Donation of kidneys after brain death

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Summary, general discussion, and future perspectives

SUMMARY AND GENERAL DISCUSSION

Since the discovery and development of the artificial kidney by Willem Kolff (1) it is possible to treat patients with end-stage chronic kidney disease. However, dialysis is associated with an high rate of morbidity and mortality (2), therefore the preferred treatment for end-stage chronic kidney disease is kidney transplantation for those in good enough health (3). Worldwide, the majority of donated kidneys are retrieved from deceased donors. Unfortunately organs from these donors are associated with inflammation (4), reactive oxygen species induced injury (5,6), and mitochondrial dysfunction (7,8), eventually resulting in inferior transplant outcomes when compared to kidneys derived from living donors (9,10). To decrease the mortality for patients with end-stage renal disease and improve the outcomes of kidney transplantation the number of available donor organs has to be increased and the quality of those donor organs has to be improved. Because there is a severe shortage of donor organs, the transplant community turns to organs from donors who were previously considered not suitable for transplantation. These extended criteria donor organs are associated with inferior transplant outcomes and therefore a high percentage of these organs are not used (11). By understanding the mechanism that affects the organ quality a better and more objective decision could be made for what donor organs should be considered for transplantation, hopefully decreasing the unnecessary discard of potential grafts. A better insight what happens to the graft quality in the harmful environment of the deceased donor could lead to new treatment strategies to improve the graft survival of these suboptimal quality organs. One strategy to improve the graft quality is by increasing the expression of protective proteins prior to, or early during, the infliction of transplant-related injury.

Chapter 2 reviews the protective properties of heat shock proteins in organ transplantation. The working mechanism from the family of heat shock proteins (HSPs) is explained on the intracellular level and their potential immune activating properties on the extracellular level. The detrimental effects occurring during organ donation and transplantation bring for opportunities to treat the donor. In the last part of this chapter we present the evidence for organ protection after heat shock protein upregulation. The evidence for protection against ischaemia reperfusion injury after treating the animals with HSP-inducing compounds is plenty, however there are no studies published about treating the deceased donor with HSF-1 inducible heat shock proteins. Protective effects have been found with transplantation of brain dead donor kidneys after HO-1 upregulation (12,13). Recently the results of a phase-II trials have been published on recipient treatment with the HO-1 inducing compound Hemin (14). Administration of this compound at the early onset of brain death could be a promising strategy to improve the graft quality and transplant outcomes.

In **Chapter 3** we assessed the effect of brain death-related stress on the renal expression of heat shock proteins in an animal model. Here we show that after four hours the expression of stress proteins HO-1 and HSP-A1A are predominantly upregulated. In this chapter we show that the amount of HSPA1A upregulation is also associated with those of other heat shock proteins regulated by the same protein, HSF-1. Transplantation of kidneys procured from deceased donors have inferior outcomes compared to those from living donors, thus we believe this protective and recuperative response is not sufficient.

In **Chapter 4** pre-treatment with the heat shock protein boosting compound geranygeranylacetone was used to improve the renal graft quality after deceased brain dead organ donation. Brain death-related injury does not induce histological changes within the four-hour period of this model, therefore the effect of treatment was objectified by assessing pro-inflammatory changes. Geranylgeranylacetone treatment decreased the expression of one of the most potent cytokines, interleukin-6. However, no effect of geranylgeranylacetone treatment was seen on the renal expression of heat shock proteins.

In **Chapter 5** the brain dead donor was treated with a more potent heat shock protein boosting compound derived from geranylgeranylacetone. Pre-treatment of rats with Nyk9354 induced renal expression of heat shock protein HSPA1A and inhibited the expression of interleukin-6, adhesion molecules (E-selectin and ICAM-1), and the influx of leukocytes. However, in this study we only assessed surrogate markers for the quality of the kidney since no transplantation was performed to determine the effect of heat shock protein upregulation on the long-term kidney function. As reviewed in chapter 2 the induction of HSPA1A is protective for ischaemia and reperfusion injury, even if geranylgeranylacetone is administered after the initial ischaemic insult (15). In our view treatment with Nyk9354 is a promising approach to prevent pro-inflammatory changes in the donor and improve the renal graft quality. To implement a compound like Nyk9354 into the clinical practice the first logical study would be to test this in a deceased brain dead transplant model.

In **Chapter 6** we review the literature in a systematic fashion for the evidence of donor treatment on short- and longer term graft outcomes in transplantation for organs procured from deceased donors. We show that the amount of studies published on donor treatment is limited and the evidence is in general fairly poor. From this systematic review we could conclude that there is no strong evidence of a protective effect from a single strategy on the long-term graft- or patient survival after transplantation of deceased donor derived organs. In this review we only focussed on treatment of the donor, however, with the rise of the machines for organ preservation there is also a possibility to treat the graft. This treatment possibility was not reviewed but it could be a window of opportunity to improve the quality of the graft.

In **Chapter 7** we induced 45 minutes of ischaemia to the kidney and evaluated the effects of reperfusion injury after four and 24 hours. Mass spectrometry was used to evaluate the effects of this injury mechanism on the protein and metabolite level. We show that ischaemia reperfusion injury impairs mitochondria and the cell energy metabolism, forcing the kidney cells to switch their energy source to consuming more lipids. However, lipids can be toxic to the cell (16,17) and might therefore not be the optimal energy source during transplantation.

Although only two time point were investigated, the changes observed are in accordance with other studies that also show enhanced lipid utilisation after ischaemia (18). As is reviewed in the previous chapter, there is currently no strong evidence that a single treatment strategy of the donor will benefit the organ and its functions after transplantation. Therefore, it is necessary to gain a better insight what is occurring in the deceased donor and during ischaemia reperfusion that affects the graft. These insights might lead to new treatment options. What

has become apparent from this chapter and the mass spectrometry work done in a deceased brain dead model (7) is that mitochondrial dysfunction occurs in the donor organ. Treating this cell organelle could enhance the energy metabolism and the cellular functions of the graft.

In **Chapter 8** we used mass spectrometry to assess what changes occur in the kidney during brain death. To investigate this effect, kidney biopsies from human kidney donors were grouped according to the outcome after kidney transplantation. Here we show that kidneys with a good transplant outcome have higher levels of anti-oxidants, while kidneys with a poor transplant outcome show higher expression levels of pro-inflammatory proteins. In this study we show that the changes that occur during brain death are associated with the graft function after transplantation. To implement a diagnostic tool for a molecular based donor risk index these results first have to be further validated. The cohort size for this study is limited and ideally these results should be confirmed in a very large cohort where donors were not preselected for transplant outcomes, something that is now possible with the national collection of kidney biopsies in the UK QUOD biobank. Furthermore, these results show the relevance of anti-oxidants in kidney transplantation, a pre-treatment approach that has shown to be protective in the donor and recipient (19).

Determination of the molecular profile of the donor kidney is associated with the graft function after transplantation, but it would be more elegant if blood could be used as a tool for graft quality assessment. Blood can be retrieved from the donor at multiple and earlier time points, and the organ does not become injured to obtain a biopsy. In **Chapter 9** we used mass spectrometry to assess the effect of long-term blood storage at room temperature on the sample quality for proteomic analysis. Here we show that storage of blood at room temperature for two days still produces good quality proteomics data for discovery study purposes. Therefore, we believe that it is feasible to screen blood of deceased organ donors for biomarkers, even though candidate samples have been stored at room temperature for variable times before long-term storage. After an initial discovery approach with mass spectrometry, validation usually occurs with enzyme-linked immunosorbent assays (ELISA). We did not validate if longer storage at room temperature affects those assays. Although we observed only limited enzymatic cleavage and protein degradation, validation techniques should be validated first before use on samples with variable storage times.

FUTURE PERSPECTIVES

The results of this thesis give further direction for new research projects to improve the outcome of deceased donor kidney transplantation. The potential of distinguishing between good and suboptimal donors could aid the physician with the decision of accepting or declining a kidney for transplantation. There is a high need for biomarkers or surrogate markers of graft quality and thus far there has not been a single biomarker that could help to predict graft quality. A panel of biomarkers indicating what protective and detrimental pathways are activated would be in our view the most feasible approach to develop a molecular donor

risk index score. Discovered biomarkers should be tested in a large and multicentre cohort, a possibility that has now been established by the QUOD biobank in the UK. Instead of using kidney biopsies it would be elegant if blood could be used for biomarker discovery, as this is an easy to obtain sample that could be analysed before the start of organ procurement.

The two protective agents, geranylgeranylacetone and Nyk9354 have been tested as a treatment of the donor. We conclude that there is no role for geranylgeranylacetone as treatment of the donor, however Nyk9354 enhanced the expression of the cytoprotective protein HSPA1A and inhibited to some extent the inflammation in the donor kidney. Upregulation of HSPA1A should be further explored before commencing a human trial. Apart from donor treatment, the graft can also be treated ex-situ during hypothermic or even normothermic machine perfusion. Administration of cytoprotective agents like anti-oxidants or heat shock protein boosting compounds could be a potential treatment strategy to protect the graft quality during the process of organ preservation and transplantation.

CONCLUSION

Heat shock proteins play an important role in kidney transplantation. Deceased organ donation is associated with upregulation of the heat shock proteins HSPA1A and HO-1. Enhancing the expression of HSPA1A improves the graft quality of the procured deceased brain dead donor kidney. Other associations for a good functioning graft are low levels of pro-inflammatory proteins and high levels of anti-oxidants. In randomised clinical trials the effect of innate immune system inhibition and anti-oxidant administration should be explored to improve allograft outcome after kidney transplantation.

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