Donation of kidneys after brain death

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Introduction and rationale

ABBREVIATIONS

BD: Brain Death
CAN: Chronic Allograft Nephropathy
CKD: Chronic Kidney Disease
DBD: Donation after Brain Death
ESRD: End-stage Renal Disease
GGA: GeranylGeranylAcetone
HO1: Heme Oxygenase-1
HSPA1A: Heat Shock Protein 70
ICU: Intensive Care Unit
IRI: Ischaemia Reperfusion Injury

RENAL FAILURE AND KIDNEY TRANSPLANTATION

Chronic kidney disease (CKD) is the progressive loss of kidney function and is recognised as a public health problem, affecting 10% of the Dutch population.(1) Major risk factors for developing CKD are diabetes mellitus, elevated blood pressure, and atherosclerosis, explaining the higher prevalence of CKD with age. Every year about 2.000 people develop end-stage renal disease (ESRD) in The Netherlands.(2) ESRD is a great burden to the patient, as it is associated with many complications e.g. cardiovascular disease, and has to be eventually treated with renal replacement therapies. The most common treatment for ESRD is dialysis with currently 6.500 people subjected to this type of treatment in The Netherlands.(2) Although patients with ESRD obviously can survive whilst on dialysis compared to no treatment, the 5-year patient survival rate is still on average only 50%.(2) To date the preferred choice of treatment for ESRD is kidney transplantation for those in good enough health, which is life prolonging, quality of life enhancing, and becomes cost-effective within two years after transplantation.(3) The availability of kidney transplantation is however limited since there is a persistent shortage of viable donor organs. The average time a patient has to wait for an organ in The Netherlands is 3.2 years, and as a consequence 10-12% of patients waiting for a kidney will die or become too ill to receive a transplant.(4) Therefore, it is of crucial importance to be able to optimise all potential donor kidneys and utilise most of them once they have been procured. Unfortunately, in a deceased donor the sequence of cerebral injury, followed by donor management, procurement and preservation will expose the graft-to-be to a series of inevitable injury mechanisms that negatively affect the graft quality and outcome after transplantation. After the period of donor management in the intensive care unit (ICU) and during procurement and cold preservation, the kidney graft becomes subjected to ischaemic injury followed by reperfusion injury at time of transplantation in the recipient. The transplanted organ will find itself in a rather hostile immune system of the recipient that is activated even more when recognising the pre-existent injury due to donation and after transplantation. Common strategies to reduce injury and increase the chance of immediate function are to improve organ storage conditions by using machine perfusion at either hypo- or normothermic temperatures, as well as administering dedicated immunosuppressive therapies to the recipient.

It is important to realise that donor organ injury does not start with preservation as viable organs can also be injured in the donor even prior to organ procurement and cold storage. Different type of organ donors are associated with different short- and long-term results, and kidneys from deceased donors after brain death (DBD) have been reported to result in inferior transplantation outcomes when compared to those derived from living donors. (5) Due to the donor organ shortage in The Netherlands, living kidney donation has been stimulated significantly in the past decade, resulting nowadays in that approximately half of the kidneys transplanted are derived from living related or unrelated donors whilst the other half are procured from deceased donors(4). This situation is quite unique to The Netherlands as in most other developed countries the majority of kidneys comes from DBD donors. (2) Unfortunately, the physiological state of brain death itself predisposes for considerable metabolic- and pro-inflammatory changes in donor kidneys prior to procurement and with the

ischaemia and reperfusion injury (IRI) still to be added after preservation.(6-8) Prevention or at least reduction of this type of early donor injury could ameliorate early graft function as well as render grafts less susceptible to chronic allograft nephropathy (CAN) with better long-term graft survival.

BRAIN DEATH AND ITS IMPACT ON THE DONOR ORGAN

Brain death is defined as an irreversible coma with absence of brain stem reflexes, and apnoea but with a functioning systemic circulation. The major causes of brain death (BD) are cerebrovascular injury, anoxia, or traumatic injury, and the clinical diagnosis of brain death should only be considered in patients that suffer from severe irreversible brain injury of an identifiable origin. Patients that are diagnosed with BD are considered dead and organs donated from these patients for transplantation are referred to as deceased BD donor organs. Although the patient remains ventilated during their stay in the ICU and a stable blood pressure is maintained, the organs procured from these donors are more susceptible to IRI, cold storage and rewarming injury, and immune mediated graft injury. During the onset of BD a cascade of events occur due to the cerebral ischaemia, cerebral oedema, and cerebral hypertension. The increased intracranial pressure results in a strong parasympathic response followed by a sympathic response with severe vasoconstriction due to endogenous catecholamine release. This haemodynamic response is named the Cushing reflex that aims to maintain adequate cerebral perfusion pressure.(9) After the onset of BD there is an increased systemic circulation of cytokines, chemokines, and adhesion molecules.(6,10-13) Eventually BD results in organ dysfunction due to a series of detrimental responses including; microthrombus formation, decreased peripheral perfusion, generation of reactive oxygen species (ROS), increased vascular permeability, leukocyte mobilisation and infiltration, and pro-inflammatory changes in the transplantable organs **(Figure 1)**.(6,7,11,13,14)

The trigger for the inflammatory response is not fully understood, but cerebral injury itself is associated with a systemic inflammatory response syndrome (SIRS).(15,16) In addition, the integrity of the blood-brain barrier is lost during brain death, resulting in release of central nervous system-derived cytokines, like matrix metalloproteinases, that may induce inflammation in the peripheral organs.(17-19) The pathological process of brain death including its systemic changes has a detrimental effect on organ quality. Kidneys having suffered from BD induced haemodynamic instability and inflammation, have been reported to have higher rates of primary non-function and poorer short- and long-term outcomes when compared to optimal living donors after transplantation.(5,20,21) Several studies have shown increased plasma creatinine levels in brain dead animals and humans, increased leukocyte influx, enhanced expression of vascular adhesion molecules, and activation of the innate immune system.(10,11,22) Also, other donor organs such as liver, heart, and lungs are affected by this pathological process of brain death demonstrating significant pro-inflammatory changes.(13,17,23) Therefore, early intervention in the donor after diagnosis of brain death could prevent the detrimental changes, potentially better preserving organ quality, and improving

transplantation outcomes.

At the same time, it should be noted that not only harmful molecules become upregulated during BD. There is also an enhanced simultaneous expression of cytoprotective proteins, especially those of the family of heat shock proteins.(10,11,24) In particular heat shock protein-72 (HSP72 or HSPA1A) and heme oxygenase-1 (HO1 or HSP32) appear to be rapidly upregulated after cellular stress and during BD. In tissue culture and animal models, elevated levels of HSPs can substantially reduce the level of cell death and has led to the insight that HSPs can protect cells or even influence the course of disease.(25-27) Enhanced expression of HSPA1A and HO1 are able to provide protection against the detrimental effects of ischaemia and reperfusion injury (IRI) in the kidney(28,29), liver(30,31), and heart(32,33). In the context of brain death it is conceivable that the balance between cytoprotective- and inflammatory proteins eventually will determines the graft quality, function, and survival.

RATIONALE

The aim of this thesis is to explore the expression and properties of protective proteins and pathways in the deceased donor kidney after brain death to improve donor quality and outcomes after kidney transplantation. It is conceivable that enhancing the expression of protective proteins may improve the capacity of the donor organ to better cope with injury during donation and transplantation, and therefore provide benefit to the recipient with improved graft quality. This approach is investigated in the first part of this thesis by exploring the expression profile and protective properties of heat shock proteins in the setting of the deceased brain dead donor. The goal of this thesis is to improve the outcome after kidney transplantation. Since donor management affects all organs, the effects of protective strategies was also considered for other transplantable-organs in chapter 2 and 6.

In **Chapter 2** the protective working mechanism of the family of heat shock proteins is explained, including HSPA1A and HO1. **Chapter 2** also contains a detailed overview of the immune-active properties of heat shock proteins, since their release in the extracellular space can elicit an immune response. Especially donor organs from deceased donors are susceptible to inflammation. Despite the ability of heat shock proteins to induce inflammation, it appears that upregulation of these proteins is protective during ischaemia reperfusion injury and organ transplantation. The evidence for these protective effects is reviewed in the final section of **Chapter 2**.

In **Chapter 3** the effect of brain death-related kidney stress on heat shock protein expression is assessed in an animal model of brain death that has been developed by our group. The expression levels of several heat shock proteins are objectified using different techniques after a period of four hours of brain death. In particular, expression levels of HO1 and HSPA1A are analysed whether they appear to be upregulated.

Not only cellular stress, but also circulating compounds can enhance the expression of

cytoprotective proteins. In **Chapter 4** we aimed to stimulate heat shock protein levels in the donor kidney to protect the renal tissue from the hostile environment during brain death. To accomplish this, animals are treated orally with the drug GeranylGeranylAcetone (GGA) at 24 hours prior to the induction of brain death. Protective effects are evaluated.

As oral administration of drugs is not the preferred route for deceased brain dead donors, since the absorption is not optimal during this pathophysiological state, in addition, a derivate of GGA with improved pharmacochemical properties will be used to investigate whether the expression of HSPA1A increases in the deceased brain dead donor kidney. In **Chapter 5** we describe the effect after intravenous administration of this compound prior to brain death induction on the inflammatory and cytoprotective response. Treating the donor prior to organ procurement could be an elegant and also clinically relevant strategy to improve the donor quality.

In **Chapter 6** the results of a systematic review and meta-analyses are reported for different treatment strategies for deceased donors tested in randomised controlled trials.

In the latter part of this thesis the injury mechanisms leading to inferior graft quality in kidney transplantation are explored on a more molecular level. The goal of these studies is to reveal injury mechanisms or pathways that may either be affected after intervention, or could assist in better assessment of the donor organ quality.

In **Chapter 7** the effect of ischaemia and reperfusion injury is investigated on the molecular level in a rat model by applying 45 minutes of ischaemia to the kidney followed by subsequent four or 24 hours of reperfusion. IRI is especially relevant to donor management since the organs from deceased circulatory dead donors endure prolonged periods of ischaemia, after which reperfusion injury occurs. Since IRI is inevitable in solid organ transplantation, a better understanding of the molecular mechanisms leading to injury after reperfusion could identify new treatment strategies to prevent detrimental effects. Mass spectrometry is used to identify the changes occurring after IRI on the protein and metabolite level.

In **Chapter 8** we analyse kidney biopsies from deceased brain dead donors with either a good or suboptimal kidney function after transplantation. Using an -Omics approach, the goal is to see whether a difference can be observed in the donor proteomic profile at time of procurement of those transplanted kidneys that were found to have either a good or a suboptimal transplant function at the long-term. With the current need to accept more older and higher risk donor kidneys to meet the demand of patients on the waiting list, clinicians often have the dilemma of uncertainty when they have the choice to accept or decline a kidney. To date, the transplant community lacks more sophisticated diagnostic tools to support evidence-based decisions whether to accept or discard a kidney for transplantation. The use of a pre-transplantation biopsy scoring combined with donor age, cold ischaemia time, donor hypertension, and latest donor creatinine plasma levels are assumed to provide the best clinical assessment predicting graft function. However, such a scoring system has been shown not be able to discriminate between good and suboptimal kidney donors as outlined in **Chapter 8**.

Identification of biomarkers in blood of the deceased donor that could help with the clinical decision-making whether to accept or decline a kidney graft for transplantation could be of significant impact for the clinician burdened with this difficult decision. An -Omics approach would be the first logical step towards identification of biomarkers in blood of deceased brain dead donors. Biobanks that store blood or urine from deceased donors are of great value and importance to answer this question. However, sample collection during transplantation usually occurs during out-of-office hours and can result in prolonged room temperature storage of samples prior to correct storage, potentially inducing pre-analytical variability with artefacts. In **Chapter 9** an -Omics approach was used to investigate the effect of sample storage at room temperature for different periods of time. In this chapter we evaluate the results and how a protocol can be developed allowing sufficiently high quality proteomics data to support identification and validation of molecular profiles and biomarkers associated with lower or higher risk kidneys and their outcomes after transplantation.

In **Chapter 10** the findings in this thesis are summarised and discussed with a brief outlook into future developments and some recommendations are provided.

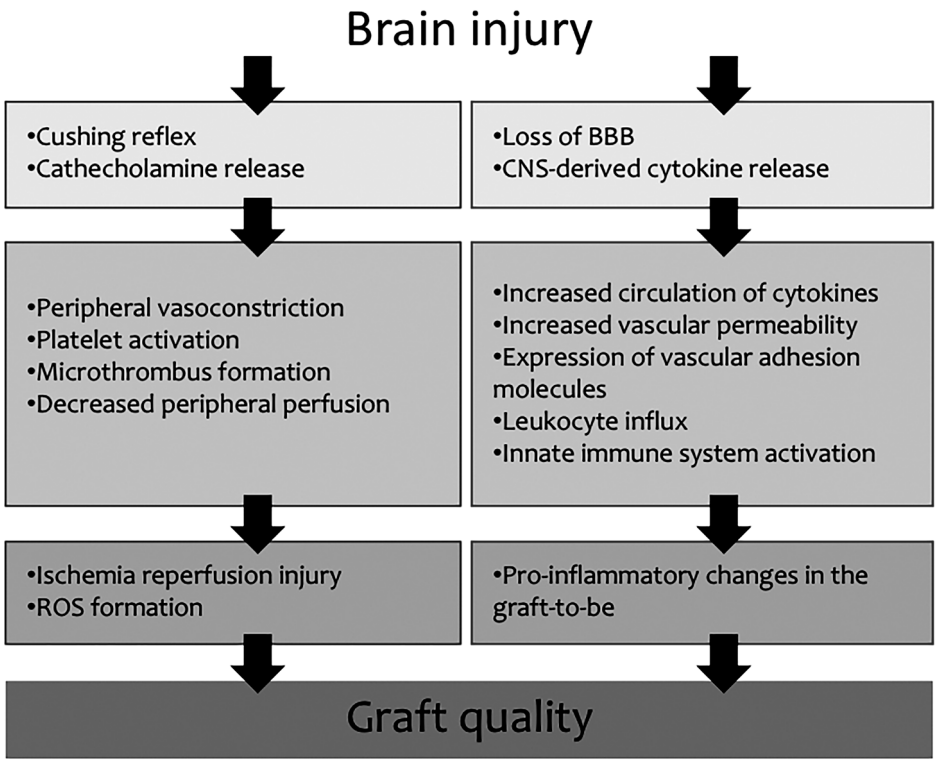

Figure 1. Brain injury results in loss of the blood brain barrier (BBB) with increased systemic circulation of central nervous system (CSN)-derived or activated cytokines, chemokines, and adhesion molecules. (6,10-13). Progression of brain injury leading to brain stem death results in a strong sympathic response with cathecholamine release, known as the Cushing reflex. Eventually, brain injury will compromise organ quality due to detrimental responses leading to reactive oxygen species (ROS) formation and pro-inflammatory changes in the graft.(6,7,11,13,14)

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