 2. Sources of cellular superoxide production and its clearance by endogenous anti-oxidants. Adapted from Wang, K. 2013. Superoxide (O2-) is formed mainly from the mitochondrial electron transport chain (ETC). Endogenous anti-oxidants such as superoxide dismutase (SOD) convert superoxide to hydrogen peroxide (H2O2) which can subsequently be converted to water (H2O) by amongst others catalase and glutathione peroxidase (GpX).

BD pathophysiology, which comprises hemodynamic, inflammatory, and metabolic changes, can lead to oxidative stress through aforementioned processes[39,41,42]. Hemodynamic changes, and the resulting ischemia, trigger mitochondrial dysfunction and the subsequent leakage of radicals from the mitochondrial respiratory chain. The influx of inflammatory cells can cause increased oxidative stress as large amounts of superoxide are released which forms part of the respiratory burst to kill pathogens. Furthermore, metabolic changes can lead to mitochondrial dysfunction and thereby increase the oxidative load. Considering all the possible sources of oxidative stress, anti-oxidative therapy could encompass many different options. Therefore, prior assessment as to what might be the most up-stream cause of oxidative stress could be useful for administration of an efficient anti-oxidative compound.
The primary aim of this thesis is to assess the detrimental effects of BD on different donor organs and to thereby put forward and test organ-specific therapies. Many studies have been conducted on the detrimental effects of brain death and many studies have focused on preventing these effects. Unfortunately, many of these interventional studies don't show positive clinical effects. Likely, increased and more in depth knowledge about organ-

specific effects of BD is necessary to gain advancements in treating brain-dead donors. Therefore, in this thesis we delved deeper into certain detrimental effects which were already touched upon on by previous studies, such as oxidative stress and metabolism. Furthermore, to gain more insight into the processes leading up to BD, we assessed the effects of different speeds of BD induction on donor organs. The speed of BD induction has shown to influence heart function and damage. Clinically, brain insults tend to progress to BD at different speeds. Therefore, we aimed to investigate the effects of speed of BD induction on other organs than the heart as this could also contribute to organ- and donor-specific interventions.

In **Chapter 2** we focused on assessing the effects of fast and slow BD induction on the two most frequently transplanted organs, the kidney and liver. In this Chapter we aimed to assess general differences between these organs elicited by fast and slow speed BD induction. Subsequently, in later Chapters, more in depth analysis could be performed based on these initial results. In Chapter 2 we used the most clinically relevant function and damage markers, such as plasma creatinine and plasma levels of ASAT and ALAT to determine the effects of speed of BD induction on the kidney and liver. Furthermore, commonly used marker for inflammation in BD, such as IL-6 and other common markers for cell death such as caspase expression and the oxidative stress marker MDA were assessed.

In **Chapter 3**, similarly to Chapter 2, we assessed the effects of fast and slow BD induction on lung parameters. Experimental data shows that the heart is negatively affected by a fast increase in intracranial pressure which is attributed to a more severe catecholamine storm. Experimental research has shown that blocking the catecholamine storm has a beneficial effect on lung function. Moreover, clinical data shows that a traumatic cause of BD, associated with a fast increase in ICP, leads to decreased lung function. No experimental data has been published on the effects of speed of BD induction on the lung. Therefore, we aimed to assess the effect of the speed of BD induction on lung function and damage. This data could help in designing donor-specific management strategies and optimal organ allocation policies.

Chapter 4 of this thesis is an expansion of the observation done in Chapter 2 that slow BD leads to increased renal oxidative stress compared to fast induction. In this Chapter, we investigated oxidative and anti-oxidative processes after fast and slow speed BD induction. These processes could explain the increased renal oxidative stress observed especially after slow BD induction. This data could lead to specific anti-oxidative therapy for brain-dead donors and thereby possibly improve transplantation outcomes.

Chapter 2 and **4** show that BD leads to increased renal oxidative stress. These results were manifested despite the maintenance of hemodynamic stability. In **Chapter 5**, we investigated how BD affects regional renal and hepatic hemodynamics and metabolic effects in these organs and how this might affect the oxidative processes we observed in previous Chapters. Changes in metabolism elicited by brain death were shown almost thirty years ago. However, no clinical relevant interventions have evolved from this pioneering work. Therefore, in this Chapter, we looked more deeply into metabolic changes in the liver and kidney. We assessed changes in levels of glucose, fatty acids, and proteins. Moreover, mitochondrial functional changes were assessed and changes in regional hepatic and renal perfusion.

The kidney is by far the most transplanted organ. Therefore, in **Chapter 6,** we undertook an interventional study by treating brain-dead rats with the goal of improving renal quality. Oxidative membrane damage in brain-dead donors correlates with delayed graft function in renal transplant recipients. Therefore, anti-oxidative therapy administered to brain-dead donors could lead to improved transplantation outcomes. Based on the observations made in Chapter 2, 4, and 5, we treated brain-dead rats with MnMTPyP, a selective superoxide dismutase mimetic, after slow BD induction. In Chapter 2 we found that oxidative stress was increased more after slow BD induction so we chose this form of induction to study the effects of MnTMPyP. In Chapter 4 we showed that superoxide is probably one of the most "upstream" oxidative processes in BD. Therefore, a superoxide dismutase mimetic could potentially eliminate this process and exert beneficial downstream effects. In Chapter 5 we showed that oxidative stress is likely influence by decreased regional renal perfusion which leads to changes in metabolism and oxidative stress. At the same time this shows that in anti-oxidative therapy should comprise a compound which can exert effect in the renal tissue since the maintenance of hemodynamic stability is not sufficient.

In Chapter 6 we showed that MnTMPyP exerts beneficial effects on the kidney as shown by decreased renal and systemic oxidative stress. However, no beneficial effects on function were seen which we attributed to the fact that BD does not lead to sufficient oxidative damage to expect beneficial effects of anti-oxidative treatment. Therefore, in **Chapter 7**, we assessed renal function of kidneys of brain-dead rats treated with MnTMPyP in an ex vivo isolated perfused kidney system.

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