CHAPTER

General Discussion & Future Perspectives
INTRODUCTION

Organs obtained from brain-dead donors show lower rates of graft survival compared to organs obtained from living donors. This may be related to the fact that brain death (BD) itself leads to a cascade of detrimental systemic events such as hemodynamic instability, inflammation, and altered metabolism which pose a significant threat to the structure and function of the future grafts. Many interventional strategies have been tested in brain-dead donors with the goal of improving transplantation outcomes. However, only a few have shown beneficial clinical effects of which the most notable involve the administration of methylprednisolone and dopamine. Clinically, the administration of methylprednisolone has shown varying effects and no beneficial effects on renal function. Therefore, dopamine is the only interventional strategy in brain-dead donors which has shown consistent beneficial effects, including on renal function. To this date, no intervention has been incorporated into standard policy in the management of brain-dead donors.

BD pathophysiology is complex which probably hinders exact determination of all detrimental pathways. In this thesis, we looked for mechanistic clues to explain the pathophysiology of BD. Our primary aim was to identify the detrimental processes taking place in the future donor grafts. Based on these findings we designed novel therapeutic interventions to attenuate or prevent graft deterioration.

OBSERVATIONS

In Chapter 2, we therefore assessed the effect of the speed at which intracranial pressure (ICP) increases in the process leading to BD on functional, damage, and inflammatory markers of kidney and liver in laboratory animals. We showed that a slower increase in ICP leads to detrimental BD effects on the kidney and liver. The effects of BD on donor organs are well documented, however, less is known about the effects of the processes leading to BD. In human conditions, cerebrovascular and traumatic causes of brain death are risk factors for renal and cardiac graft survival, respectively. Besides donor characteristics, the difference in survival could be related to the nature of the brain insult as the speed of ICP increase after traumatic and cerebrovascular causes of brain death can vary greatly. Traumatic brain insults tend to cause faster rises in ICP than cerebrovascular causes. However, the speed at which ICP increases may even vary when the nature of the brain insult is the same. Therefore, assessing the effects of the speed at which ICP increases on organs is warranted. We acknowledge that the underlying pathophysiology of increased ICP is different for traumatic and cerebrovascular causes and that different experimental models can be used to study these processes. For example, cerebral infarctions will lead to increased ICP trough cellular swelling whereas a traumatic cause typically leads to increased ICP through the rupture of a blood vessel. Nevertheless, the central hallmark of these processes is increased ICP and we used this common endpoint to study the effect of speed of ICP increase within the confines of a single model. In our model, increased ICP was manifested by in

Chapter 4, we expanded on the findings described in Chapter 2, in which we showed a higher increase in renal MDA levels after slow compared to fast BD induction. In this Chapter, we examined possible underlying renal oxidative and anti-oxidative processes which could explain the increased MDA levels. Of these processes, the high increase in superoxide levels was the most notable and indeed, these levels were affected more by slow BD induction. Superoxide is increased early during BD and could form an initiating factor in the process leading to increased MDA levels. Therefore, preventing superoxide formation in brain-dead donors could lead to better transplantation outcomes since donor-related MDA levels correlate with DGF, acute rejection and short and long-term allograft survival in renal transplant recipients. The increased renal superoxide levels are
not caused by ischemic phases through major hemodynamic changes as rats are kept hemodynamically stable during the BD period. Therefore, we postulate that superoxide scavenger therapy is key in decreasing renal superoxide levels in addition to the maintenance of hemodynamic stability. Our results indicate that anti-oxidative treatment of brain-dead donors is probably especially valuable in donors progressing to BD slowly.

The idea mentioned above, to counteract superoxide levels through anti-oxidative therapy besides the maintenance of hemodynamic stability was reinforced by the findings done in Chapter 5. In this Chapter, we showed that increased renal oxidative stress formation during BD is accompanied by decreased regional renal blood flow despite the maintenance of normotension. Hepatic perfusion is not affected and therefore ischemia probably only plays a detrimental role for the liver at the onset of BD through the effects of the catecholamine storm. Hepatic oxidative stress is not increased during BD as MDA levels remain comparable to sham levels. However, we did observe increases in hepatic HO-1 expression indicating a hepatic anti-oxidative response. We believe this response could be caused by the decreased activity of complex I which was observed in Chapter 5 which could cause the leakage of superoxide anions. Since no increase in MDA levels were observed, the hepatic anti-oxidative response is probably sufficient to protect the liver from oxidative stress. Therefore, anti-oxidative therapy in brain-dead donors seems beneficial for the kidney and not the liver. Since modifying regional perfusion could be difficult to accomplish clinically, we postulate that anti-oxidative therapy is key in preventing renal oxidative damage in addition to the maintenance of hemodynamic stability. Preventing increased MDA levels in brain-dead donors could result in decreased I-R injury as MDA levels correlate with DGF.

**INTERVENTIONS**

In Chapter 6 we administered MnTMPyP, a superoxide dismutase mimetic, to brain-dead rats with the goal of decreasing MDA levels. BD-related MDA levels correlate with DGF in renal transplant recipients and therefore reducing these levels could result in better transplantation outcomes. MnTMPyP treatment resulted in decreased MDA levels which shows that superoxide plays a key role in BD-related oxidative processes leading to increased MDA levels. MnTMPyP treatment did not lead to increased renal function in the brain-dead donor rat. It has been shown that BD predisposes livers to worse I-R injury. Similarly, increased lipid peroxidation, measured by MDA levels, could predispose renal cells to worse I-R injury. Lipid peroxidation leads to membrane dysfunction and could render the cells more vulnerable to the influx of oxygen radicals. Therefore, in Chapter 7, we subjected kidneys of brain-dead rats treated with MnTMPyP to reperfusion injury to assess beneficial effects on organ function. To simulate I-R injury, we used an isolated perfused kidney (IPK). We found that MnTMPyP treatment of brain-dead rats led to increased renal function in the brain-dead donor which leads to decreased I-R injury. However, the beneficial effects could also be attributed to the prevention of superoxide radicals formed during reperfusion in the IPK. We cannot ascertain that MnTMPyP was still active in the renal cells at the time. The reperfusion injury mimicked in our IPK system shares common features with clinical I-R injury as the kidneys suffered a period of ischemia and were subsequently exposed to the introduction of oxygen in the perfusion fluid. However, our model does not incorporate other processes such as leukocyte infiltration. This was not incorporated into the model as we wanted to solely assess the effects of MnTMPyP on renal function in an isolated system without other possible altering factors. Considering the promising effects of MnTMPyP on renal function and therefore possibly on DGF and graft survival, future research should be conducted on testing this compound in a transplantation model.

**FUTURE PERSPECTIVES**

The experimental data in this thesis pave the way for similar experiments in human organs. The observational and interventional studies we performed can be conducted in human brain-dead tissue and donors. The observational studies can be undertaken in human brain-dead donor tissue to assess whether oxidative stress markers are upregulated and whether there are differences between donor types. Interventional studies can be undertaken in human brain-dead donors after they have first been tested in higher animal models.

Ethical concerns are raised when the question arises to treat brain-injured patients in which death is not yet evident. Brain-injured patients can suffer hypotension and hypertension which leads to ischemia and probably already predisposes to worse I-R injury. Also, we have seen that organ damage is probably already imminent during the onset of BD through for example the catecholamine storm. To combat these effects, administration of protective compounds should ideally be administered to brain-injured patients when BD is not yet evident. Many of these interventions have beneficial effects for the patient, regardless of the effect on possible future donor grafts. For example, hypotensive phases in brain-injured patients could lead to AKI which could be attenuated with the prophylactic administration of anti-oxidants. Perhaps, in the future, with the combined effects of altered legislation regarding the treatment of brain-injured patients and the advent of compounds with no harmful side effects, improved transplantation results could be realized.

Its apparent from this thesis that organs are differentially affected by the effects of BD. Future donor management strategies could encompass different interventions with the goal of preserving numerous organs within one donor. This would require one intervention to not affect the other which seems plausible considering that the optimal environment for an organ is one which resembles normal homeostasis. This poses the ICU doctor the challenge of undertaking numerous interventions such as the maintenance of normotension, preventing inflammation, and the administration of compounds which target local processes that take place independently of hemodynamic and inflammatory changes.
REFERENCES