Early effects of brain death on kidney injury and outcome after transplantation

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CHAPTER 9

Summary & Future perspectives

- Summary -

In the last decades, kidney transplantation has become the treatment of choice for end-stage renal failure. The main source for donor organs in kidney transplantation has traditionally been heart-beating brain dead patients (donation after brain death, DBD). Due to the persistent shortage of donor organs, however, living (un)related donors and, to a lesser extent, kidneys from non-heart-beating donors have been increasingly accepted for transplantation. Transplantation with well-matched living donor-recipient combinations was known to have superior results compared to those with DBD and NHB donor kidneys. During the past fifteen years it became obvious that fully mismatched living-unrelated grafts also have better survival outcomes than kidneys retrieved from DBD donors with a very reasonable match for HLA antigens. This difference in results cannot be fully attributed to prolonged cold ischemia times in grafts procured from DBD donors, since no significant effect of cold ischemia time on kidney transplantation outcome was seen with preservation times up to 24 hr. Thus, other risk factors must be responsible and should explain the difference in success rates between living and cadaveric kidney transplantation.

In the past years, our group has focused on the unphysiologic state of brain death as a key player and potential risk factor that influences graft survival. Brain death has been known for many years to affect the circulatory and hormonal state of the donor. Attempts to neutralize these effects by monitoring blood pressure and by hormone suppletion therapy have, unfortunately, not been able to reverse these adverse effects. In this thesis, we have focused on the progressive pro-inflammatory response to brain death in the kidney and the detection of these effects prior to transplantation. We have now studied the characteristics of this inflammatory response, hypothesized about its origin and also investigated mechanisms of repair. Protective mechanisms during brain death may lead to a better outcome after transplantation, so we have evaluated the effect of the duration of brain death. The early detection of kidney injury due to cerebral injury was described. It was also our aim to test novel more specific biomarkers for their ability as early markers of kidney injury. Finally, we have included an intervention study using a modified form of erythropoietin to improve kidney quality during brain death.

In the **Introduction** of this thesis, the current knowledge and concepts as regards the nature of brain death and its effects on organ quality is described. Irreversible cerebral injury leading to brain death is usually the result of intracranial hemorrhage or traumatic brain injury. Both lead to a mass effect in the brain and compression of brain tissue, ultimately forcing the brain stem to herniate with loss of brain stem reflexes and finally complete respiratory arrest. During the increase of intracranial pressure, a Cushing reflex is observed due to catecholamine release, followed by hypotension due to progressive sympathetic deactivation. Most patients suffer from complete dysfunction of the hypothalamo-pituitary axis, as reflected by the onset of diabetes insipidus, as well as dysfunction of the hypothalamic-pituitary-thyroid axis.

Whether substitution with triiodothyronine, cortisol, and insulin has beneficial effects on donor condition still remains questionable. In addition, shortly after brain death, high levels of proinflammatory cytokines such as interleukin-1β and interleukin-6, soluble cytokine receptors like sIL-2 receptor and sTNF receptor II, and vasoactive substances like endothelin-1 can be detected in the peripheral blood of the donor. Release of these substances from the injured brain, as well as ischemia or vascular shear stress due to haemodynamic instability may be responsible for the effects seen in periferal organs. In the heart, systemic as well as local release of catecholamines leads to myocyte necrosis, and endothelial cell injury is a starting point for allograft vasculopathy. In the lung, brain injury results in neurogenic pulmonary edema, aggravated by the effects of mechanical ventilation. IL-8 is upregulated and PMNs are recruited to invade into the lung tissue. In the liver, an increase of transaminases is seen during brain death. Also, endothelial activation and influx of PMNs and macrophages can be seen. In an animal model, graft survival decreased significantly by using a liver from a brain dead donor, even without cold ischemia. In the kidney, a progressive inflammatory response with endothelial activation is found, as well as a decrease in kidney function. On the other hand, HO-1 and Hsp70 are significantly elevated in DBD donors. HO-1 and Hsp70 encode proteins with several cytoprotective properties against ischemia, heat and toxins. DNA microarray analysis of kidney tissue showed upregulation of several genes in brain death, which can be categorized in three groups: genes involved in inflammation and coagulation, in cell division and fibrosis, and in protection and repair processes. These findings in brain death of an inflammatory response at organ level together with a first attempt of the organ to protect itself offer new opportunities in donor treatment and subsequent preservation. When focusing on specific blocking or stimulation of these processes, graft survival of kidneys retrieved from DBD donors may be enhanced. This Introduction is followed by a general **Rationale** outlining the chapters of this thesis.

In **Chapter 3**, we focused on the effects of BD on inflammatory response and stress-related heat shock proteins in the kidney. Kidney biopsy specimens and serum were obtained during organ retrieval from DBD and living organ donor controls. With these biopsy specimens immunohistochemistry and semiquantitative reverse transcriptase-polymerase chain reaction were performed. These data, as well as clinical and laboratory parameters from DBD donors were recorded and related to outcome data of recipients of those kidneys. Immunohistochemistry studies showed an increase of E-selectin and interstitial leukocyte invasion in DBD donors compared with controls. RT-PCR showed a threefold increased heme oxygenase-1 and Hsp70 gene expression after BD. Levels of monocyte chemotactic protein-1 and transforming growth factor-β were twice as high after brain death but did not reach significance. Transplantation outcome was influenced by several donor variables: positively most notably by donor treatment with desmopressin and negatively by high serum urea levels during brain death and by high intercellular adhesion molecule and vascular cell adhesion molecule expression in the kidney. Heme oxygenase-1 proved to have a protective function, but only in kidneys from living donors. The presence of interstitial leukocytes and the early

adhesion molecule E-selectin in DBD donor kidneys indicates an early-phase inflammatory process during organ retrieval. Elevated levels of monocyte chemotactic protein-1 and transforming growth factor-β suggest a role for monocytes/macrophages in this phase. This leads to our conclusion that BD causes a stress-related response against which protective heat shock proteins are formed in the graft-to-be. This stress response in the DBD donormay be too severe to be fully counteracted by elevated heat shock proteins. Heat shock proteins may however be a promising starting point for interventions in the donor.

The upregulation of cytokines and adhesion molecules in the kidney may be aggravated by disturbances of the donor's milieu intérieur by other organs. It has been known that many DBD donors have a higher endotoxin load and more bacterial translocation from the gut compared to living donors. Endotoxemia could trigger a decrease of vascular integrity leading to increased permeability in the intestine or lung by influencing the Angiopoietin-Tie ligand-receptor system. A decreased Angiopoietin 1/Angiopoietin 2 ratio is associated with increased morbidity and mortality during sepsis, while an increased ratio can maintain vascular integrity and dampen the inflammatory response. Therefore, in **Chapter 4** we studied Ang-1 and Ang-2 in donor serum, as well as the amount of Lipopolysaccharide Binding Protein (LBP) as a quantification of endotoxemia. Also, VEGF was studied as Ang-2 primes endothelium to respond to VEGF. DBD donors had higher serum levels of LBP, Ang-2 and VEGF than living kidney donors, leading to a decreased Ang-1/Ang-2 ratio indicative of pro-inflammatory activation. In the univariate regression analysis, high Ang-2 levels in DBD donors predicted the chance of rejection in the first year after kidney transplantation. This indicates that Ang-2 is a potential marker for organ quality assessment in the donor.

Possible leakage of detrimental substances from the brain into the circulation of DBD donors, as well as the rise in cytokine and chemokine expression of potential donor organs during brain death suggests that early organ retrieval in DBD donors may be beneficial. On the other hand, the expression of protective heat shock proteins could be seen as a mechanism by which the body tries to repair present damage. Only little data is available but in two publications it is suggested that a longer BD period might be better for donor organ quality. In **Chapter 5** of this thesis, we report a large retrospective analysis using data from the OPTN database to study the effects of duration of brain death (BDdur) on outcome after kidney transplantation. 20,773 kidney donor and recipient couple data were studied. BDdur was calculated as the period between declaration of brain death and aortic cross clamping. The effect of BDdur on delayed graft function (DGF), acute rejection and one- and three-year graft survival was calculated using binary logistic regression and Cox regression models. We found a median BDdur of 23.8h. BDdur had a small positive effect on the chance of immediate function (odds for DGF 0.995) and 1- and 3-year graft survival (hazard ratios 0.995 and 0.996), but not on acute rejection. These effects were not independent in a multivariate analysis: donor age and acute rejection were confounding factors. Yet, in a multivariate subgroup analysis of donors aged ≤ 55 years BDdur was an independent predictor of imme-

diate function. With each extra hour of BDdur the risk of DGF decreased with 0.4%. This analysis showed that a longer BDdur seems to be slightly beneficial in DBD donors ≤ 55 years, reducing the chance of DGF in the recipient. We concluded that long BDdur is not detrimental for kidney graft quality, as long as donor management is sufficient to adequately support the circulation of the donor. This fact may have major impact on the logistics of organ retrieval procedures in Western Europe as it implies that there is no need to hurry these procedures.

Although longer duration of brain death does not have a detrimental effect on organ quality after transplantation, it is still difficult to predict organ quality at the moment of donor evaluation and management. An early prediction of the chance of transplantation success by specific markers would not only allow organ allocation in a fair and succesfull way, e.g. by programmes like the 'old-for-old programme', but also a better evaluation of interventional strategies in the donor or during preservation, aiming at improved graft quality. In **Chapter 6** we have tested several interesting novel urine markers to discover kidney specific injury. We evaluated urinary levels of lactate dehydrogenase (LDH), kidney injury molecule-1 (KIM-1), heart-type fatty acid binding proteins (H-FABP), alanine aminopeptidase (AAP), malondialdehyde (MDA), N-acetyl-β-D-glucosaminidase (NAG) and interleukin-18 (IL-18). These markers were compared to urinary creatinine and urinary protein as classic parameters of kidney function. Two different populations were studied: urine samples were collected from 40 kidney donors during donation, and another set from 29 transplant recipients during the first 10 days after kidney transplantation. Biomarker levels were related to delayed graft function, acute rejection and kidney function up to one year after transplantation using regression analyses. We found that most biomarkers were elevated in urine samples from deceased donor kidneys compared to living donor kidney controls. In the urine samples collected during donation, AAP predicted short-term kidney function, and H-FABP and KIM-1 predicted 3-month's function independently. LDH in donor urine predicted delayed graft function (DGF), defined as the need for dialysis in the first week after transplantation. In the urine samples collected during the first days after transplantation, urinary creatinine, LDH and NAG predicted kidney function at 3 months and one year after transplantation, although sample collection was not complete due to lack of urine production in several recipients with DGF. Our analysis suggests that determination of urinary biomarkers at time of donation and kidney transplantation can be useful for an early evaluation of the kidney graft.

Kidney Injury Molecule-1 was studied in more detail in **Chapter 7**. We were especially interested in KIM-1 as a urinary biomarker because of its apparent high sensitivity for kidney injury and early upregulation during kidney injury. Also, KIM-1 gene expression had been shown to be upregulated during brain death in our microarray study. Therefore, we studied Kim-1 expression in a standardised rat brain death model, where brain death is induced by intracranial balloon inflation. Sham operated animals were compared to animals that had

been brain dead for 0.5, 1, 2 or 4h. In the kidney, real-time PCR revealed a 46-fold Kim-1 gene upregulation after 4h of brain death. In situ hybridisation showed proximal tubular Kim-1 localization, which was confirmed by immunohistochemistry. Also, Luminex assay showed a 6.6-fold Kim-1 rise in urine after 4h of brain death. Data were confirmed in human DBD donors, where a 2.5-fold KIM-1 gene upregulation was seen in kidney biopsy specimens and two-fold higher urine KIM-1 levels were found compared to living kidney donors. Multiple regression analysis showed that urinary KIM-1 after diagnosis of brain death was a positive predictor of recipient serum creatinine on day 14 day and 1 year after kidney transplantation. This suggests that KIM-1 is a promising novel and non-invasive marker for early brain death-induced kidney damage.

The previous chapters of this thesis are merely descriptive, characterizing the effects of brain death. Obviously, the goal is to intervene during brain death and aim at a better balance between injury and repair, hopefully resulting in better graft survival and transplantation. In **Chapter 8**, the administration of (carbamylated) erythropoietin in brain death in a rat model is studied. In this experiment we used our standardised brain death animal model and after retrieval we evaluated kidney function with the Isolated Perfused Kidney (IPK) model. Animals were treated with EPO, carbamylated EPO (cEPO, which is a modified molecule without the erythropoietic properties of EPO), or a vehicle, and compared with sham operated animals. Both substances, but especially cEPO, were able to decrease the expression of several proinflammatory genes and to reduce infiltration of PMNs in the kidney. No effect on tubular injury parameters was seen. During organ evaluation in the IPK setup, kidney function decreased by approximately 50% after brain death, but was fully restored in the groups treated with both cEPO and EPO. These results are promising, and further studies are necessary to find out whether this inhibition of the inflammatory response and restoration of kidney function will lead to better results in clinical kidney transplantation.

- Future perspectives -

The research questions described in this thesis have been performed at an interface of laboratory science and clinical practice. As the Groningen Junior Scientific Masterclass motto states: from bed to bench and back. In the future, this starting point will become increasingly important, as our aim is to improve clinical outcome in transplantation.

Improving early organ evaluation and monitoring interventions
This thesis describes the evaluation of urinary biomarkers for an early detection of kidney injury in organs from DBD donors. The studies presented are pilot studies, and should be extended. Also, several studies have suggested recently that more than one marker may be necessary to obtain sufficient sensitivity and specificity for the early detection of acute kidney injury. This may lead to the creation of 'biomarker panels' for more accurate evaluation. Organ specific injury markers may not only be valuable for a better evaluation of organ quality. They may also serve as a means to evaluate the therapeutic effect of interventions. In this respect, the development of easy-to-use bedside tests, such as the new KIM-1 dipstick test that was described recently could be very helpful for monitoring the effect of donor treatment. In contrast to serum markers, urinary markers may not only be used in the donor during brain death, but also during the period of machine preservation of a kidney when 'urine' production during perfusion may be collected from the ureter and analyzed as a reflection of organ viability.

Improvements in intervention and study of its therapeutic effects
In this thesis, one intervention study in brain death is described. The results are promising, but we need to know whether cEPO administration does have an affect on outcome after transplantation. Our animal models are demanding and technically complex, but we will have to improve our models and our skills, to be able to test our interventions in the donor by subsequent evaluation in the (allogeneic) recipient.
When thinking about new ways of intervention in brain death, there are several possibilities. As positive data are reported concerning corticosteroid administration in the donor, more specific stimulation or inhibition of the immune system may be effective, e.g. using tacrolimus, complement inhibitors or interleukin ligands. Another approach may be a focus on (high fat) enteral feeding, increasing vascular integrity, and decreasing intestinal permeability and endotoxemia. The natural ability of kidneys in DBD donors to protect themselves by expressing heat shock proteins could also be a starting point for therapy, e.g. by geranylgeranylacetone administration.

One mechanism which may contribute to the inflammatory response during brain death was recently described in the thesis of Simone Hoeger, which she called the cholinergic anti-inflammatory pathway. Normally, afferent signals carried in the vagus nerve can activate an efferent response that inhibits cytokine release. In Hoeger's hypothesis, this so-called inflammatory reflex is disrupted by the brain damage of the DBD donor, which leads to cytokine production without central inhibition. Vagus stimulation in the donor by nicotine administration may be a way to counteract this problem.