The impact of cerebral injury in donation and transplantation

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Chapter 3 **Increased Intestinal Permeability in Deceased Brain Dead Rats**

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Abstract

Background: Deceased brain death (DBD) induces inflammation in the rat intestine as evidenced by upregulation of adhesion molecules and influx of granulocytes. We hypothesised that increased intestinal permeability, induced during brain death, causes a systemic inflammatory state which may result in distant organ failure and subsequent inferior transplant outcome.

Methods: This hypothesis was tested using an experimental model in which rats were either exposed for 4 hr to experimental brain death (n=6) or sham operated (n=6). Changes in intestinal barrier function were assessed by serum endotoxins, lipopolysaccharide binding Protein (LBP) and gene expression of LBP was assessed in intestine and liver.

Results: In the serum of DBD rats we found higher LPS and LBP levels, indicative of endotoxemia. The LBP mRNA expression in intestine and liver was significantly increased in liver and intestine in the DBD rats.

Conclusions: Our results support the hypothesis that brain death induced intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial translocation, provoking cytokine release. This vicious circle may contribute to the inflammatory reaction in potential donor organs which results in distant organ failure and inferior transplant outcome.

Introduction

Following kidney and liver transplantation, organs recovered from deceased brain dead (DBD) donors have a significant higher rate of acute rejection and chronic transplant dysfunction compared with organs from living donors, resulting in inferior transplant outcome (1). This inferior survival of deceased donation cannot be attributed to differences in immunogenicity alone (2). Matzinger's Danger model proposes that the immune response is primary concerned with entities that cause damage, rather than distinguishing between self and non-self. The hypothesis that injury is most important can be applied on the better survival outcome after transplantation between living compared and deceased donors. Antigen-presenting cells respond to danger signals from injured cells, such as those exposed to pathogens, toxins and mechanical damage (3;4). One of the potential danger signals in DBD donors is endotoxemia. Bacterial translocation and endotoxemia occur frequently in deceased brain dead organ donors (5). In other conditions, such as severe burns, brain injury and acute respiratory distress syndrome (ARDS), an enhanced intestinal permeability is associated with distant organ injury (6-8).

We hypothesized that an increased intestinal permeability, induced during brain death, causes a systemic inflammatory state which results in distant organ failure and inferior transplant outcome.

Material and Methods

ANIMALS

Adult male Fisher 344 rats (260-300g, Harlan, Horst, The Netherlands) were housed under standard conditions at the animal research facility of the University Medical Center Groningen with free access to drinking water and rat chow. The experiments were in accordance with institutional and legislatory regulations and were approved by the local Committee for Animal Experiments.

EXPERIMENTAL PROTOCOL AND STUDY DESIGN

Rats were randomly allocated to one of two experimental groups. In one group, brain death was induced (n=6) and the rats were sacrificed after four hours. The control group consisted of sham operated rats in which a trepanation was performed without inserting the balloon catheter (n=6). Sham operated rats remained ventilated with oxygen and isoflurane 2% during the entire experiment.

BRAIN DEATH INDUCTION

Brain death induction was performed, accordingly the method described by Kolkert et al. (9). Briefly, the rats were anesthezised with isoflurane and then intubated. Through a frontolateral trepanation lateral of the bregma, trepanned with a micro drill, a balloon catheter was inserted. The balloon was slowly inflated over a time period of average 30 minutes with 0.5 ml water using a Syringe pump. Brain death was confirmed by the absence of corneal reflexes and an apnoea test. Directly

after brain death induction anesthesia was stopped and all animals were ventilated with O2/air. If necessary, when mean arterial pressure (MAP) dropped below 80mmHg, animals received hemodynamic support by intra venous infusion of 10% hydroxyethylstarch (HAES) only, to achieve normotension. Ten minutes before retrieval of organs, the rats were ventilated with O2 /ISO 0.5%, to allow muscle relaxation and a laparotomy. Just before termination of the experiment, blood was collected. Jejunum, ileum and liver tissue were retrieved after a flush with saline through the abdominal aorta. Tissue samples were either stored in formaline (4%) or frozen in -80°C.

SERUM ENDOTOXINS

The Cambrex Limulus Amebocyte Lysate (LAL) kinetic-QCL® was used for the determination of endotoxin in serum. This test is validated and conform the United States Pharmacopeia. The principle of this colorimetric assay is that Gram negative bacterial endotoxin catalyzes the activation of a pro-enzym in the LAL. The initial rate of activation is determined by the concentration of endotoxin present. The activated enzyme catalyzes the splitting of p-nitroaniline (pNA) from a colorless substrate. Its reaction time of the forming of pNA, which is depending on the amount of endotoxin in the sample, is measured at 405 nm. The sample consisted of serum. Therefore the proteins had to be removed by adding perchloric acid to the 1:1000 diluted sample and then the pH was brought to pH = 7.0 by sufficient pyrogen free bicarbonate buffer.

SERUM LPS BINDING PROTEIN

Human LBP enzyme-linked immunosorbent assay (ELISA) test kit (HyCult Biotechnology, Uden, The Netherlands) were performed, according to the manufacturer's instructions, to evaluate LBP in serum samples from all rats included in the study. All samples were tested in duplicate and read at 450 nm for LBP, in microplate reader (Victor3, 1420 multilabel counter, Perkin Elmer).

SERUM CYTOKINE MEASUREMENTS

Serum cytokine levels of MCP-1 and IL-6 were analyzed via multiplex bead technology using the 13-plex kit (LINCOplex: HCYTO-60K, Linco, St. Louis, MO). The cytokine protein values were expressed in pg/ml.

REALTIME PCR

Realtime PCR was performed with SYBR green as previously described (8). The primers were designed using Primer Express 2 software (Applied Biosystems, Foster city, USA) , used for the LBP the forward primer used were 5'-AGAAGGCGCAAGTGAGCTGAT-3' and reverse primer 5'-TAGTTGAGGAATGCCTGGAACA-3', the length of the product is 75 bp.

STATISTICAL ANALYSES

All data are presented as median and [25-75 percentiles]. The Mann-Whitney U test was applied for comparison of two groups. A P-value of less than 0.05 was considered significant.

Results

All rats from the DBD group had a higher endotoxin concentration compared to the control group. The median [25-75 percentiles] were 6.2 [5.6-6.4] EU/ml in the DBD group compared to 5 [4.7-5.5] EU/ml in the control group ($P<0.05$) (Figure 1A). In accordance with the endotoxin measurement, serum LBP was significantly elevated in the DBD group 53 [45-59] ng/ml compared to control group 2.3 [1.4-3.0] ng/ml ($P<0.05$) (Figure 1B). m-RNA levels of LPS binding protein are significantly elevated in intestine and liver of DBD rats compared to living controls. In the liver in DBD rats the median fold induction is 17 [14-22] compared to 1[1-1] in the control group ($P<0.05$). In the intestine, the observed fold induction in the jejunum was 1.4 [1.2-4.5] compared to 0.9 [0.6-1.3] in the controls ($P<0.05$). In the ileum a fold induction of 3.0 [1.9-3.6] was calculated compared to 0.8 [0.8-1.3] in the controls ($P<0.05$). Serum IL-6 and MCP-1 were strongly elevated in the DBD rats. Serum IL-6 was 7300 [1880-26392] pg/ml in DBD rats compared to 225 [127-550]pg/ml in control rats. Serum MCP-1 was 8941 [6332-21841] pg/ml in the DBD group compared to 397 [252-479] pg/ml in the controls ($P<0.05$) (Figure1C+D). In addition to our previous experiments using an identical experimental brain death model, we observed inflammation in the donor intestine, characterised by an increase in the adhesion molecules E-Selectin, Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Adhesion Molecule 1 (VCAM-1), granulocytes and apoptosis on the tip of the intestinal villi, we now show high levels of LBP and LPS in DBD rats, an acute phase protein which has an important role in the response to LPS. In line with the serum data, LBP mRNA levels in the liver, the major LBP producing organ as well as in the intestine are markedly elevated.

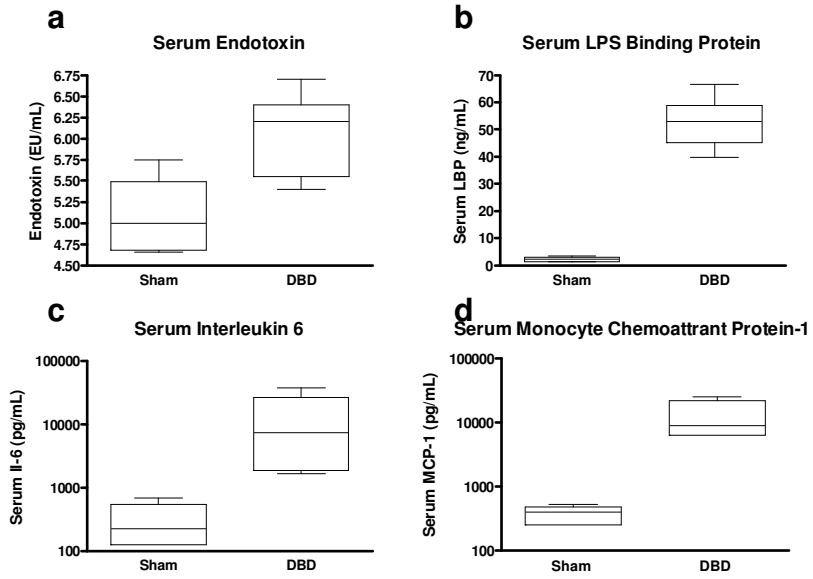

Figure 1

Serum levels of: (A) endotoxins EU/mL and (B) Lipopolysaccharide Binding Protein in ng/mL (C) Interleukin 6 (IL-6) pg/mL and (D) Monocyte Chemoattractant Protein 1 (MCP-1) ng/mL in deceased brain dead rats. The controls are represented by sham-operated animals. Box plots of six animals, the boundary of the box closest to zero indicates the 25th percentile, the line within the box marks the median of six measurements and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles

Discussion

This study shows an increase in intestinal permeability after experimental brain death induction in rats evidenced by increased serum levels of LPS and LBP compared to controls. Further, we show elevated circulation levels of the cytokines IL-6 and MCP-1, indicating a systemic inflammatory state. These potent inflammatory cytokines are key players in inflammation and chemo-attractants of granulocytes, monocytes and other inflammatory cells (10). In line with the serum data, LBP mRNA levels in the liver, the major LBP producing organ as well as in the intestine are markedly elevated.

In previous experiments using an identical experimental brain death model, we observed inflammation in the donor intestine, characterised by an increase in the

adhesion molecules E-Selectin, Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Adhesion Molecule 1 (VCAM-1), granulocytes and apoptosis on the tip of the intestinal villi (11;12). Induction of inflammation during brain dead has also been reported in other potential donor organ such as kidney, liver and lung (12-16).

In this paper we show high levels of LBP, an acute phase protein which has an important role in the response to LPS, in DBD rats. Binding of LPS to LBP is the first step in the recognition of bacterial products by the innate immune system, which leads to a disruption of LPS aggregates (17). LBP catalyzes the transfer of LPS from bacteria or micelles to CD14. LPS can also enter the cytosol directly. Many LPS responses require a complex of MD2 with Toll-like receptor 4 (TLR4) (17;18). Studies of other conditions commonly associated with endotoxemia, such as sepsis, bacterial infection and liver cirrhosis, show evident higher LBP levels (19-21).

Organ injury observed in DBD donors resembles distant injury to the lung and heart seen after severe burns. The intestine plays an important role in mediating this burn induced injuries (8;22;23). Furthermore, acute kidney injury is common in burn patients. It develops shortly after the burn and parallels other dysfunctioning organs. Although reversible, in more severe cases it correlated to mortality (24). In addition, after traumatic brain injury the intestinal permeability is increased and expected to play a role in and to contribute to multiple organ failure (6;25). Based on the observation in this study, combined with the published literature about intestinal permeability and distant organ injury (6-8;22;23;25), we consider the intestine of the DBD donor a critical player in the development of distant organ injury, which reflects organ quality. Kidneys and livers recovered from brain dead donors suffer indeed from injury and show inferior function after transplantation (26-28).

Both in the systemic inflammatory response syndrome (SIRS) and the state of brain death, the normal homeostatic balance is in dysbalance (29). When homeostasis is not restored, SIRS can result in multiple organ dysfunction syndrome (MODS). In parallel, livers derived from brain dead donors show higher mortality than livers from living donors (26;28). Also, a high LPS concentration in 14 DBD liver donors predisposed to graft loss (30). The exact mechanisms, however, by which translocating bacteria or endotoxins, and antigenic components or cytokines generated in the gut set about causing SIRS, sepsis and MODS remains unclear (31).

Summarizing, our results support the hypothesis that intestinal inflammation leads to enhanced intestinal permeability, which causes bacterial translocation, provoking cytokine release. This vicious circle enhances the inflammatory state of potential donor organs. We propose that the disturbed permeability of the intestine is responsible for the elevated endotoxin levels and thereby contributes to the inflammatory reaction in potential donor organs. Our findings indicate that protection of the intestine is a novel strategy to improve donor organ quality.

Reference List

1 Terasaki PI, Cecka JM, Gjertson DW, Takemoto S. High survival rates of kidney transplants from spousal and living unrelated donors. N Engl J Med 1995 Aug 10;333(6):333-6.
2 Futagawa Y, Waki K, Gjertson DW, Terasaki PI. Living-unrelated donors yield higher graft survival rates than parental donors. Transplantation 2005 May 15;79(9):1169-74.
3 Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991-1045.
4 Matzinger P. Friendly and dangerous signals: is the tissue in control? Nat Immunol 2007 Jan;8(1):11-3.
5 van GH, Rosman C, Grond J, Kooi K, Wubbels GH, Bleichrodt RP. Translocation of bacteria and endotoxin in organ donors. Arch Surg 1994 Oct;129(10):1063-6.
6 Hang CH, Shi JX, Li JS, Wu W, Yin HX. Alterations of intestinal mucosa structure and barrier function following traumatic brain injury in rats. World J Gastroenterol 2003 Dec;9(12):2776-81.
7 Sambol JT, Xu DZ, Adams CA, Magnotti LJ, Deitch EA. Mesenteric lymph duct ligation provides long term protection against hemorrhagic shock-induced lung injury. Shock 2000 Sep;14(3):416-9.
8 Deitch EA, Shi HP, Lu Q, Feketeova E, Skurnick J, Xu DZ. Mesenteric lymph from burned rats induces endothelial cell injury and activates neutrophils. Crit Care Med 2004 Feb;32(2):533-8.
9 Kolkert JL, 't Hart NA, van DA, Ottens PJ, Ploeg RJ, Leuvenink HG. The gradual onset brain death model: a relevant model to study organ donation and its consequences on the outcome after transplantation. Lab Anim 2007 Jul;41(3):363-71.
10 Kubes P, Ward PA. Leukocyte recruitment and the acute inflammatory response. Brain Pathol 2000 Jan;10(1):127-35.
11 Koudstaal LG, 't Hart NA, van den BA, Olinga P, van GH, Ploeg RJ, et al. Brain death causes structural and inflammatory changes in donor intestine. Transplant Proc 2005 Jan;37(1):448-9.
12 Koudstaal LG, 't Hart NA, Ottens PJ, van den BA, Ploeg RJ, van GH, et al. Brain death induces inflammation in the donor intestine. Transplantation 2008 Jul 15;86(1):148-54.
13 Takada M. Effects of explosive brain death on cytokine activation of peripheral organs in the rat. Transplantation 1998;65(12):1533-42.
14 Zweers N, Petersen AH, van der Hoeven JA, de Haan A, Ploeg RJ, de Leij LF, et al. Donor brain death aggravates chronic rejection after lung transplantation in rats. Transplantation 2004 Nov 15;78(9):1251-8.
15 van der Hoeven JA, Molema G, Ter Horst GJ, Freund RL, Wiersema J, van Schilfgaarde R, et al. Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased immunologic activation of donor kidneys. Kidney Int 2003 Nov;64(5):1874-82.
16 Der Hoeven JA, Ter Horst GJ, Molema G, de Vos P, Girbes AR, Postema F, et al. Effects of brain death and hemodynamic status on function and immunologic activation of the potential donor liver in the rat. Ann Surg 2000 Dec;232(6):804-13.
17 Jerala R. Structural biology of the LPS recognition. Int J Med Microbiol 2007 Sep;297(5):353-63.

18 Kaisho T, Akira S. Critical roles of Toll-like receptors in host defense. Crit Rev Immunol 2000;20(5):393-405.
19 Albillos A, de la HA, Gonzalez M, Moya JL, Calleja JL, Monserrat J, et al. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. Hepatology 2003 Jan;37(1):208-17.
20 Sakr Y, Burgett U, Nacul FE, Reinhart K, Brunkhorst F. Lipopolysaccharide binding protein in a surgical intensive care unit: a marker of sepsis? Crit Care Med 2008 Jul;36(7):2014-22.
21 Ubenauf KM, Krueger M, Henneke P, Berner R. Lipopolysaccharide binding protein is a potential marker for invasive bacterial infections in children. Pediatr Infect Dis J 2007 Feb;26(2):159-62.
22 Magnotti LJ, Xu DZ, Lu Q, Deitch EA. Gut-derived mesenteric lymph: a link between burn and lung injury. Arch Surg 1999 Dec;134(12):1333-40.
23 Watkins AC, Caputo FJ, Badami C, Barlos D, Xu dZ, Lu Q, et al. Mesenteric lymph duct ligation attenuates lung injury and neutrophil activation after intraperitoneal injection of endotoxin in rats. J Trauma 2008 Jan;64(1):126-30.
24 Steinvall I, Bak Z, Sjoberg F. Acute kidney injury is common, parallels organ dysfunction or failure, and carries appreciable mortality in patients with major burns: a prospective exploratory cohort study. Crit Care 2008;12(5):R124.
25 Feighery L, Smyth A, Keely S, Baird AW, O'Connor WT, Callanan JJ, et al. Increased intestinal permeability in rats subjected to traumatic frontal lobe percussion brain injury. J Trauma 2008 Jan;64(1):131-7.
26 Weiss S, Kotsch K, Francuski M, Reutzel-Selke A, Mantouvalou L, Klemz R, et al. Brain death activates donor organs and is associated with a worse I/R injury after liver transplantation. Am J Transplant 2007 Jun;7(6):1584-93.
27 Nijboer WN, Schuurs TA, van der Hoeven JA, Fekken S, Wiersema-Buist J, Leuvenink HG, et al. Effect of brain death on gene expression and tissue activation in human donor kidneys. Transplantation 2004 Oct 15;78(7):978-86.
28 van der Hoeven JA, Lindell S, van Schilfgaarde R, Molema G, Ter Horst GJ, Southard JH, et al. Donor brain death reduces survival after transplantation in rat livers preserved for 20 hr. Transplantation 2001 Nov 27;72(10):1632-6.
29 Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. Crit Care Med 1996 Jan;24(1):163-72.
30 Zipfel A, Schenk M, You MS, Lauchart W, Bode C, Viebahn R. Endotoxemia in organ donors: graft function following liver transplantation. Transpl Int 2000;13 Suppl 1:S286-S287.
31 Gatt M, Reddy BS, MacFie J. Review article: bacterial translocation in the critically ill--evidence and methods of prevention. Aliment Pharmacol Ther 2007 Apr 1;25(7):741-57.