Brain dead donor graft deterioration and attenuation with N-octanoyl dopamine preconditioning
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CHAPTER 8

Slow brain death induction leads to more pronounced donor kidney and liver graft deterioration in comparison to a fast induction model

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Abstract

Donor brain death (BD) is an independent risk factor for graft survival in recipients. The time-interval between cerebral insult and the onset of BD varies greatly among patients. This time-interval may be a possible risk factor for renal and hepatic dysfunction. This study was conducted to assess the effect of different durations for BD induction on the donor kidney and liver. Brain death induction was performed in 64 mechanically ventilated male Fisher rats. Rats were observed for 0.5 h, 1 h, 2 hrs, or 4 hrs following BD induction. For slow BD induction an epidurally placed Fogarty catheter was inflated at a speed of 0.015 ml/min until an increase of mean arterial pressure was noted. For fast BD induction 0.45 ml was infused in 1 minute. After the observation period plasma, kidney and liver tissue were collected for analysis.

During slow brain death induction 10 minutes of severe hypotension were recorded in contrast to a brief hypertensive period in the fast model. In the slow BD model creatinine levels were higher at all time points compared to the fast model, as well as systemic IL-6 during the first hour after BD induction. At 4 hrs after BD induction this was associated with an increased renal mRNA expression of IL-6 and renal malondialdehyde (MDA) values. Hepatic mRNA expression of TNF-α, Bax-/Bcl-2 ratio, and protein expression of caspase-3 were elevated 4 hrs after slow BD induction compared to the fast BD induction. Polymorph nuclear leukocyte infiltration did not differ.

Markers for acute kidney injury (creatinine, IL6) were elevated in the slow BD model compared to the fast model. A potential double hit of acute kidney injury and BD may have induced distant organ injury, leading to the more pronounced liver deterioration in the slow BD induction model.
Introduction

Brain dead donors continue to remain the major source of donor organs used for transplantation. Unfortunately, brain death has been identified as an allogen independent risk factor leading to a higher incidence of rejection and delayed graft function compared to living donors [1]. Moreover, the etiology of brain death has been identified as risk factor for graft function in some organs [2-4].

Brain death (BD) is a complex pathophysiological condition, characterized by hemodynamic imbalance, hormonal impairment, and a systemic inflammatory response. The hemodynamic imbalance comprises changes elicited by brainstem herniation, followed by a catecholamine storm, and neurogenic shock due to ischemia of the spinal cord. The systemic inflammation is characterized by increased levels of circulating cytokines including interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β) and monocyte chemotactic protein 1 (MCP-1) [5-7]. This systemic inflammatory environment promotes the migration of immune competent cells into the endorgans triggering a local inflammatory and (pro-) apoptotic response [8, 9]. Furthermore, BD affects the pituitary function causing endocrine alterations which are considered to exacerbate the graft deterioration [10-12].

There is a correlation between the extent of catecholamine storm and the duration of intracranial pressure (ICP) increase. A sudden increase in ICP leads to higher levels of circulating catecholamines than a prolonged increase. The more pronounced release of catecholamines with subsequent hemodynamic changes is more detrimental for cardiac and pulmonary graft [4, 13]. Clinically correlating, traumatic brain injury, the typical cause for a rapid progression to BD, is a risk factor for mortality in heart recipients [14]. In contrast, a cerebrovascular origin of brain death, the typical cause for prolonged progression to BD, is a risk factor for renal and hepatic graft dysfunction. However, this phenomenon is not believed to be associated with a slower increase in ICP. It has rather been considered that donor characteristics such as obesity, age, and the presence of cardiovascular disease are the underlying cause [3, 15, 16]. The aim of this study was to assess whether the difference in duration of ICP increase to induce brain death has a differential effect on the renal and hepatic function or immune response in brain dead donor rats.
Materials and Methods

Ethical statement
All animals received care in compliance with the guidelines of the local animal ethics committee according to the Experiments on Animals Act (1996) issued by the Ministry of Public Health, Welfare and Sports of the Netherlands.

Surgical procedure and BD induction
Sixty four male Fisher F344 rats (270-300 g) were randomly assigned to one of 8 experimental groups (n = 8):
- Slow/fast BD induction 0.5 hrs
- Slow/fast BD induction 1 hrs
- Slow/fast BD induction 2 hrs
- Slow/fast BD induction 4 hrs

Animals were anaesthetized using 2-5% isoflurane with 100% oxygen. Initially, 2 ml saline were administered s.c. to prevent dehydration. Animals were intubated via a tracheostomy and ventilated (Tidal Volume: 6.5 ml/kg of body weight, PEEP: 3 cm of H2O at an initial respiratory rate of 120 was adjusted after BD Induction to maintain the ETCO2 in a hypocapnic range) throughout the experiment. Cannulas were inserted in the femoral artery and vein for continuous mean arterial pressure (MAP) monitoring and volume replacement. Through a fronto-lateral bore hole in the skull, a 4F Fogarty catheter (Edwards Lifesciences Co, Irvine, CA) was placed in the epidural space and inflated with saline using a syringe pump (Terufusion, Termo Co., Tokyo, Japan). Slow and fast BD induction were achieved by inflating the catheter at a speed of 0.015 ml/min or 0.45 ml/min, respectively. For the slow BD induction, inflation of the balloon was terminated when the MAP increased above 80 mmHg. For the fast BD induction, the catheter was inflated over a period of one minute. BD was confirmed by the absence of corneal reflexes half an hour after brain death induction and discontinuation of anesthesia. The mean arterial pressure was maintained above 80 mmHg during the brain death period. If necessary, colloid infusion with 10% polyhydroxyethyl starch (HAES) (Fresenius Kabi AG, Bad Homburg, Germany) was given in boli of 0.5 ml (limited to a maximum of 1 ml/h) to maintain the MAP above 80 mmHg. Insufficient MAP stabilization with HAES indicated the need of an intravenous noradrenaline (NA) drip (1 mg/mL), carefully adjusted as needed. A homeothermic blanket control system was used throughout the experiment to maintain the body temperature between 37 and 38 °C. At the end of the experimental period, blood and urine were collected, and the animals were systemically flushed with cold saline. After the flush, organs were harvested and
tissue samples were snap frozen in liquid nitrogen (stored at -80 °C) or fixated in 4% paraformaldehyde. Plasma samples and urine were also snap-frozen and stored. One animal was discarded in the slow BD induction 2 h group, two animals in the fast BD induction 2 h group and one in the fast BD induction 4 h group due to unknown amounts of noradrenaline administration. One animal was discarded in the fast BD induction 4 h group due to an apnea test conducted during the BD period. In the fast BD induction model 6 animals were replaced since hemodynamic stabilization failed immediately after BD induction.

**Biochemical determinations**

Plasma levels of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) and creatinine were determined at the clinical chemistry lab of the University Medical Centre Groningen according to standard procedures. Plasma IL-6 was determined by a rat enzyme-linked immunosorbent assay (IL-6 ELISA) kit (R&D Systems Europe Ltd. Abingdon, Oxon OX14 3NB, UK), according to the manufacturer’s instructions. All samples were analyzed in duplicates and read at 450 nm.

**RNA isolation and cDNA synthesis**

Total RNA was isolated from whole liver and renal sections using TRIzol (Life Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. Briefly, RNA samples were verified for absence of genomic DNA contamination by performing RT-PCR reactions in which the addition of reverse transcriptase was omitted, using Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primers. For cDNA synthesis 1 μl T11VN Oligo-dT (0.5 μg/μl) and 1 μg mRNA were incubated for 10 min at 70 °C and cooled directly after that. cDNA was synthesized by adding a mixture containing 0.5 μl RnaseOUT® Ribonuclease inhibitor (Invitrogen, Carlsbad, USA), 0.5 μl RNase water (Promega), 4 μl 5 x first strand buffer (Invitrogen), 2 μl DTT (Invitrogen), 1 μl dNTP’s and 1 μl M-MLV reverse transcriptase (Invitrogen, 200U). The mixture was held at 37 °C for 50 min. Next, reverse transcriptase was inactivated by incubating the mixture for 15 min at 70 °C. Samples were stored at −20 °C.

**Real-Time PCR**

Fragments of several genes were amplified with the primer sets outlined in table 1. Pooled cDNA obtained from brain-dead rats was used as an internal reference. Gene expression was normalized with the mean of β-actin mRNA content. Real-Time PCR was carried out in reaction volumes of 15 μl containing 10 μl of SYBR Green
mastermix (Applied biosystems, Foster City, USA), 0.4 μl of each primer (50 μM), 4.2 μl of nuclease free water and 10 ng of cDNA. All samples were analyzed in triplicate. Thermal cycling was performed on the Taqman Applied Biosystems 7900HT Real Time PCR System with a hot start for 2 min at 50 °C followed by 10 min 95 °C. Second stage was started with 15 sec at 95 °C (denaturation step) and 60 sec at 60 °C (annealing step and DNA synthesis). The latter stage was repeated 40 times. Stage 3 was included to detect formation of primer dimers (melting curve) and begins with 15 sec at 95 °C followed by 60 sec at 60 °C and 15 sec at 95 °C. Primers were designed with Primer Express software (Applied Biosystems) and primer efficiencies were tested by a standard curve for the primer pair resulting from the amplification of serially diluted cDNA samples (10 ng, 5 ng, 2.5 ng, 1.25 ng and 0.625 ng) obtained from brain-dead rats. PCR efficiency was $1.8 < \varepsilon < 2.0$. Real-time PCR products were checked for product specificity on a 1.5% agarose gel. Results were expressed as $2^{-\Delta\Delta Ct}$ (CT: Threshold Cycle).

### Table 1. Primer sequences used for Real-Time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
<th>Amplification size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>5′-CCAACCTTCCAATGCTCTCCTAATG-3′ 5′-TTCAAGTGCTTTCAAGAGTTGGAT-3′</td>
<td>89</td>
</tr>
<tr>
<td>TNF-α</td>
<td>5′-GGCTGCCTTGGTTCAGATG-3′ 5′-CAGGTGGGAGCAACCTACAGTT-3′</td>
<td>79</td>
</tr>
<tr>
<td>BAX</td>
<td>5′-GCGTGTTGGCCTCTTCTAC-3′ 5′-TGATCAGCTCGGCGCCTTATG-3′</td>
<td>74</td>
</tr>
<tr>
<td>Bcl2</td>
<td>5′-CTGGGATGCCCTTTGTGGA-3′ 5′-TCAGAGACGACCCAGGAGAAATCA-3′</td>
<td>70</td>
</tr>
</tbody>
</table>

### Tissue MDA

Kidney and liver tissue was homogenized with a pestle and mortar in PBS containing 5mM butylated hydroxytoluene. MDA was measured fluorescently after binding to thiobarbituric acid. Of the tissue homogenate 100 μL were mixed with 2% SDS followed by 400 μL 0.1 N HCl, 50 μL 10% phosphotungstic acid and 200 μL 0.7% TBA. The mixture was incubated for 30 min at 97 °C. Of 1-butanol 800 μL were added to the samples and centrifuged at 960 g. Subsequently, 200 μL of the 1-butanol supernatant were fluorescently measured at 480 nm excitation and 590 nm emission wavelengths. Samples were corrected for the total amount of protein.
**Cleaved caspase-3 and HIS 48 staining**

To detect caspase-3 positive cells in liver and kidney, immunohistochemistry was performed on 3 μm sections of paraffin embedded liver and kidney samples. Sections were deparaffined in a sequence of xylene, alcohol and water. As an antigen retrieval method for caspase-3 samples EDTA (1mM, pH 8.0) buffer was used. For HIS-48 (anti-granulocyte antibody) 4 μm cryosections were cut and acetone fixed. Next, endogenous peroxidase was blocked using H₂O₂ 0.3% in phosphate-buffered saline for 30 min. First, sections were stained with caspase-3 (Cell Signaling cat. nr. 9661, 1:100) or HIS-48 (Department of Pathology and Microbiology, University Medical Center Groningen, 1:2), using an indirect immunoperoxidase technique. After thorough washing, for caspase-3 sections were incubated with a peroxidase-conjugated goat anti-rabbit as a secondary antibody for 30 min (Dako, Glostrup, Denmark. cat. nr. P0448), followed by a peroxidase-conjugated rabbit anti-goat as a tertiary antibody for 30 min (Dako, Glostrup, Denmark. cat. nr. P0449). For the HIS-48 staining a secondary peroxidase-conjugated rabbit anti-mouse antibody (1:100, DAKO, Glostrup, Denmark) and a tertiary peroxidase-conjugated goat-anti-rabbit antibody (1:100, DAKO, Glostrup, Denmark) were used. To the secondary and tertiary antibodies 1% normal rat serum was added and incubated for 30 min. Subsequently the peroxidase activity was visualized by ten minutes incubation in 3,3-diaminobenzidine tetrachloride or aminoethylcarbazole for respectively paraffinized- or cryosections. Sections were counterstained using Mayer hematoxylin solution (Merck, Darmstadt, Germany). Negative antibody controls were performed. Localization of immunohistochemical staining was assessed by light microscopy. For each tissue section, positive cells per field were counted by a blinded researcher in 10 microscopic fields of the tissue at 10x magnification. Results were presented as number of positive cells per field.

**Statistical Analyses**

Statistical analysis was performed between both experimental groups using a nonparametric test (Mann-Whitney) for every time point (Prism 5.0, GraphPad Software, CA, USA). Hazard curves for the probability of NA and HAES utilization were compared using Mantel-Cox test. All statistical tests were 2-tailed and p < 0.05 was regarded as significant. Results are presented as mean ± SD (standard deviation).
Results

As an internal control we compared the catheter volume after brain death induction and the blood pressure pattern during the induction phase. The final catheter volume was similar between the slow and fast induction model (0.41 ± 0.03 ml vs 0.41 ± 0.02). During BD induction, the MAP showed different characteristic patterns due to fast and slow inflation of the Fogarty catheter (Figure 1). Slow inflation was characterized by a decrease in blood pressure 10 min before the end of the BD induction, the minimum pressure registered was 51.17 ± 10.76 mmHg. In contrast, the fast inflation was characterized by a sudden and short increase in MAP which was typically observed at the end of the balloon inflation (167.39 ± 37.85 mmHg).

![Mean Arterial Blood Pressure](image)

Figure 1. Course of MAP during BD induction and during 4 hrs BD donor stabilization in fast and slow BD inducted rats. T = 0 represents the start of the BD stabilization period.

The amount of HAES needed for a stable MAP was similar in the two brain death models. While the amount of administered NA was significantly higher in the fast brain death induction model compared to the slow model at 0.5 h and 1 h after BD induction (Table 2). We estimated the chance of noradrenaline and HAES utilization using hazard curves. Slow BD induction led to a 17.05% probability of NA use during the first hour of BD, while fast induction led to a 54.84% probability. Additionally, we compared both curves using Mantel-Cox test. The curves for NA use were significantly different (p = 0.0004). HAES was used mainly in the first minutes after...
BD induction. In the slow BD model probability of HAES use in the first 10 min after BD induction was 48.39% while it was an 84.38% probability in the fast model. Curve comparison was found significant using Mantel-Cox test (p = 0.0091. Figure 2).

Table 2. Total Noradrenaline (1 mg/ml) and HAES 10% infusion requirements.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Fast Induction</th>
<th>Slow Induction</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noradrenaline (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.35 ± 0.42</td>
<td>0.10 ± 0.24</td>
<td>0.0188*</td>
</tr>
<tr>
<td>1</td>
<td>0.55 ± 0.76</td>
<td>0.05 ± 0.14</td>
<td>0.0238*</td>
</tr>
<tr>
<td>2</td>
<td>1.1 ± 1.6</td>
<td>0.13 ± 0.25</td>
<td>0.1515</td>
</tr>
<tr>
<td>4</td>
<td>0.33 ± 0.58</td>
<td>0.23 ± 0.42</td>
<td>0.8564</td>
</tr>
<tr>
<td>HAES 10% (ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0.44 ± 0.18</td>
<td>0.31 ± 0.26</td>
<td>0.5692</td>
</tr>
<tr>
<td>1</td>
<td>0.56 ± 0.18</td>
<td>0.50 ± 0.0</td>
<td>0.9999</td>
</tr>
<tr>
<td>2</td>
<td>0.50 ± 0.0</td>
<td>0.38 ± 0.35</td>
<td>0.2000</td>
</tr>
<tr>
<td>4</td>
<td>0.56 ± 0.50</td>
<td>0.56 ± 0.42</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

Figure 2. Hazard curves for Noradrenaline and HAES utilization in rats after fast and slow BD induction. * indicates p < 0.05.

Plasma creatinine levels were measured in order to estimate the kidney function. Creatinine was significantly higher after slow BD induction compared to fast BD induction at every time point. Plasma urea levels were increased due to slow BD induction compared to 4 hrs after explosive BD (p = 0.0308, Figure 3).

ALAT and ASAT plasma levels were measured as liver cell injury markers. No differences were found in ALAT levels between the two models. The ASAT plasma levels were increased in the fast model compared to the slow model in the groups 0.5 and 2 hrs after BD induction (p = 0.0225 and p = 0.0088, Figure 3).
Plasma IL-6 levels were measured as a marker for systemic inflammation. At 0.5 and 1h after BD induction IL-6 plasma levels were significantly increased due to slow BD induction compared to fast BD induction (p = 0.0014 and p = 0.0002, Figure 4).
We assessed tissue inflammation by measuring the relative expression of pro-inflammatory genes in the kidney and liver. Relative TNF-α gene expression in the kidney showed no differences between the two models. In contrast, the relative IL-6 gene expression was significantly increased at 0.5 h and 4 hrs after slow BD induction compared to fast BD induction (p = 0.0348 and p = 0.0270, Figure 5A). The hepatic TNF-α gene expression was significantly increased 4 hrs after slow BD induction compared to fast BD induction (p = 0.0293). No difference was found in the relative gene expression of IL-6 between the two brain death models (Figure 5B).

PMN infiltration in renal and hepatic tissue was assessed by HIS-48 staining. There was no difference in HIS-48 positive staining in the renal cortex and hepatic tissue between the two BD induction models (Figure 6).
In order to study apoptotic pathways in renal and hepatic tissue, we calculated the ratio of relative Bax and Bcl-2 expression. No difference was found in renal tissue between the two models. In contrast, hepatic gene expression of the Bax/Bcl-2 ratio was significantly higher at 4 hrs after slow brain death induction compared to fast BD induction (p = 0.0293, Figure 7).
Slow brain death causes pronounced abdominal organ deterioration.

Figure 7. Ratio of the relative BAX/Bcl-2 expressions in the kidney and liver after 0.5 h, 1 h, 2 hrs, and 4 hrs of BD induction in the slow and fast model. The fold induction represents the relative expressions of these genes as compared to the expression level of the household GAPDH gene. * indicates p < 0.05.

Additionally, 4 hours after slow BD induction the hepatic cleaved caspase-3 protein expression was significantly increased compared to fast BD induction (p = 0.001, Figure 8).
Figure 8. Cleaved-caspase 3 expression in renal and hepatic tissue 4 hrs after BD induction. Kidneys after A) fast BD induction and B) slow BD induction. Livers after C) fast BD induction and D) slow BD induction. * indicates p < 0.01.

Oxidative stress was assessed by measuring lipid peroxidation. MDA levels were significantly higher in renal tissue due to slow balloon inflation compared to fast inflation 4 hrs after induction (p = 0.01). Hepatic MDA levels were comparable between the two models 4 hrs after BD induction (p = 0.48, Figure 9).
Slow brain death causes pronounced abdominal organ deterioration

Figure 9. MDA levels in renal and hepatic tissue from the fast and slow model 4 hrs after BD induction. * indicates p < 0.05.

Discussion

This study aimed to investigate the consequences of difference in time interval for intracranial pressure increase leading to BD on the donor kidney and liver. During brain death induction for both models a typical change in mean arterial pressure was noted. Slow BD induction led to more decrease in kidney function compared to the fast ICP increase, accompanied by initially increased systemic IL-6 levels. While after 4 hrs of slow BD induction in the kidney IL-6 gene expression and MDA levels were higher compared to fast BD induction. In the liver the same occurred with TNF-α gene expression and apoptosis. Nevertheless, the liver function remained comparable in the models during the observation period.

The time period between cerebral insult and progression to BD varies greatly in ICU patients, even with the same nature of cerebral insult. After infarction of the middle cerebral artery (MCA) for example BD can typically manifest itself anywhere between 24 hours and a week [17]. This broad range in time intervals is partly the origin of the complexity of pathophysiological processes leading to BD and is reflective of the duration for ICP increase [18].

A typically observed difference, as a result of the two speeds for ICP increase, was the pattern of mean arterial pressure during brain death induction in the two models. As described before by Marshman and our group [19, 20], slow brain death induction leads to approximately 10 minutes of severe hypotension in rats. Kidney ischemia might have been prolonged by a hypotension induced dysfunction of the renal blood flow autoregulation [21]. Nevertheless, even a few minutes of hypotension have been associated with an increased risk for acute kidney injury (AKI) [22]. The onset of AKI is reflected by a decrease in kidney function and increase in systemic Interleukin 6 release [23-25], as observed here. The extent of IL-6 release can predict the outcome
in patients and determine the degree of kidney injury [26, 27]. But, systemic IL-6 levels described in this study became comparable between the two BD models at 2 and 4 hrs after BD induction. Therefore, the hypotension as a single hit would have probably not led to a chronic renal dysfunction in a non-brain dead patient [23, 28]. However, the combination of acute kidney injury and the second hit by brain death might have led to the respectively more pronounced local IL-6 production in the kidney at 4 hours after BD induction [29]. The more pronounced MDA levels in the kidney support the idea of ischemic injury in the slow model. Surprising however is the time point of increased lipid peroxidation in the brain death model [30]. But also direct measurements of reactive oxygen species, in a comparable set up, were elevated at later time points, although one would expect an immediate elevation after the potential ischemic event [30, 31]. The described oxidative stress in liver tissue associated to AKI described in literature is probably the result of neutrophil granulocyte infiltration which was at least not pronounced compared to the fast BD induction model [32, 33].

Nevertheless, AKI may lead to distant organ injury. AKI can increase in the liver TNF-α gene expression and apoptosis [32, 34], which may have led to the difference at 4 hours after BD induction between the slow and fast model. That we did not find a difference for apoptosis in the kidney might be since in ischemic injury apoptosis and necrosis share a common pathway [35, 36]. In the kidney, ischemia may have resulted in the depletion of ATP and therefore pronounced necrosis should occur, which was not investigated here [35, 37]. In contrast, in the liver cell death was probably the result of AKI induced distant organ injury, mediated by cytokines, and therefore led in the presence of ATP to apoptosis [38, 39]. Nevertheless, also fast BD induction may cause an induction of apoptosis in the liver which would again suggests a double hit in the slow model [40].

No difference was observed for PMN infiltration between the two models for neither the donor kidneys nor the livers which could be due to the fact that 4 hrs were not sufficient to reveal a difference. This is supported by the delayed local proinflammatory gene expression at 4 hours after BD induction compared to the fast BD induction model [28, 29, 41, 42]. On the other hand, brain death also leads to a systemic induction of proinflammatory gene expression and infiltration of immune cells [43] and possibly because of this no difference was found. In non-brain dead animals others have described an infiltration at 6 hours after AKI compared to sham animals however less severe than at 36 hours [44]. This infiltration correlated with MDA levels in the kidney, dependent on IL-6 [27].

ALAT levels, reflective of liver cell injury, showed no differences between both BD induction models. However, ASAT levels were higher at 0.5 h and 2 hrs after fast BD
induction compared to slow BD induction. This is probably not a reflection of liver damage due to the incongruence with ALAT levels and the pathological changes in the liver induced by slow brain death induction. Moreover, ASAT is found in many tissues including the heart and lung. It has been shown that fast BD induction causes early heart and lung damage probably as a result of greater catecholamine release compared to gradual brain death [4, 45], but also exogenous catecholamine administration may lead to the increase in injury marker [46].

Apoptosis levels in the slow BD induction model were increased in contrast to the fast model even though this group received half an hour longer isoflurane which has been shown to limit distant organ injury induced liver apoptosis [47, 48].

Previously, it has been shown that hemodynamic instability of brain dead donors leads to an exacerbation of organ deterioration [49]; this study is the first to describe the detrimental effect of hypotension before the onset of brain death. While most commonly cerebral insult is associated with hypertensive periods there are a number of reports that associate cerebral insult in almost 50% of the patients with hypotensive periods [50, 51], and there is a particularly high risk for iatrogenic induced hypotension [52, 53]. While in the fast BD induction more hemodynamic support was needed to stabilize the mean arterial pressure, despite the usage of noradrenaline, kidney injury was more pronounced in the slow BD induction model [46]. While correction of the instable animals probably prevented the second hit in the fast BD induction model [46, 49]. A decrease in renal function can be caused by the usage of the colloid HAES for hemodynamic support [54], but the administered amount here did not differ between the models.

In conclusion, slow brain death induction leads to a hypotensive period which probably leads to the development of acute kidney injury. The double hit of AKI and brain death leads to a more pronounced immune response in the kidney and liver in the slow BD induction model compared to the single hit for the abdominal organs in the fast model. Patients with cerebral insult and possibly additional trauma are at a high risk to develop periods of hypotensive crisis. The long term effect and possible intervention strategies need to be identified.
References

Slow brain death causes pronounced abdominal organ deterioration


