

University of Groningen

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

Hottenrott, Christina Maximilia Valentina

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:
2016

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Hottenrott, C. M. V. (2016). *Brain dead donor graft deterioration and attenuation with N-octanoyl dopamine preconditioning: Emphasis on lung and kidney grafts*. University of Groningen.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

CHAPTER 4

Brain dead donor preconditioning with N-octanoyl dopamine to attenuate ischemia/reperfusion injury in a rat lung transplantation model

Maximilia Hottenrott
Johannes Wedel
Arjen Petersen
Jan Doff
Henri Leuvenink
Charalambos Tsagogiorgas
Benito Yard
Michiel Erasmus

Manuscript in preperation

Abstract

Brain death and ischemia/ reperfusion cause graft deterioration, thereby limiting transplantation outcome substantially. N-octanoyl dopamine (NOD) has been shown to prevent graft deterioration in kidney and heart transplantation in rats. For this reason we aimed to investigate the effect of NOD donor preconditioning on the ischemia/ reperfusion injury after lung transplantation.

Fischer rats were randomly assigned into four groups: 1) non brain dead donors, 2) NaCl treated- 3) NOD treated BD donors or the recipient group. Lungs were harvested after four hours and the left lung was orthotopically transplanted into 4) syngeneic recipients. Recipients were sacrificed after 6 hours of continuous ventilation of the transplant. Physiological parameters were noted. Lung tissue was obtained to analyze changes in gene expression on RNA level and to determine histological lung injury before and after transplantation.

The only significant difference between BD NaCl and NOD donor preconditioning was the difference in Icam1 gene expression six hours after reperfusion. However, gene expression for the cytokines, Tnf and Cinc1 was only in the NOD preconditioned transplanted lungs significantly reduced compared to the non-transplanted donor lung.

The differences in gene expression suggest not beneficial effect of NOD donor preconditioning in lung transplantation on the immediate outcome. However, future studies are needed to determine the exact molecular mechanism and the effect of NOD on an alloimmune response model.

Introduction

The onset of brain death induces in potential organ donors hemodynamic changes and intensifies the systemic release of pro-inflammatory mediators, initiated at cerebral insult [1, 2]. These systemic changes are accompanied by alveolar hemorrhage, capillary leakage and pulmonary edema formation, exaggerating the immune response [1, 3]. In recipients with excessive cytokine production in the lung primary graft dysfunction develops [4, 5]. Primary graft dysfunction is the main reason for perioperative and early mortality in lung transplantation [6], and is a major risk factor for chronic rejection [7]. Implementation of strict donor management protocols, protective ventilation strategies and administration of high dose steroids, limiting the immune response, have already resulted in increased numbers of suitable donor lungs for transplantation [8-11]. Nevertheless, the success in lung transplantation remains limited, due to chronic rejection [6, 12]. For that reason, pharmacological preconditioning strategies in brain dead donors have been tested in the clinic [13], to reduce inflammation and graft deterioration, as well as to increase their resilience to injury [1, 14]. Dopamine is a successful pharmacological preconditioning agent in kidney and heart transplantation [15, 16]. Since hemodynamic side effects led to discontinuation of preconditioning in 12.5% of the donors and subsequent loss of potential protection of dopamine, a non-hemodynamic derivative, N-octanoyl dopamine (NOD) was developed [15, 17]. NOD was proven to be superior compared to dopamine in preventing cold ischemia induced cell injury [18]. In addition, only NOD was capable of limiting acute kidney injury in an ischemia reperfusion injury model, possibly due to inhibition of NF κ B [19, 20]. Brain dead donor preconditioning with N-octanoyl dopamine resulted in a renal- and heart transplantation model in a reduced expression of adhesion molecules. Additionally, NOD was associated in the renal graft with superior graft function after transplantation and lower acute rejection scores compared to untreated rats. In correlation to this, systemic LDH levels were reduced in heart transplantation, without any adverse side effects [21]. To test whether NOD may also attenuate the inflammatory response in lung transplantation we investigated if NOD preconditioning of brain dead donor animals limits lung injury and cytokine expression after transplantation.

R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39

Materials and Methods

Animals

Rats were kept under clean conventional conditions, with a 12/12h light-dark cycle at 22 °C and were fed standard rat chow *ad libitum*. All animals received humane care in compliance with the Principles of Laboratory Animal Care (NIH Publication No.86-23, revised 1985) and the Dutch Law on Experimental Animal Care. The experiments were approved by the local animal care committee.

Experimental groups

Male Fisher (F344; Janvier (USA)) rats, weighing 230 - 300 g, were randomly assigned into three donor groups or the recipient group for isogenic left lung transplantation. The three donor groups were: 1) untreated non brain death (control) group (n=8), 2) acute traumatic brain death (BD NaCl) with continuous 0.9% NaCl infusion (1 ml/h) (n=7) and 3) brain death with continuous NOD (BD NOD) treatment (1 ml/h) (n=8).

Brain death model

The donor rats were anesthetized by intraperitoneal (IP) injection of ketamine hydrochloride (50 mg/kg) and xylazine (2 mg/kg). In the brain deaths groups 1/3 of the initial dose was given i.v. after catheterization. In the control group anesthesia was continued with a ketamine-perfusor (20 mg/ml) and every 30 minutes 1/3 of the initial xylazine dosage i.v. To prevent hypovolemia 2 ml of saline were administered subcutaneously before the start of surgery.

The right femoral artery was cannulated for continuous monitoring of mean arterial pressure (MAP) and to collect blood samples. The femoral vein was used to stabilize the MAP above 80 mmHg with fluid boli of colloidal (HAES-steril 10%, Fresenius Kabi, Bad Homburg, Germany) and crystalloid solution (NaCl 0.9%, Baxter B V, Utrecht, Netherlands) as needed, with the limitation of 2 ml HAES and 5 ml NaCl per hour. The amount of i.v. administered fluid was recorded. No vasopressors were used.

In prone position, the suturae of the skull were exposed and a fronto-lateral burr hole was drilled (Dremel 3000, Breda, Netherlands; boring head 9905 Dremel, Breda, Netherlands), after placing lidocaine on the periosteum. A thin osseous lamina was left, only in the two brain death groups a 4F Fogarty catheter (Edwards Lifesciences LLC, Irvine, U.S.A.) was introduced subdurally.

After this, the rats were placed in the final supine position, tracheotomized, intubated with a 14G polyethylene tube (Kliniject, KLINIKA Medical GmbH, Usingen, Germany) and mechanically ventilated (Babylog 8000, Draeger, Luebeck,

Germany) using a pressure-controlled mode with a PEEP of 3 cm H₂O, inspiratory / expiratory ratio (I:E) of 1:1 in the ViVe Mode (1:4), a fraction of inspired oxygen (FiO₂) of 0.5 and a tidal volume of about 6 - 7 ml/kg of body weight by adjustment of the end-inspiratory pressure (P_{insp}). After intubation a recruitment maneuver was performed (PEEP of 10 cmH₂O and a max P_{insp} of 20 cmH₂O) until a drop in blood pressure was noted, followed by a hyperventilation period of 10 minutes at a frequency of 145/min. before brain death induction to normalize oxygen saturation and was reduced to 120/min. in the brain death groups, in the control group to 135/min. Brain death was induced by continuous inflation of the 4F fogarty catheter with 0.45 ± 0.5 ml over a period of 100 ± 10 seconds, manually. In both brain death groups, immediately after brain death induction, a perfusor was started giving through the venous femoral line 1 ml of saline per hour. In the BD NOD group 10 µg NOD (Novaliq, Heidelberg, Germany)/g of body weight per hour were added in the infusate. A rectal probe was placed to monitor and keep the body temperature between 37 °C and 38 °C with heating pads. After 15 minutes a second recruitment maneuver was conducted. Brain death was confirmed with the absence of corneal reflexes 30 minutes after brain death induction and subsequent anesthesia discontinuation.

After 4 hours of ventilation a median laparo-thoracotomy was performed, after taking blood gas sample for the iSTAT handheld analyser (Abbott) and measurements with EG7+ cartridges. The left rib cage was removed and the hilus area exposed. Before lung procurement 750 IU heparin were administered directly into the right ventricle. After dissection of the pulmonary vein the lung was flushed through a pulmonary cannula (18G) via the right ventricle with cold saline at a constant pressure of 15 cmH₂O. Afterwards the heart-lung package was allocated in cold saline to the recipient and prepared for transplantation (Figure 1). The left lung lobe was used for transplantation after positioning it between plastic foil to prevent drying. The right lower lung lobe was divided along the bronchus, the lateral part was snap frozen in liquid nitrogen and the medial part fixed in formalin 4%.

R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39

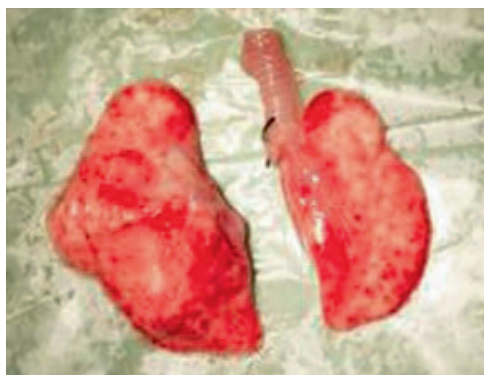


Figure 1. Brain dead donor lungs prepared for transplantation. The left lung was placed on the thin foil and subsequently covered with the foil to prevent dehydration of tissue. The right lung lobe was not used for transplantation, but used for analysis after separation from the left lung.

Recipient procedure

Anesthesia in the recipient was initiated, an hour before lung procurement from the donor, with 5% of isoflurane and 95% of oxygen. Anesthesia was continued, after oral intubation with a 14G polyethylene tube with 2% of isoflurane and 50% of oxygen. Subcutaneously 0.05 mg/kg of body weight atropine and 0.03 mg/kg of body weight buprenorfine were administered. Volume controlled ventilation was initiated with 8 ml/kg of body weight at a frequency of 80 and FiO_2 of 0.5. Via the tail vein 3 ml/h of 0.9% NaCl and 0.3 mg/kg/h rocuronium were given intravenously, after an initial dose of 0.06 mg/kg. Once the left lung was removed as previously described [22] the tidal volume was set at 10 ml/kg, with 30% of volume subtracted for the removed left lung. Frequency was set between 80 and 90/min. The body temperature was maintained between 37 °C and 38 °C.

The recipient rat was placed and fixed on the right side for the transplantation procedure. The rib cage was exposed and the third and fourth rib were removed. After preparation of the hilus area a clamp was placed on the pulmonary vein and artery. Once the original left lung was removed the operation area was covered with a wet compress until the donor lung was harvested and prepared for transplantation. The left lung was transplanted orthotopically by anastomosis of the vein followed by the artery. Hereafter a small silicon cannula was placed in the pulmonary vein and fixed with a tobacco-pouch suture (Figure 2). The left bronchus was ventilated via a 14G cannula at a frequency of 60 and 30% of the original tidal volume of 6 ml/kg of body weight (donor animal) with 100% oxygen. Recruitment maneuvers were performed until the lung was fully inflated with a maximum pressure of 25 mmHg, followed by removal of the clamp for the reperfusion of the graft. For blood gas analysis (EG7+, iSTAT, Abbott) 0.2 ml of blood was withdrawn from the pulmonary

vein cannula 5 minutes after reperfusion and after 6 hrs. of ventilation. After 6 hrs. of ventilation the left lung was removed and divided into four longitudinal sections. The second section from the cranial direction was formalin fixed the other parts were snap frozen. Both, brain death induction (M.C.H.) and lung transplantation were each performed by a single operator (A.J.P) to reduce variability.



Figure 2. View on operating field after implantation of the donor lung. The recipient's left pulmonary artery and vein were clamped for removal of the left lung of the recipient. After implanting of the left brain dead donor lung a pulmonary vein cannula was inserted for blood gas analysis after start of reperfusion and after 6 hrs.

RNA isolation and reverse transcriptase Polymerase-Chain Reaction

Total RNA was isolated from snap frozen lung tissue of the brain dead donor lung and the transplanted lung using Trizol (Invitrogen Life Technologies, Breda, Netherlands) according to the manufacturer's instructions. Guanidine thiocyanate contaminated samples, identified by E260/E230 ratio below 1.6 with the nanodrop 1000 spectrophotometer, were purified before continuation. The integrity of total RNA was analyzed by gelelectrophoresis. To remove genomic DNA total RNA was treated with DNase I (Invitrogen, Breda, Netherlands). 1 μ g RNA was transcribed into cDNA using M-MLV (Moloney murine leukemia virus) Reverse Transcriptase (Invitrogen, Breda, Netherlands), after an initial incubation with Oligo-dT primers (Invitrogen, Breda, Netherlands) in the presence of dNTPs (Invitrogen, Breda, Netherlands).

Gene expression analyses were performed at mRNA level by TaqMan low density array (TLDA). Designed primer sets were loaded with 5 μ l cDNA (2 ng/ μ l) and SYBR green (Applied Biosystems) into 384 wells of TLDA cards. Amplification and detection were performed with the ABI Prism 7900-HT Sequence Detection System (Applied Biosystems) measuring the emission of SYBR green. Each sample was measured in triplicate and a pool of sample CDNA served as calibrator. PCR reaction

R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39

consisted of 40 cycles at 95 °C for 15 sec. and 60 °C for 60 sec. after initiation for 2 min. at 50 °C and 10 min. at 95 °C. A dissociation curve analysis was performed for each reaction to ensure the amplification of specific products.

The designed qRTD-PCR primers are shown in table 1. Gene expression was normalized with the mean of the housekeeping gene Ppia and Eif2b1 and gene expression values were calculated with the $\Delta\Delta C_t$ method [23].

Table 1. RTD-Primer Overview

| Primer | Gene | Primer | Amplicon (bp) |
|-------------|---|--|---------------|
| Tnf | Tumor necrosis factor | 5'-AGGCTGTCGCTACATCACTGAA-3' 5'-TGACCCGTAGGGCGATTACA-3' | 67 |
| Il6 | Interleukin 6 | 5'-CCAACTTCCAATGCCTCCTAATG-3' 5'-TTCAAGTGCTTTCAAGAGTTGGAT-3' | 89 |
| Ccl1 | Chemokine (C-x-C motif) ligand 1 | 5'-TGGTTCAGAAGATTGTCCAAAAGA-3' 5'-ACGCCATCGGTGCAATCTA-3' | 78 |
| Ccl1 (Mcp1) | Chemokine (C-C motif) ligand 2 | 5'-CTTTGAATGTGAACTTGACCCATAA-3' 5'-ACAGAAGTGCTTGAGGTGGTTGT-3' | 78 |
| Icam1 | Intercellular adhesion molecule 1 | 5'-CCAGACCCTGGAGATGGAGAA-3' 5'-AAGCGTCGTTTGATCCTCC-3' | 251 |
| Vcam1 | Vascular adhesion molecule 1 | 5'-TGTGGAAGTGTGCCCGAAA-3' 5'-ACGAGCCATTAACAGACTTTAGCA-3' | 84 |
| Gapdh | Glyceraldehyde-3-phosphate dehydrogenase | 5'-GTATGACTCTACCCACGGCAAGTT-3' 5'-GATGGGTTTCCCGTTGATGA-3' | 79 |
| B-actin | Actin, beta | 5'-GGAAATCGTGCGTGACATTTAAA-3' 5'-GCGGCAGTGGCCATCTC-3' | 74 |
| Ho1 | Heme-oxygenase 1 | 5'-CTCGCATGAACACTCTGGAGAT-3' 5'-GCAGGAAGGCGGTCTTAGC-3' | 74 |
| Ppia | Peptidylprolyl isomerase A | 5'-TCTCCGACTGTGGACAACTCTAATT-3' 5'-CTGAGCTACAGAAGGAATGGTTTGA-3' | 76 |
| Eif2b1 | Eukaryotic translation initiation factor 2B | 5'-ACCTGTATGCCAAGGGCTCATT-3' 5'-TGGGACCAGGCTTCAGATGT-3' | 77 |

Histological scoring and Immunohistology

The right lower lobe and the second upper part of the left transplanted lung were fixed in 4% formalin, embedded in paraffin and cut in three- μ m-thick slices. Slides were stained with hematoxylin-eosin and scored based on a semiquantitative scoring system, as previously described [24]. The amount of intra- and extra-alveolar hemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace and overinflation graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. Examination was performed in a blinded fashion by 2 investigators, using a conventional light microscope at a magnification of 200 across 10 random, non-coincident microscopic fields.

Statistical Analysis

Data are expressed if not mentioned otherwise as the mean \pm standard error of the mean. Differences in measured variables between brain dead treated groups were assessed using t-test, and in the presence of significant variances a post hoc Welch's correction was performed. Statistical difference was accepted at $p < 0.05$.

Results

Surgical procedure

Immediately after brain death induction one animal was lost to circulatory collapse in the BD NOD group and after three and a half hours one in the BD NaCl group. In the recipient group, six rats bled out after the first hour after transplantation. Two received a Non-BD, one a BD NaCl and three a BD NOD donor lung. The origin of bleeding could in all of these cases not be located. Lost animals were replaced.

Although in the living donor group only two animals were lost, due to hemodynamic instability, a common problem in this group was the unexpected development of pleural effusion with subsequently high ventilation pressures. We therefore decided to exclude this group from further analysis.

NOD preconditioning does not alter respiratory function of the lung graft

During four hours of brain death an increase in pulmonary inspiratory pressure, in both brain dead donor groups, was observed ($p < 0.05$). However, blood gas analyses at the end of the brain death period were within physiological range for both groups (Table 2).

After reperfusion, the pulmonary inspiratory pressure also increased in the recipients over time, unaffected by NOD. Other parameters determined by blood gas analysis were outside of physiological range without significant differences between the groups (Table 3).

R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39

Table 2. Physiological data at baseline and 4 hours of brain death.

| Donor-Animals | Baseline | | End | |
|--|------------------|-----------------|------------------|-----------------|
| | BD NaCl n = 7 | BD NOD n = 8 | BD NaCl n = 7 | BD NOD n = 8 |
| Pinsp (cm H ₂ O) | 12.0 ± 1.2 * | 11.6 ± 0.5 # | 15.6 ± 1.1 | 15.9 ± 0.5 |
| PaO ₂ /FiO ₂ (mmHg) | - | - | 402 ± 51 | 381 ± 53 |
| PaCO ₂ (mmHg) | - | - | 43.7 ± 1.4 | 43.1 ± 3.7 |
| pHa | - | - | 7.35 ± 0.03 | 7.35 ± 0.02 |
| MAP (mmHg) | 100 ± 5 | 106 ± 12 | 106 ± 12 | 105 ± 3 |
| Volume (ml) | - | - | 11.2 ± 1.3 | 11.9 ± 0.7 |

The presented values are the mean ± standard error of the mean.

Baseline– before induction of brain death; BD– brain dead donor group; END– after 4 hrs. of ventilation and stabilization; MAP– mean arterial pressure; NaCl- 0.9% saline continuously for four hours (1ml/h); NOD–N-octanoyl solved in 0.9% NaCl continuously given for four hours (1ml/h); PaCO₂ – partial pressure of carbon dioxide; PaO₂/FiO₂ – ratio of partial pressure of arterial oxygen and fraction of inspired oxygen; pHa– arterial pH; Pinsp– end-inspiratory pressure (* vs. BD - NaCL END, p < 0.05 and # vs. BD - NOD END, p < 0.05)

Table 3. Physiological data at reperfusion and after 6 hours of ventilation after reperfusion.

| Lung transplant | Baseline | | End | |
|--|------------------|-----------------|------------------|-----------------|
| | BD NaCl n = 7 | BD NOD n = 8 | BD NaCl n = 7 | BD NOD n = 8 |
| Weight Diff. (g) | - | - | 12 ± 3 | 10 ± 0 |
| Pinsp (cmH ₂ O) | 13.6 ± 1.5 * | 10.4 ± 1.1 # | 20.2 ± 1.8 | 16.4 ± 1.3 |
| PaO ₂ /FiO ₂ (mmHg) | 292 ± 68 | 170 ± 43 | 258 ± 76 | 237 ± 56 |
| PaCO ₂ (mmHg) | 65.4 ± 5.5 | 54.8 ± 2.7 | 67.6 ± 8.8 | 66.6 ± 7.2 |
| pHa | 7.26 ± 0.03 | 7.28 ± 0.01 | 7.22 ± 0.03 | 7.23 ± 0.03 |
| Hb (g/dl) | 13.0 ± 0.3* | 11.6 ± 0.5 | 9.2 ± 1.3 | 8.4 ± 1.1 |

The presented values are the mean ± standard error of the mean.

Baseline– 3 minutes after reperfusion; BD– brain dead donor group; END– after 6 hrs. of ventilation and perfusion of the left transplanted lung; MAP– mean arterial pressure; NaCl- 0.9% saline continuously given to the donor for four hours (1ml/h); NOD–N-octanoyl solved in 0.9% NaCl continuously given to the donor for four hours (1ml/h); PaCO₂ – partial pressure of carbon dioxide; PaO₂/FiO₂ - ratio of partial pressure of arterial oxygen and fraction of inspired oxygen; pHa– arterial pH; Pinsp– end-inspiratory pressure; Sham– untreated non brain dead donor group (* vs. BD END, p < 0.05 and # vs. NOD END, p < 0.05)

NOD attenuates intragraft cytokine and adhesion molecule expression after transplantation

Next, we assessed the expression of the key cytokines and adhesion molecules in the lungs before and after reperfusion (Table 4). Before transplantation, NOD preconditioning did not significantly affect cytokine or adhesion molecule expression. However, six hours after reperfusion NOD significantly reduced Icam1 expression compared to BD NaCl ($p < 0.05$). By comparison of cytokine and adhesion molecule expression over the time course (before vs. after reperfusion within the group), NOD significantly lowered Tnf, Cinc1, Icam1 and Vcam1, while in the BD NaCl group only Vcam1 expression was significantly altered, and Tnf expression had a trend towards significance ($p < 0.078$).

Table 4. Real time quantitative detection PCR after 4 hours of brain death and 15 minutes of warm ischemia, as well as after transplantation and 6 hours of ventilation and reperfusion.

| RTD-PCR | Baseline | | End | |
|---------|------------------|-----------------|------------------|-----------------|
| | BD NaCl n = 7 | BD NOD n = 8 | BD NaCl n = 7 | BD NOD n = 8 |
| Tnf | 1.72 ± 0.2 | 2.25 ± 0.43 | 1.07 ± 0.19 | 0.75 ± 0.12 |
| Il6 | 1.56 ± 0.56 | 1.52 ± 0.38 | 2.87 ± 0.89 | 1.39 ± 0.26 |
| Cinc1 | 2.05 ± 0.32 | 1.85 ± 0.25 | 1.78 ± 0.53 | 0.82 ± 0.17 |
| Mcp1 | 1.25 ± 0.17 | 1.33 ± 0.3 | 1.21 ± 0.36 | 0.92 ± 0.19 |
| Icam1 | 3.29 ± 0.97 | 3.97 ± 0.22 | 0.97 ± 0.15 | 0.6 ± 0.04 |
| Vcam1 | 2.12 ± 0.22 | 2.29 ± 0.44 | 0.46 ± 0.09 | 0.65 ± 0.09 |
| Gapdh | 1.39 ± 0.13 | 1.51 ± 0.06 | 1.44 ± 0.14 | 1.11 ± 0.09 |
| B-actin | 1.68 ± 0.18 | 2.25 ± 0.43 | 1.06 ± 0.17 | 0.74 ± 0.12 |
| Ho1 | 1.68 ± 0.19 | 2.75 ± 0.47 | | |

The presented values are the mean ± standard error of the mean.

BD- brain dead donor group; before transplantation- 4 hrs. of ventilation and stabilization after brain death induction; Ccl2- Chemokine (C-C motif) ligand 2, also known as Mcp1 (Monocyte chemotactic protein 1); Cxcl1- Chemokine (C-X-C motif) ligand 1, also known as Cinc1 (Cytokine induced neutrophil chemo-attractant 1); GAPDH- Glycerinaldehyd-3-phosphat dehydrogenase; Ho1- Hemeoxygenase1; Icam1- Intercellular adhesion molecule 1; Il6- Interleukin 6; NaCl- 0.9% saline continuously for four hours given in the donor animal (1ml/h); NOD- N-octanoyl solved in 0.9% NaCL continuously given for four hours in the donor animal (1ml/h); RTD-PCR- Real time quantitative PCR; Tnf- Tumor necrosis factor α ; Vcam1- Vascular adhesion molecule 1 (# before vs. after transplantation, $p < 0.05$ and † vs. BD NOD after transplantation, $p < 0.05$).

NOD preconditioning does not alter histological lung tissue damage

To assess tissue damage, a lung injury score was applied on hematoxylin-eosin stained histological slides as described in the Method section (Figure 3). The total lung injury significantly increased after reperfusion (3.56 ± 0.28 (BD NaCl) vs. 6.34 ± 0.32 (BD NaCl aLTX), $p < 0.05$ and 4.21 ± 0.77 (BD NOD) vs. 6.36 ± 0.56 (BD NOD aLTX), $p < 0.05$) (Figure 1). The increase in injury could be attributed to increased

inflammation (2.52 ± 0.16 (BD NaCl) vs. 3.58 ± 0.07 (BD NaCl aLTX); $p < 0.05$ and 2.56 ± 0.28 (BD NOD) vs. 3.39 ± 0.16 (BD NOD aLTX); $p < 0.05$) and increased hemorrhagic infarction (0.65 ± 0.17 (BD NaCl) vs. 1.88 ± 0.22 (BD NaCl aLTX), $p < 0.05$ and 1.07 ± 0.36 (BD NOD) vs. 2.28 ± 0.3 (BD NOD aLTX), $p < 0.05$). There was no difference in overinflation (0.17 ± 0.08 (BD NaCl) vs. 0.12 ± 0.06 (BD NaCl aLTX) and 0.18 ± 0.07 (BD NOD) vs. 0.11 ± 0.05 (BD NOD aLTX)) and intra-alveolar edema formation (0.21 ± 0.09 (BD NaCl) vs. 0.76 ± 0.27 (BD NaCl aLTX) and 0.39 ± 0.23 (BD NOD) vs. 0.59 ± 0.15 (BD NOD aLTX)). Nevertheless, there were no significant differences between treatment groups at any time point.

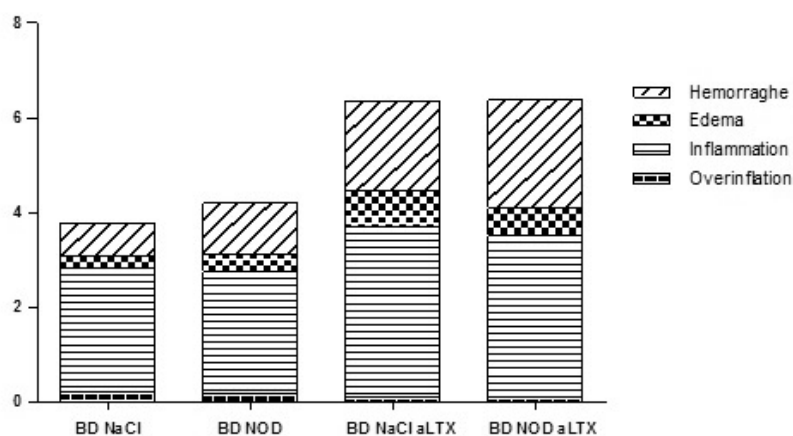


Figure 3. Histological total lung injury score. Lung tissue before (right lung) and after 6 hrs of reperfusion (left lobe) were haematoxylin-eosin stained and scored for the extent of intra- and extra-alveolar hemorrhage, intra-alveolar edema, inflammatory infiltration of the interalveolar septa and airspace, and overinflation. The pathological findings were graded as negative = 0, slight = 1, moderate = 2, high = 3, and severe = 4. The sum of variables gives the total lung injury score. Morphological examination was performed in a blinded fashion by 2 investigators, using a conventional light microscope at a magnification of 200 across 10 random, non-coincidental microscopic fields. All values are presented as mean. aLTX- after lung transplantation and 6 hrs of ventilation; BD- brain dead donor group; D- right donor lung before transplantation; NaCl- 0.9% saline continuously given to the donor for four hours (1ml/h); NOD-N-octanoyl solved in 0.9% NaCl continuously given to the donor for four hours (1ml/h).

Discussion

After the favorable effect of N-octanoyl dopamine preconditioning was shown in rat kidney and heart transplantation model [21], we investigated in this study its effect on lung transplantation. NOD preconditioning of brain dead donor rats significantly reduced Icam1 gene expression six hours after reperfusion compared to BD NaCl. Additionally, there was a general decrease in the investigated cytokine

and adhesion molecule gene expression at six hours after transplantation compared to before transplantation. However, this reduction was only significant in the NOD group with the exception for Vcam1 gene expression that was significantly reduced in both groups. Secondly, there was only an increasing ratio of partial arterial oxygen pressure and fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) observed in the NOD preconditioned group, though the values did not reach statistical significance and remained below BD NaCl values after transplantation.

Brain death is the result of a progression of ischemia after cerebral insult [25], initiating a proinflammatory immune response [26, 27]. Hemodynamic changes upon brain death intensify the immune reaction [1], and make the lung more susceptible to further injury [28]. Some markers before procurement of the lung graft have been identified to determine the risk for primary graft dysfunction and survival in the early phase after transplantation [4, 29]. This is probably the result of a synergistic effect of brain death and ischemia reperfusion injury, and the exaggeration of the inflammation [30]. In this study we investigated gene expression levels of cytokines upregulated in brain death and potential key cytokines in ischemia reperfusion injury in lung transplantation [1, 14, 29, 31]. Their common regulator is the transcription factor $\text{NF}\kappa\text{B}$ [32-34]. The anti-inflammatory effect N-octanoyl dopamine has been shown to be $\text{NF}\kappa\text{B}$ related [20]. The downregulation of $\text{NF}\kappa\text{B}$ might be the result of an induction of the unfolded protein response [35, 36]. The more pronounced down-regulation of cytokines and adhesion molecules in the NOD treated group in contrast to the brain death NaCl group suggests a possible down regulatory effect of NOD on $\text{NF}\kappa\text{B}$, which we so far did not investigate directly.

Though the decrease of cytokines found in our study was anticipated for the majority of cytokines after reperfusion in difference to the ischemic phase in the BD NaCl group [37] we also expected a pronounced upregulation for Il6 and possibly Il8 (Cinc1 is an Il8 like cytokine in rats) [1, 37, 38]. However, while there was a rather increasing trend in the BD NaCl group for Il6 comparing before to after transplantation levels it did not reach significance, for the BD NOD group stable levels were found. For Il8 we found a decrease possibly as a result of usage of a syngeneic model. Though a different time point might have been more beneficial for an increased immune response 6 hours after transplantation was chosen since NOD induced downregulation of gene expression requires a few hours [20]. Secondly, for a later time point the usage of an allogeneic model would have been preferable [39], however we choose to investigate the isolated effect of NOD on ischemia/reperfusion in the absence of an alloimmune reaction.

Inflammatory changes in the lung are accompanied by a reduction in lung graft dysfunction, defined by the ratio of arterial partial oxygen pressure and fraction of

R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39

R1 inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) [7, 40]. Dependent on the extent of the lung function
R2 the extent of lung injury may be defined [40]. In lung transplantation, the trend
R3 of $\text{PaO}_2/\text{FiO}_2$ during the first hours after transplantation is predictive for a 90 day
R4 mortality in humans [41]. Interestingly, while the $\text{PaO}_2/\text{FiO}_2$ ratio suggested a
R5 moderate lung injury in both groups after transplantation in this experimental set
R6 up [40] only in the BD NOD group an increase of the $\text{PaO}_2/\text{FiO}_2$ during the six hours
R7 after transplantation was observed.

R8 The lung graft deterioration is accompanied by typical histological changes. Upon
R9 induction of brain death inflammatory changes, hemorrhagic infarction and edema
R10 formation are commonly present in the donor [3, 11]. This has been suggested to be
R11 the result of integrity loss of the capillary-alveolar membrane [1, 3]. These changes
R12 are similar to the injury found after transplantation in the clinic [42]. The histological
R13 changes found in this study are comparable and correlate to our findings of moderate
R14 lung injury according to the $\text{PaO}_2/\text{FiO}_2$.

R15 This study has several limitations. Firstly, we excluded the non brain dead control
R16 group. These lungs were meant to give insight into the brain death induced changes in
R17 this model. Secondly, the time point of observation after transplantation was not ideal.
R18 To determine the effect of brain death and ischemia reperfusion on the inflammatory
R19 immune response after lung transplantation animals are usually sacrificed two hours
R20 or in allogeneic models more than 24 hours after reperfusion. The six hour follow
R21 up in this study was selected since N-octanoyl dopamine treatment in vitro only
R22 reached its maximal anti-inflammatory effect after a few hours of treatment. While
R23 the mechanism of its anti-inflammatory and organ function improving effect has not
R24 been elucidated yet [19-21, 43], it might be the result of a mitochondrial uptake [17]
R25 and a delayed regenerative effect [19]. Last but not least, this study was designed to
R26 investigate the effect of NOD on brain death- and ischemia/reperfusion associated
R27 injury. However in a clinical setting transplantations are generally performed in
R28 an allogeneic setting. This study showed some anti-inflammatory potential in the
R29 pulmonary grafts after transplantation. However, the potential of NOD to prolong
R30 graft survival can only be assessed in allogeneic transplantations.

R31 In conclusion, while we could only detect a mild anti-inflammatory potential of
R32 N-octanoyl dopamine in this study, it did not cause additional injury. As we have
R33 previously reported beneficial effects in allogeneic transplantations [21], this study
R34 clearly warrants further studies to address this potential anti-inflammatory effect in
R35 allogeneic lung transplantation models.
R36
R37
R38
R39

References

1. Avlonitis, V.S., et al., *The hemodynamic mechanisms of lung injury and systemic inflammatory response following brain death in the transplant donor*. Am J Transplant, 2005. 5(4 Pt 1): p. 684-93.
2. Takada, M., et al., *Effects of explosive brain death on cytokine activation of peripheral organs in the rat*. Transplantation, 1998. 65(12): p. 1533-42.
3. Novitzky, D., et al., *Pathophysiology of pulmonary edema following experimental brain death in the chacma baboon*. Ann Thorac Surg, 1987. 43(3): p. 288-94.
4. Fisher, A.J., et al., *Elevated levels of interleukin-8 in donor lungs is associated with early graft failure after lung transplantation*. Am J Respir Crit Care Med, 2001. 163(1): p. 259-65.
5. Mathur, A., et al., *Cytokine profile after lung transplantation: correlation with allograft injury*. Ann Thorac Surg, 2006. 81(5): p. 1844-9; discussion 1849-50.
6. Trulock, E.P., et al., *Registry of the International Society for Heart and Lung Transplantation: twenty-fourth official adult lung and heart-lung transplantation report-2007*. J Heart Lung Transplant, 2007. 26(8): p. 782-95.
7. Lee, J.C. and J.D. Christie, *Primary graft dysfunction*. Clin Chest Med, 2011. 32(2): p. 279-93.
8. Salim, A., et al., *Aggressive organ donor management significantly increases the number of organs available for transplantation*. J Trauma, 2005. 58(5): p. 991-4.
9. Mascia, L., et al., *Effect of a lung protective strategy for organ donors on eligibility and availability of lungs for transplantation: a randomized controlled trial*. JAMA, 2010. 304(23): p. 2620-7.
10. Follette, D.M., S.M. Rudich, and W.D. Babcock, *Improved oxygenation and increased lung donor recovery with high-dose steroid administration after brain death*. J Heart Lung Transplant, 1998. 17(4): p. 423-9.
11. Krebs, J., et al., *Open lung approach with low tidal volume mechanical ventilation attenuates lung injury in rats with massive brain damage*. Crit Care, 2014. 18(2): p. R59.
12. Burton, C.M., et al., *Long-term survival after lung transplantation depends on development and severity of bronchiolitis obliterans syndrome*. J Heart Lung Transplant, 2007. 26(7): p. 681-6.
13. Rosendale, J.D., et al., *Aggressive pharmacologic donor management results in more transplanted organs*. Transplantation, 2003. 75(4): p. 482-7.
14. de Perrot, M., et al., *Ischemia-reperfusion-induced lung injury*. Am J Respir Crit Care Med, 2003. 167(4): p. 490-511.
15. Schnuelle, P., et al., *Effects of donor pretreatment with dopamine on graft function after kidney transplantation: a randomized controlled trial*. JAMA, 2009. 302(10): p. 1067-75.
16. Benck, U., et al., *Effects of donor pre-treatment with dopamine on survival after heart transplantation: a cohort study of heart transplant recipients nested in a randomized controlled multicenter trial*. J Am Coll Cardiol, 2011. 58(17): p. 1768-77.
17. Losel, R.M., et al., *N-octanoyl dopamine, a non-hemodynamic dopamine derivative, for cell protection during hypothermic organ preservation*. PLoS One, 2010. 5(3): p. e9713.
18. Vettel, C., et al., *Dopamine and lipophilic derivatives protect cardiomyocytes against cold preservation injury*. J Pharmacol Exp Ther, 2014. 348(1): p. 77-85.
19. Tsagogiorgas, C., et al., *N-octanoyl-dopamine is an agonist at the capsaicin receptor TRPV1 and mitigates ischemia-induced [corrected] acute kidney injury in rat*. PLoS One, 2012. 7(8): p. e43525.
20. Hottenrott, M.C., et al., *N-octanoyl dopamine inhibits the expression of a subset of kappaB regulated genes: potential role of p65 Ser276 phosphorylation*. PLoS One, 2013. 8(9): p. e73122.
21. Spindler, R.S., et al., *N-Octanoyl Dopamine for Donor Treatment in a Brain-Death Model of Kidney and Heart Transplantation*. Transplantation, 2015.
22. Marck, K.W. and C.R. Wildevuur, *Lung transplantation in the rat: I. Technique and survival*. Ann Thorac Surg, 1982. 34(1): p. 74-80.
23. Schmittgen, T.D. and K.J. Livak, *Analyzing real-time PCR data by the comparative C(T) method*. Nat Protoc, 2008. 3(6): p. 1101-8.
24. Krebs, J., et al., *Open lung approach associated with high-frequency oscillatory or low tidal volume mechanical ventilation improves respiratory function and minimizes lung injury in healthy and injured rats*. Crit Care, 2010. 14(5): p. R183.

- R1
R2
R3
R4
R5
R6
R7
R8
R9
R10
R11
R12
R13
R14
R15
R16
R17
R18
R19
R20
R21
R22
R23
R24
R25
R26
R27
R28
R29
R30
R31
R32
R33
R34
R35
R36
R37
R38
R39
25. Smith, M., *Physiologic changes during brain stem death--lessons for management of the organ donor*. J Heart Lung Transplant, 2004. 23(9 Suppl): p. S217-22.
26. McKeating, E.G., et al., *Transcranial cytokine gradients in patients requiring intensive care after acute brain injury*. Br J Anaesth, 1997. 78(5): p. 520-3.
27. Fisher, A.J., et al., *Enhanced pulmonary inflammation in organ donors following fatal non-traumatic brain injury*. Lancet, 1999. 353(9162): p. 1412-3.
28. Avlonitis, V.S., et al., *Early hemodynamic injury during donor brain death determines the severity of primary graft dysfunction after lung transplantation*. Am J Transplant, 2007. 7(1): p. 83-90.
29. Kaneda, H., et al., *Pre-implantation multiple cytokine mRNA expression analysis of donor lung grafts predicts survival after lung transplantation in humans*. Am J Transplant, 2006. 6(3): p. 544-51.
30. Kusaka, M., et al., *Genomewide expression profiles of rat model renal isografts from brain dead donors*. Transplantation, 2007. 83(1): p. 62-70.
31. Skrabal, C.A., et al., *Organ-specific regulation of pro-inflammatory molecules in heart, lung, and kidney following brain death*. J Surg Res, 2005. 123(1): p. 118-25.
32. Kusaka, M., et al., *Activation of inflammatory mediators in rat renal isografts by donor brain death*. Transplantation, 2000. 69(3): p. 405-10.
33. Zanotti, G., et al., *Novel critical role of Toll-like receptor 4 in lung ischemia-reperfusion injury and edema*. Am J Physiol Lung Cell Mol Physiol, 2009. 297(1): p. L52-63.
34. Ohmori, K., et al., *Attenuation of lung injury in allograft rejection using NF-kappaB decoy transfection--novel strategy for use in lung transplantation*. Eur J Cardiothorac Surg, 2005. 27(1): p. 23-7.
35. Stamellou, E., et al., *N-octanoyl dopamine treatment of endothelial cells induces the unfolded protein response and results in hypometabolism and tolerance to hypothermia*. PLoS One, 2014. 9(6): p. e99298.
36. Kitamura, M., *Control of NF-kappaB and inflammation by the unfolded protein response*. Int Rev Immunol, 2011. 30(1): p. 4-15.
37. De Perrot, M., et al., *Interleukin-8 release during early reperfusion predicts graft function in human lung transplantation*. Am J Respir Crit Care Med, 2002. 165(2): p. 211-5.
38. Hall, D.J., et al., *Immediate postoperative inflammatory response predicts long-term outcome in lung-transplant recipients*. Interact Cardiovasc Thorac Surg, 2012. 15(4): p. 603-7.
39. Zweers, N., et al., *Donor brain death aggravates chronic rejection after lung transplantation in rats*. Transplantation, 2004. 78(9): p. 1251-8.
40. Force, A.D.T., et al., *Acute respiratory distress syndrome: the Berlin Definition*. JAMA, 2012. 307(23): p. 2526-33.
41. Prekker, M.E., et al., *Early Trends in PaO(2)/fraction of inspired oxygen ratio predict outcome in lung transplant recipients with severe primary graft dysfunction*. Chest, 2007. 132(3): p. 991-7.
42. Chaparro, C., et al., *Acute lung injury in lung allografts*. J Heart Lung Transplant, 1995. 14(2): p. 267-73.
43. Wedel, J., et al., *N-acyl dopamine derivatives as lead compound for implementation in transplantation medicine*. Transplant Rev (Orlando), 2014.