Brain dead donor graft deterioration and attenuation with N-octanoyl dopamine preconditioning
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Transplantation

In the second half of the last century the first successful kidney transplantation was performed [1]. It took more than two decades before the first successful lung transplantation followed, only enabled by the introduction of adequate immunosuppression [2]. With the introduction of immunosuppression and advances in the operation technique first year graft survival tremendously improved by preventing acute rejection. Thereafter transplantation quickly became the treatment of choice for patients with chronic diseases [3, 4]. Unfortunately, the success in short term survival was expected to also influence the long-term graft survival, but the effect was little to none, as illustrated in figure 1 for kidney graft survival [5-7].

![Figure 1. Long term graft survival has not improved after the introduction of immunosuppression.](image)

Superior graft survival over a decade are the result of an improved early 6 month's outcome after transplantation. After this period no differences are found in the trend of long-term graft survival, represented by the parallel progression of the survival curves in kidney transplantation [7].

Important for the understanding of the unchanged long-term graft survival in organ transplantation was the finding that even though kidneys procured from brain dead donors were better cross-matched, the outcome was inferior compared to transplantations using organs from living donors [8]. The main difference between these donors is cerebral insult leading to brain death with subsequent organ deterioration, and furthermore extended cold ischemia time [8-11]. However, also brain death and transplantation associated factors such as the need for ventilation [12, 13], inadequate hemodynamic stabilization [14] or hormonal resuscitation [15] contribute to the inflammatory response and subsequent reduction of organ function. The decrease in organ function, after the onset of brain death and cold ischemia/
Introduction

reperfusion, have been associated with an increased risk for primary graft dysfunction (PGD) in the recipient [16-18]. PGD is yet another independent risk factor for chronic rejection and poor survival [19]. Therefore lung donors, with substantially impaired lung function, determined by the ratio of partial arterial oxygen pressure and fraction of inspired oxygen (PaO$_2$/FiO$_2$) < 300 mmHg, are not considered eligible for lung transplantation [20]. Also, in case of other transplantable organs, organ function prior to donation is a reason to refuse these organs. For example kidneys from donors with increased creatinine levels are declined for kidney transplantation [21]. Interestingly, the risk for PGD between organs of one multiorgan donor strongly correlates [17]. This suggests that strict donor management protocols are beneficial for all grafts if implemented as early as possible.

Brain injury and initiation of graft deterioration

With the rare exception of living lung lobe donors, lung grafts are almost exclusively derived from donors with some sort of brain injury. The majority are donors sustaining brain death. But even in case of donors after circulatory death (DCD; Table 1), brain injury is present. For instance, in DCD category 3, ventilation is switched off in patients with infaust neurologic prognosis. Also in DCD category 2 where resuscitation failed, the brain suffers from ischemia [22].

<table>
<thead>
<tr>
<th>Categories</th>
<th>Description</th>
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<tr>
<td>Category 1</td>
<td>Dead on arrival</td>
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<tr>
<td>Category 2</td>
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<td>Controlled</td>
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Table 1. Different donor categories after circulatory death, adapted from Detry et al. [22]

Brain injury causes the release of cytokines and pro-inflammatory subcellular fragments into the blood stream [23, 24]. This initiates a systemic immune response with increased neutrophil activation and migration into the parenchyma [25]. Neutrophil granulocyte accumulation, their release of cytokines and oxidative burst contribute to parenchyma injury and development of acute respiratory distress syndrome (ARDS) [26]. ARDS is found in 15-20% of the brain injured patients [27]. Interestingly, the rate of acute kidney failure is comparable to the occurrence of ARDS in brain injury [28-30], suggesting a common denominator or cross-talk between the two organ systems.
Additionally, many brain injured potential organ donors develop neurogenic pulmonary edema (NPE) [31-33], enhanced by inflammation [34] and loss of epithelial integrity [35]. At the occurrence of NPE high ventilation pressures are needed to ventilate the lung and ensure oxygenation of the other organs. The high pressure ventilation results in enhanced lung injury [36], with a further increase in cytokines. Enhanced local cytokine release may cause a spill over into the circulatory system affecting distant organs [37], in particular the kidney [38].

**Brain death and amplification of graft injury**

Extensive brain injury may lead to brain death. As a result of venous engorgement and brain swelling the intracranial pressure increases to values above systemic pressure. Subsequently, the brain stem is forced towards the skull opening, the foramen magnum. This causes as a consequence of arterial compression tissue ischemia and infarction [39]. The injury leads to brain swelling and increase of intracranial pressure until intracranial circulation ceases [40]. The culmination of progressive rostral-to-caudal ischemia leads to brain death [41]. As a consequence of brain death four substantial pathophysiological changes are initiated i.e. (1) hemodynamic, (2) endocrinologic, (3) metabolic and (4) immunologic.

(1) The rostral-to-caudal ischemia at onset of brain death [41] is characterized by a typical sequence of hemodynamic changes, though they may vary between the individual donors and depend on the cause of brain death [42].

At first the pons becomes ischemic, causing the ‘Cushing response’, characterized as bradycardia and hypertension. This is followed by massive hypertension and tachycardia, as a result of ‘catecholamine storm’ upon ischemia of the medulla oblongata [40]. During this ‘catecholamine storm’ a massive increase of catecholamines in the blood occurs and is accompanied by a significant increase in systemic vascular resistance (SVR) [39, 43-45]. During this hyperdynamic phase arrhythmias occur [42], with increase in cardiac enzymes and ventricular dysfunction [46-49] influencing the post-transplantation phase after cardiac transplantation [42, 47].

The sudden increase in systemic vascular resistance with the short sudden hypertensive period is associated with rupture of the capillary-alveolar membrane [50]. As a result of cessation of the aortic blood flow and slight increase of the central venous pressure (CVP), a large proportion of the total blood volume is pooled in the cardio pulmonary vasculature [51]. The increased hydrostatic pressure causes pulmonary edema [52] and explains commonly found focal areas of erythrocytes in the pulmonary parenchyma [51].
Finally, total sympathetic denervation occurs [40, 53], resulting in hypotension and cardiac dysfunction [42, 51]. This autonomic dysregulation causes insufficient perfusion and oxygen delivery to the peripheral organs [54-56]. Supportive fluid loading and catecholamines are applied to prevent ischemia and organ injury. However, these mainly kidney protective measures can particularly harm the lung [14, 57-60].

(2) + (3) Endocrinologic and metabolic changes are evident in brain death but an in-depth discussion is beyond the scope of this thesis. Briefly, after the onset of brain death circulating hormones - triiodothyronin, cortisol and insulin as well as antidiuretic hormone (ADH) are significantly reduced [39, 61-63]. This hypothalamic-pituitary dysfunction is the consequence of altered brain perfusion in individual donors after the onset of brain death, with different extent [64-68]. This endocrinologic dysregulation is associated with perfusion perturbation, mismatch of demand and oxygen supply ultimately accompanied by a shift from aerobic to anaerobic metabolism [55, 66, 69]. In the anaerobic state ATP is depleted. This depletion of ATP results in loss of barrier function, intensifying tissue injury [70].

Both, decreasing insulin levels and subsequently reduced cellular glucose uptake, as well as reduced metabolism arising from lack of thyroid hormones, eventuate in hypothermia [44, 71]. Additionally, the induced pro-inflammatory changes contribute to the dysbalance of thyroid hormones [72]. Evidence in other disorders of thyroid dysbalance suggest that the hormones are involved in regulation of systemic vascular resistance. Therefore, a fall of thyroid hormones could quickly lead to increased systemic vascular resistance, enhancing ischemia, and possibly intensifying brain death induced cardiac impairment [73] and ensuing endorgan injury.

(4) The inflammatory immune response initiated by the cerebral insult is exaggerated by the hemodynamic, endocrinologic and metabolic changes [74]. But even without previous cerebral injury, blood of brain dead animals administered to healthy animals induces an immune response, with deterioration of the peripheral organs [75]. This suggests that there is a release of potent, pro-inflammatory molecules into the blood upon brain death induced injury. The latest findings suggest damage associated molecular pattern (DAMP) molecules [24], or even more potent mitochondrial DAMPs (mtDAMPs) [76], released during sudden or extensive tissue injury [77], contributing to severe lung parenchyma injury [78]. Recently, it has been shown that upon stimulation with mtDAMPs the DAMP high-mobility group box-1 (HMGB1) is expressed [79]. In lung transplantation the systemic level of HMGB1 is one of the DAMPs associated with lung dysfunction before and after transplantation [80]. It is predictive for transplantation outcome [81]. Comparing living and deceased kidney
donor transplantation HMGB1 was only found in kidney parenchyma of deceased donors [82].

Related to the systemic release of mtDAMPs and HMGB1 neutrophil granulocytes are recruited. But also brain death induced shear stress and subsequent chemokines and adhesion molecules expression on the endothelial cells lead to migration of neutrophil granulocytes [83-85]. The extent of chemokine IL-8 release correlates with the number of migrated neutrophil granulocyte into the donor lung [86] predicting the impairment of graft function, development of early graft dysfunction and early mortality in the recipient after transplantation [86].

The combination of endothelial activation, subcellular fragment release and hemodynamic induced microcirculatory changes also exaggerate hemostasis [34, 77, 84, 87]. Hemostasis is initiated after the cerebral insult by release of pro-inflammatory cytokines and passage of blood through the injured cerebral tissue [88]. Clot formation occurs as a consequence of a dysbalance between an activated coagulation system and dysfunctional fibrinolysis in brain dead patients [89]. This may explain the relatively high incidences of pulmonary embolism (PE) in brain dead donors, with a respective risk of PGD for the recipient of the lung after transplantation [90]. The removal of these emboli reduces the risk for PGD in the lung recipient [91]. However many PE remain undetected in the donor [90].

All in all, the inflammatory response induced in the brain dead donor determines the success of transplantation [11, 92]. Thus, until alternative sources for organ transplants are found, it is of utmost importance to elucidate and target deleterious processes in brain death. Furthermore, lungs should carefully be selected for transplantation.

Cold ischemia and reperfusion

For allocation of lungs and other solid organ systems grafts are cold preserved at approximately 4 °C in order to reduce their metabolic rate and subsequently decrease their energy requirements [93]. Ironically, this protective measurement remains a significant, allogen-independent [9] cause of early morbidity and mortality in any solid organ transplantation [94], particularly after lung transplantation [18]. In approximately 20% of the lung recipients it leads to primary graft dysfunction [95], with a 30 day mortality of 50% [96]. The clinical and histological picture of primary graft dysfunction is comparable to ARDS and is associated with an increased risk of acute and chronic rejection [18, 97].
Cold ischemia leads to ATP depletion and reactive oxygen species (ROS) accumulation. The failure of the sodium-potassium ATPase with subsequent intracellular sodium accumulation leads to cell swelling and cytosolic calcium arises [98-105]. Along with the activation of mitochondrial transition pores and the release of free iron molecules cell death is induced [106-108]. The lack of oxygen forces cells to switch from aerobic to anaerobic metabolism, resulting in acidosis [100]. These changes are deleterious but may be limited using adequate preservation solutions and limiting cold ischemic time. In the lung this can be achieved using a low-potassium dextrane solution and inflating the lungs with oxygen which both guard and preserve cell integrity [18]. However ischemia induced injury may be exaggerated by reperfusion resulting in necroptosis, which has been associated with the release of damage associated molecular patterns (DAMPs) provoking a strong inflammatory immune response [109].

The first minutes after reperfusion are considered to be crucial for the extent of reperfusion injury [110, 111], partially as result of increased endothelial permeability [110, 112] and because of the previously induced changes. It led to the development of controlled reperfusion strategies. This concept of controlled reperfusion pressure resulted in substantial attenuation of lung injury, even clinically [113-115]. However controlled reperfusion is commonly combined with leukocyte depletion, because leukocytes are considered to be the key players in progression of lung reperfusion injury [116, 117]. Leukocytes are believed to be the major source of oxidative stress and origin of cellular injury [118]. This injury seems to consist of two different phases, an earlier macrophage transmitted injury and a later neutrophil granulocyte transmitted injury [119-121]. Passenger macrophages [121] and DAMPs [80, 122, 123] initiate the pro-inflammatory response to reperfusion [120]. In combination with the activation of the endothelium [124, 125], it leads to migration of host neutrophil granulocytes- the second phase of ischemia reperfusion injury [126]. However de Perrot et al. [127] have convincingly shown that, independent of neutrophil granulocytes, also the host T lymphocytes strongly contribute to the second phase of ischemia reperfusion injury.

The initial insult by brain death is considered to increase susceptibility to the second insult i.e. ischemia reperfusion injury [128], however both are inevitable in the current transplantation process. The limitation or prevention of inflammation should have priority in protocols for reperfusion since the immune cell activation leads to the release of pro-inflammatory molecules [129] and oxidative stress [18] with systemic impact [130]. Ventilation strategies might be part of such a protocol [96].
Ventilation: Pre- and post-transplantation phase

As mentioned before, both during brain death and ischemia reperfusion, acute lung injury arises [131, 132]. This injury is on a histological level characterized by infiltration of neutrophil granulocytes, protein rich intra-alveolar exudate and alveolar-capillary disruption [86, 132], resulting in the deterioration of lung function and increasing airway resistance [133].

Until the mid 80ies, in case of acute lung injury, lungs were ventilated with high tidal volumes or high pressure favoring better pH and reduced PaCO₂, considering a homogenously stiff lung. However, with the introduction of computer tomography in the clinic in the mid 80ies, it became obvious that acute respiratory distress syndrome effects lung parenchyma in-homogenously [134]. Around the same time it became evident that application of high pressure, independent of pre-existing injury, severely impairs lung parenchyma with subsequent loss of function [36]. The first study comparing high tidal volume (traditional approach) to low tidal volume (new approach) ventilation clearly showed that mortality, days on the ventilator and needed inspiratory pressure were significantly reduced under the new approach [135]. Importantly, with the shortage of eligible lung donors for transplantation the change from traditional ventilation to lung protective ventilation strategy resulted in a significantly higher rate of lung transplants suitable for transplantation [12].

In the short term, 6 month survival was not different between the ventilation strategies [12]. De Perrot et al. [13] showed that during the early phase of ischemia reperfusion injury conventional mechanical ventilation impairs the lung function and exacerbates the immune response compared to protective lung ventilation. This finding is particularly important since the majority of recipients requires mechanical ventilator support for the first few hours after transplantation. The pronounced immune reaction may impair other organs [38] and might explain increased morbidity and mortality in patients with primary graft dysfunction [129].

Preconditioning

At any time point in the transplantation process, graft quality determines the fate of transplantation. Takada et al. [75] were one of the first describing that graft quality is impaired in peripheral organs after onset of brain death. The pro-inflammatory immune response induced by brain death is considered to be the origin of the inferior outcome after transplantation compared to living organ donation [8]. For that reason the limitation of the inflammatory response and maintenance of organ
quality in brain dead donors has become the focus of intensive research. In particular, while before the typical donor was a healthy young donor with acute traumatic injury leading to brain death [136], today the typical donor is an old donor with spontaneous cerebral hemorrhage and preexisting comorbidities [137, 138] with an increased risk for primary graft dysfunction [139-141]. This is possibly the result of the pro-inflammatory nature of the comorbidities [142].

One important consideration for prevention of organ deterioration is to stabilize brain dead donors close to physiological values [136]. For that reason strict donor management protocols were implemented to stabilize donors adequately, which increased the number of organs suitable for transplantation tremendously [15]. Part of these protocols is sometimes hormonal resuscitation treatment, reversing the brain death induced endocrinologic changes. This resulted in superior hemodynamics and aerobic metabolism in the donor [69, 143, 144], and improved outcome in the recipient [145]. Conversely, there have also been reports that hormonal resuscitation is not effective, possibly as a result of higher dosage [146] and the dosage dependent effect of the hormones on mitochondria [147-149].

One successful treatment, particularly in lung transplantation, is the administration of high dose steroids before the procurement of the lung, commonly performed in the clinic [150]. Not only is the lung function under this treatment improved, but also more transplants become available by suppressing pro-inflammatory mediated cellular injury [150]. Although more preconditioning strategies are available, the most noted one is ischemic preconditioning [151], based on the work of Murry et al. [152]. He showed that repetitive short periods of ischemia reduce the extent of ischemia induced myocardial tissue injury [152].

Alternatively, another preconditioning strategy, is pharmacological preconditioning, f.i. with dopamine [153]. Dopamine is one of many catecholamines commonly used for cardiovascular support in hypotensive brain dead donors [71]. However it lost preference in the clinical routine after it failed to improve the outcome or mortality of patients with acute renal failure in the ICU [154, 155]. There were even implications for a negative systemic impact [156]. In contrast were the findings of Schnuelle et al. [153, 157, 158] that showed that brain dead donor dopamine preconditioning improved early kidney function and improved graft survival, particularly in grafts with extended cold ischemia times. In experimental studies, dopamine has an anti-inflammatory [159] but also cryoprotective effect [160]. The effect of dopamine may be either mediated by the induction of hemeoxygenase 1 [161, 162], its suggested influence on mitochondria by its redox activity [163, 164] or via the induction of H₂S production [165]. This may explain the clinical success of low dose dopamine preconditioning in kidney [166] and heart transplantation [167]. However, 12.5%
brain dead donors develop adverse side effects under treatment with dopamine. As a consequence the treatment needs to be discontinued and the potentially beneficial effect is lost [166, 167]. To circumvent this, a non-hemodynamic dopamine derivative, N-octanoyl dopamine (NOD) was developed by the group Yard et al. [163], with an increased cellular affinity as a result of its lipophilicity [163]. The cryoprotective effect of this dopamine derivative, sharing many similarities with other cryoprotective N-acyl dopamine derivatives, is superior compared to dopamine (Figure 2) [163, 168]. It is therefore likely that NOD will exert similar or even improved protection in transplantation.

![Figure 2. Dopamine (left structural formula) and N-octanoyl dopamine (right structural formula) with the lipophilic octanoic acid [163].](image)

**Aim of this Thesis**

The aim of this thesis consists of two parts. Part 1 (Chapter 1-4) focusses on the effect of NOD in transplantation and related processes, while the second part (Chapter 5-8) aims to elucidate which effect two commonly used ventilation strategies and the cause of brain death have on the donor lung and kidney quality.

In contrast to the improved outcome after dopamine preconditioning in transplantation, it failed to improve outcome or mortality in acute kidney failure in the ICU. However, its synthetic lipophilic derivative N-octanoyl dopamine belongs to N-acyl-dopamine derivatives that have been described to potentially prevent and attenuate ischemia/reperfusion injury [169]. For that reason, the effect of N-octanoyl dopamine in comparison to dopamine was tested in an acute kidney injury model in *Chapter one*. Nevertheless, dopamine, the hemodynamic active precursor of NOD, was beneficial in kidney and heart transplantation as preconditioning agent [166, 167]. In *Chapter two*, it was tested whether the effect was due to prevention of cold storage induced injury and whether this was adrenceptor independently. Because NOD has a higher cell-affinity it was expected to lead to superior results compared to dopamine [163]. *Chapter three* aimed to evaluate the potential anti-inflammatory effect of N-octanoyl dopamine and to elucidate the underlying mechanism. In *Chapter two and three* it was investigated, which of the structural properties of NOD are needed for its cryoprotective and anti-inflammatory effect.
Independently of this thesis, it has been shown that NOD, as brain dead donor preconditioning, exerts in experimental kidney and heart transplantation an anti-inflammatory and cell protective effect, positively influencing acute kidney rejection [170]. Thus, Chapter four investigated whether NOD brain dead donor preconditioning inhibits the immune response before and after transplantation, preventing graft deterioration in a syngeneic rat lung transplantation model, since the effect of NOD might differ in various organ systems.

Recently, it has been shown that lung protective mechanical ventilation increases the number of eligible lung transplants compared to conventional mechanical ventilation [12]. However, in the comparable lung disorder acute respiratory distress syndrome [86], high tidal volume ventilation had a substantial impact on the lung and kidney [38, 135]. Since the effect in brain death is unknown Chapters five and six elucidate the effect of high tidal volume ventilation low PEEP ventilation versus low tidal volume best PEEP ventilation (considered as lung protective) during six hours of brain death. While Chapter five focusses on the direct effect of different ventilation modalities on the lung, Chapter six focuses on the indirect effect of ventilation modalities on donor kidneys. Nevertheless, ventilation strategies are not the only possible confounders of organ function but also the etiology of brain death in kidney transplantation [171, 172]. However, there are some differences between brain dead donors which may bias these findings as for e.g. age and pre-existing health conditions. Thus, Chapter seven and eight were intended to explore whether a sudden or prolonged increase of intracranial pressure leading to brain death have a different effect on the donor lung, kidney and liver. Chapter seven focusses on the effect of the two brain death induction models on the lung graft, while Chapter eight elucidates the effect on kidney and liver grafts and investigates possible underlying mechanisms. All the results are summarized in Chapter nine, followed by a discussion and a future perspective.
References

Introduction

Introduction

Introduction


Introduction


