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Time-course of plasma inflammatory mediators in a rat model of brain death

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ABSTRACT

Background: Brain death (BD) is a donor-associated risk factor that negatively affects transplantation outcome. The inflammation associated with BD appears to have a negative effect on organ quality. Complement activation, apoptosis, and pro-inflammatory cytokine and chemokine expression are significantly increased after BD. To better understand this process, we investigated plasma chemokine and cytokine levels for 8 h after BD in a rodent model.

Methods: Thirteen healthy adult male Sprague Dawley rats were intubated and mechanically ventilated. After induction of BD, animals were kept hemodynamically stable for 8 h. A panel of immune response factors, including cytokines and chemokines, were measured immediately prior to the induction of BD and at 1, 4, and 8 h after BD by multiplex analyses in 10 rats.

Results: In the early phase of BD, we observed an increase in heart rate and a decrease in mean arterial pressure. Only limited fluctuations were noted in the partial pressure of O_2, O_2 saturation, and HCO_3. Monocyte-/macrophage- and lymphocyte-derived mediators (IL-2, IL-4, and IFN-γ) increased steadily during the 8-hour monitoring period.

Conclusions: The increase in immune responses, particularly pro-inflammatory responses, after BD is time-dependent. Cytokines and chemokines from donors and recipients require further investigation to determine the optimal time frames for organ transplantation in rodent models and humans.

1. Introduction

Organ availability for transplantation is decreasing. However, the number of organ transplants using allografts from brain-dead donors has increased over the past decade. Brain death (BD) has negative effects on organ quality [1–3] and is associated with increased complement activation, apoptosis, and pro-inflammatory cytokine and hemodynamic instability [1–5]. Circulatory, hormonal, metabolic, and immunological changes after BD play an important role in graft rejection [4–10]. BD may occur after cerebral haemorrhage, trauma, or anoxia. In the acute phase, BD results in an autonomic storm, causing vasoconstriction, pulmonary congestion, late falls in temperature, and a change from aerobic to anaerobic metabolism [11,12]. Autonomic storms increase lymphokine and cytokine expression in all tissues and up-regulate major histocompatibility complex class I and II antigens. In addition, the co-stimulatory molecule B7 is up-regulated in peripheral organs including the kidney, heart, liver, and spleen [13]. Takada et al. showed that macrophage- and Th1-associated cytokine expression increases progressively in a time-dependent manner during an autonomic storm [14].

Chemokines are a group of small chemoattractant proteins that are produced by activated macrophages, monocytes, neutrophils, endothelial cells, epithelial cells, platelets, and various parenchymal cells [14]. Chemokines and cytokines help to control the selective migration and activation of inflammatory cells in injured renal tissue [14–16]. The role of inflammatory cytokines and chemokines in ischemia/reperfusion injury and ischemia/reperfusion-related diseases has been well studied [17,18]. Kusaka et al. showed that IL-1β and MCP-1 expression is transiently up-regulated in the kidneys for 6 h after BD [19]. Furthermore, inflammation was progressively activated by intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein 1 (VCAM-1) and leukocytes infiltrated the kidney for 6 h after BD [20–22]. A clinical study showed that IL-13 expression also increases after BD [23]. IL-13, tissue inhibitor of metalloproteinase 1 (TIMP-1),

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and CXCL3 are important cytokines, but their plasma concentrations have not been examined after BD. The aim of this experimental study was to measure chemokine and cytokine responses for an extended period of 8 h after BD.

2. Materials and methods

A total of 13 healthy adult male Sprague Dawley rats (Janvier, France) were purchased at 14 weeks of age and maintained in our animal colony in a 12-hour light:12-hour dark cycle at 22 °C for 2–3 weeks prior to experimentation. Two animals were housed per cage on cottonwood bedding (PK-3, LASvendi, Germany) and given food (ROD 16A, LASvendi, Germany) and water *ad libitum*.

2.1. Study design

We used 13 Sprague Dawley rats with a body weight of 350–400 g. All rats were intubated and mechanically respirated for 8 h after BD was induced. During the BD phase, respiratory and hemodynamic changes were monitored continuously. Only rats with a stable mean arterial blood pressure (MAP) of > 70 mm Hg were analysed.

2.2. Anaesthesia and ventilation

Anaesthesia was achieved by inhalation of 3–4 vol% of sevoflurane. After intubation of the trachea, all animals were mechanically ventilated (Small Animal Ventilator, RUS 1321 RA, Hugo Sachs Elektronik, Germany) with a mixture of 70% N_2O, 30% O_2, and 2–3 vol% of sevoflurane. The inspiratory peak pressure was 8–12 cm H_2O. The breathing rate was approximately 60/min and was manipulated to maintain normocapnia (assessed by blood gas analyses) and prevent an increase in positive end-expiratory pressure. Polyethylene catheters were inserted into the left femoral artery and vein. The MAP was measured and an electrocardiogram (Servomed, Hellige, Germany) was recorded by two subcutaneous needle electrodes. The tympanic temperature was maintained within a range of 36.9 to 37.8 °C by placing the animals on a heating plate (Hot Plates Type 062, Labotect, Germany).

2.3. BD induction

A 14 G Fogarty catheter (Baxter Health Corp., CA, USA) was inserted through a frontolateral trepanation into the brain parenchyma using an electric drill. Intracranial pressure was increased by slow inflation with 400–700 μl of saline. Inflation of the balloon catheter was initiated with 100 μl saline. After 1 min, the balloon catheter was inflated with a further 100 μl saline. This was repeated until BD was confirmed [24] by the absence of corneal reflexes, maximally dilated fixed pupils, and 60 s of apnoea. Brain-dead animals were kept on life support for 8 h. During this period, blood pressure, heart rate, blood gases, and core body temperature were kept within normal ranges.

2.3.1. Multiplex analysis of plasma immune factors

Levels of interleukin (IL)-1α, IL-1ß, soluble IL-1 receptor antagonist (sIL-1RA), IL-2, IL-4, IL-6, IL-10, IL-13, IL-18, interferon-gamma (IFN-γ), tumour necrosis factor-α (TNF-α), granulocyte macrophage colony-stimulating factor, vascular endothelial growth factor (VEGF), L-selectin, TMIP-1, ICAM-1, CXCL2 (macrophage inflammatory protein 2-alpha [MIP-2α]) and CXCL3 (MIP-2β) were measured by multiplex analysis. Multiplex analysis was performed using commercially available kits (R & D systems, Wiesbaden, Germany). This approach uses multi-analyte profiles based on powerful Luminex xMAP® technology (Luminex Corporation, Austin, TX, USA) to discover biomarker patterns from very small sample volumes.

Blood samples were drawn before BD was induced (baseline) and at 1, 4, and 8 h after BD. Within 2 h of blood sampling, plasma was separated from blood cells by centrifugation at 2550 × *g* for 15 min, snap frozen, and stored at − 30 °C until testing. After 40 plasma samples had been collected, plasma cytokines were measured simultaneously with the Fluorokine multi-analyte profile test. Plasma samples were thawed only once.

2.3.2. Assay of plasma biochemical parameters

Haemoglobin (Hb), haematocrit (Hct), Na^+, K^+, PO_2, PCO_2, PH, O_2 saturation, and HCO_3^+ levels were measured (RAPIDLab 348EX Blood-Gas-Analysis-System, Siemens, Munich, Germany).

2.4. Ethical statement

All procedures and housing of the animals were carried out according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health, revised in 1985. The study protocol was approved by the German Committee for Animal Care, Karlsruhe, Germany (AZ: 35-9185.81/G-15/13). Animals were maintained according to the institutional guidelines established for the Animal Care Facility at the University of Heidelberg. Following completion of the experimental protocol, the animals were sacrificed under deep anaesthesia.

2.5. Statistical analyses

All statistical analyses were performed using Statistical Package for the Social Sciences software (SPSS, 18.0; SPSS Inc., Chicago, IL, USA). Variable analysis was performed using the Friedman test. All immune parameters were expressed as median ± standard error of the mean. All p values < 0.05 after Bonferroni correction were considered statistically significant.

3. Results

Out of the 13 animals tested, 10 rats were analysed. One rat was excluded because it died from asystole 10 min after BD was induced. The other two were excluded because of unstable/low MAP.

Before BD was induced, no differences were observed in heart rate and MAP. The heart rate increased and the MAP decreased in the early phase of BD (Table 1). The animals were infused with Gelofusin (0.039 ml/min) if the MAP remained under 80 mm Hg. Normotension was achieved by fluid administration 3–10 min after Gelofusin infusion was initiated. No norepinephrine administration was used to achieve normotension. Serum sodium and potassium concentrations increased slowly after BD induction, whereas Hb and Hct concentrations decreased (Table 2). There were limited fluctuations in partial pressure of O_2 (Pa O_2), O_2 saturation (O_2 Sat) and HCO_3 (Table 2). No significant changes were observed in body temperature (≥ 36.5 °C in all animals), because a blanket was used (Table 1).

Expression of monocyte/macrophage-derived (IL-1 β, IL-6, and

Table 1
Mean arterial blood pressure (MAP) and pulses after induction of brain death in rats.

t[a]	MAP (mean ± SD)	Pulse (mean ± SD)	Temperature
0	102 ± 21	337 ± 29	37.0 ± 0.9
1	84 ± 9	377 ± 34	37.2 ± 0.2
2	102 ± 21	403 ± 50	37.2 ± 0.3
3	88 ± 12	387 ± 48	37.2 ± 0.2
4	90 ± 19	429 ± 55	37.2 ± 0.2
5	84 ± 9	450 ± 47	37.2 ± 0.1
6	84 ± 14	463 ± 50	37.2 ± 0.3
7	81 ± 16	441 ± 49	37.1 ± 0.2
8	83 ± 12	421 ± 62	37.1 ± 0.2

[a] Time after brain death in hours.