The impact of cerebral injury in donation and transplantation

Koudstaal, Lyan Giela

Chapter 7 **Summary and Perspectives**

Lyan Koudstaal

Summary

Organ transplantation is a life saving therapy for patients with end stage organ failure. In general, patients who received a solid organ transplant live longer and have a better quality of life compared to those on organ replacement therapies. Nowadays, due to improved organ preservation methods, better surgical techniques, new immunosuppressant drugs and regimens, severe rejection is less frequent. Because of these major achievements, the number of patients eligible for transplantation has increased. Despite the inclusion of patients with more co-morbidities, over the last years transplant survival has stabilised. The worldwide increasing demand for organs forced a gradual shift towards accepting suboptimal donor organs from for instance older brain dead donors and even donation after cardiac death donors. The aim of this thesis was to study the role of the intestine in brain death induced injury. Both experimental and clinical studies were focussed on inflammation in relationship with intestinal permeability.

In **chapter 1**, the background leading to the hypothesis that intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial translocation, provoking cytokine release, is explained. This chapter concludes with the aims of this thesis. The effects of brain death on the donor intestine in an experimental rat model were studied in **chapter 2**. Brain death was induced by inflation of a subdural placed balloon catheter. We observed that intestinal inflammation and apoptosis occurred early after brain death induction, characterized by an increased polymorphonuclear cell influx in the intestine, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-Selectin, and interleukin-6. Caspase-3 positive cells were found in jejunum and ileum in the brain dead rats. These events may ultimately have a negative influence on the outcome of intestinal transplantation. In **chapter 3**, we hypothesised that increased intestinal permeability occurs during brain death. Therefore changes in intestinal barrier function were investigated, in a similar animal model as described in **chapter 2**. In the serum of brain dead rats we found higher lipopolysaccharide and lipopolysaccharide binding protein levels, indicative of endotoxemia. The lipopolysaccharide binding protein (LBP) mRNA expression was significantly increased in liver and intestine in the brain dead rats. The results support the hypothesis that brain death induced intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial translocation, provoking cytokine release. This vicious circle may contribute to the inflammatory reaction in potential donor organs which results in distant organ failure and inferior transplant outcome.

The first clinical study of this thesis (**chapter 4**) describes whether the inflammatory response present in deceased brain dead donors could be explained by the leakage of pro-inflammatory proteins from the injured brain into the circulation. In deceased brain dead donors, glial fibrillary acidic protein (GFAP) as a marker of blood-brain barrier dysfunction, and interleukin-6 as a key pro-inflammatory cytokine were measured, at the beginning and the end of the brain death period. Our results show increased levels of GFAP in DBD donors at the declaration of

brain death compared to living donors. During the brain death period, GFAP levels were markedly elevated in the majority of deceased brain dead donors, indicating a distinct dysfunction of the blood-brain barrier. In **chapter 5**, we studied serum vascular endothelial growth factor (VEGF), angiopoietin-1 and angiopoietin-2 in deceased brain dead donors using living kidney donors as controls. We postulated that the anti-inflammatory angiopoietin-1 and the pro-inflammatory angiopoietin-2 is modulated progressively towards inflammation during the period of brain death prior to organ recovery. Deceased brain dead donors had higher median serum LBP, VEGF and angiopoietin-2 levels compared to living donors. Higher angiopoietin-1 levels were observed just after brain death diagnosis. Importantly, serum angiopoietin-2 levels in the deceased brain dead donor predicted the chance on rejection in the first year after kidney transplantation. We studied the potential of angiopoietin-2 in donor serum as a biomarker for transplant outcome (**chapter 6)**. Donor-derived biomarkers that have predictive value for post transplant outcome are useful to prevent unnecessary discard of donor organs and to fine-tune post-operative treatment of the recipient. We show that angiopoietin-2 measured in donor serum prior to donation is an independent predictor of kidney graft survival. From 297 deceased kidney donors included in an international prospective randomized controlled trial, serum was analyzed for angiopoietin-1 and angiopoietin-2. Using multivariate models, we tested whether donor serum angiopoietins were independently associated with delayed graft function, primary non-function and graft survival. Serum angiopoietin-2 concentration was significantly associated with graft survival: higher values in donor serum were predictive of a lower risk of graft failure.

Perspectives

The first question in this thesis was if brain death itself had an effect on the intestine, similar to the kidney, the liver and the lung. In **chapter 2**, we described inflammation and apoptosis in an experimental brain death model in the rat. In **chapter 3**, we extended these findings and described an enhanced permeability in brain dead rats.

The exact pathogenesis of intestinal damage however remains to be established. Future research should therefore focus on the role of the intestine in systemic inflammation. Because of the unique structure of the intestine, and its importance in the lymphatic system, experimental studies could focus on the role of mesenteric lymph in the cascade of brain death induces injury. With cannulation of the mesenteric lymph vessel, the cytotoxity of lymph from brain dead rats could be assessed. At the same time, this experimental model gives insight in the contribution of mesenteric lymph flow in the inflammatory cascade to distant organs, such as heart, lung, liver and kidney. For example in burn induced lung injury, mesenteric lymph flow does significantly contribute to inflammation in the lung. If obstruction of the mesenteric lymph flow does prevent the inflammatory cascade to the distant organs, this could be implemented in the clinical situation. Therapies, such as adequate enteral feeding, or medication such as 2-Mercaptopropionylglycine which enhance the intestinal integrity could be applied.

Also substances, such as LBP, which capture hazardous lipopolysaccharide could have a beneficial effect on the pro-inflammatory state in de deceased donor and subsequent graft quality.

The second important observation in this thesis is that the anti-inflammatory protein angiopoietin-1 and the pro-inflammatory angiopoietin-2 are modulated progressively towards inflammation during the period of brain death prior to organ recovery (**chapter 5**). Moreover, donor serum angiopoietin-2 has the potential to independently predict transplant outcome in the recipient (**chapter 6**). To confirm the clinical validity of serum angiopoietin-2, these measurements should be repeated in another cohort of donors. Also the potential of angiopoietin-1 and angiopoietin-2 to predict outcome in other transplants such as the liver, lung, heart, pancreas and intestine should be investigated. Before implementing angiopoietin-2 in the clinical setting, a fast, easy, and validated test must be developed. Furthermore, also after transplantation, angiopoietins in the recipient could have an additive effect on outcome. Also intervening in the angiopoietin-1 vs. angiopoietin-2 balance could therefore be a target to improve organ quality, both prior and after donation.