The impact of cerebral injury in donation and transplantation

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Chapter 4 **Dysfunction of Blood-Brain Barrier in Deceased Brain Dead Donors**

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Submitted

Abstract

Background: In deceased brain dead donors (DBD) an inflammatory response is seen. This response may explain the lower survival rates of DBD renal grafts compared to grafts from living donors after transplantation. We investigated whether this inflammatory response could be explained by the leakage of pro-inflammatory proteins from the injured brain into the circulation.

Patients and Methods: In DBD donors, we measured serum glial fibrillary acidic protein (GFAP) as a marker of blood-brain barrier dysfunction, and interleukin-6 (IL-6) as a key pro-inflammatory cytokine, at the beginning and the end of the brain death period.

Results: GFAP levels and IL-6 levels were increased in DBD donors compared to controls without brain injury. Both GFAP and IL-6 levels were not influenced by additional non-cranial injury. Further, in the majority of DBD donors GFAP and IL-6 levels increased during brain death. Using the Spearman coefficient, correlations between GFAP and IL-6 were $\rho=0.58$ after declaration of brain death; ($P<0.001$) and $\rho=0.63$ just before organ retrieval ($P<0.001$).

Conclusion: Our results show increased levels of GFAP in DBD donors compared to living donors at the declaration of brain death. During the brain death period, GFAP levels were markedly elevated in the majority of DBD donors, indicating a distinct dysfunction of the blood-brain barrier.

Introduction

Organs retrieved from a deceased brain dead donors (DBD) have an inferior outcome after transplantation compared to those obtained from living donors (1). It has been demonstrated that brain death induces pro-inflammatory and pro-coagulatory responses in potential donor kidneys, which enhance the immunogenicity of the graft-to-be and affect the allograft response in the recipient (2;3). The exact causes and mechanisms of brain death leading to decreased organ viability have not been determined.

Recently, it has been reported that brain injury itself has an effect on the immune system. Brain injury has been known to be an independent risk factor for infectious complications, both after traumatic brain injury (TBI) and following stroke (4;5). The initial response to brain damage is local inflammation accompanied by a more systemic response with features of the systemic inflammatory response syndrome (SIRS) (6). In addition, CNS injury has been shown to significantly increase susceptibility to infection by systemic down regulation of innate and adaptive immunity, the so called CNS-injury-induced immunodepression (CIDS) and infection (7;8).

There is strong evidence that immunomodulatory molecules produced by the injured brain may be secreted into the circulation through a defect blood-brain barrier leading to a pro-inflammatory systemic response after brain injury (9-12). Brain death, defined as the irreversible loss of function of the brain including the brainstem, is obviously the 'ultimate form' of brain injury. The mechanism described above could explain the pro-inflammatory systemic response seen in brain death.

In this study, we investigated whether this inflammatory response could be explained by the leakage of pro-inflammatory proteins from the injured brain into the circulation. We analyzed serum samples from DBD donors obtained at different time points prior to organ procurement for the presence of glial fibrillary acidic protein (GFAP) and interleukin-6 (IL-6). GFAP was studied to evaluate the function of the blood-brain barrier. It is a monomeric intermediate filament protein expressed exclusively in astrocytes in the CNS, and forms the major part of the astrocyte cytoskeleton. GFAP is an established brain injury marker and an indicator of cell destruction (13;14). IL-6 was studied as it is an important pro-inflammatory cytokine and one of the mayor physiological mediators of the acute phase reaction (15).

Patients and Methods

Starting from 2004, serum samples were routinely prospectively obtained during organ recovery procedures from a consecutive series of DBD donors in our region (N=30). As all samples were collected after declaration of brain death, no informed consent was needed according to Dutch law. Donors who had stated their objection to participate in transplantation research in the Dutch Donor Registry

were not included. Also, donors whose kidneys were discarded for transplantation after retrieval were not included in this analysis. As a control group, 20 living kidney donors were asked informed consent for two blood samples.

Serum samples were collected at two different time points. Baseline samples were collected just after the time of brain death diagnosis (T0). A second sample (T1) was obtained during organ retrieval just before perfusion. In the control group, a baseline sample was obtained just before the donor operation (T0) and a second sample (T1) was obtained at the time of kidney retrieval during the donor operation. All samples were kept on ice until return to our laboratory, where samples were centrifuged for 20 minutes at 1500 x g and stored at -80°C until analysis.

In all serum samples, creatinine was determined using the Jaffé reaction to assess kidney function in DBD donors during organ recovery. To determine GFAP levels in serum, a sandwich enzyme immunoassay was used (Human GFAP ELISA, Biovendor, Modrice, Czech Republic) following the manufacturer's instructions. To determine IL-6 levels, serum samples were measured using a multiplex bead sandwich immunoassay (Biosource, Invitrogen, Carlsbad, CA) which was analyzed using a Luminex 100 instrument (Luminex, Austin, TX).

Statistical analysis was performed using the computer program SPSS version 14.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as medians and 25 and 75 percentiles. As serum levels were skewed, statistical comparisons between unpaired groups were performed using the Mann-Whitney test. Correlation between GFAP and IL-6 at each time point was determined using the Spearman coefficient. All differences were considered to be significant at $P<0.05$.

Results

30 DBD donors were included. In their past medical history, 19 patients had a cerebrovascular accident, 8 a head trauma and one a meningitis. Of these 30 donors, five donors suffered also non-head injury, caused by trauma (4/5) and reanimation after cardiac decompensation (1/5). Donor characteristics are presented in Table 1. As shown in Table 2 and Figure 1, GFAP and IL-6 levels were markedly elevated compared to the control group of living donors.

We calculated the change of GFAP and IL-6 during brain death by subtracting T0 values from T1 values. For GFAP an increase was observed during the brain death period in 56% of DBD donors. For IL-6, an increase was observed in 62% of DBD donors. In the control group, GFAP levels remained just around detection limit, but IL-6 levels did increase in all donors. Using the Spearman coefficient, correlations between GFAP and IL6 at T0 were $\rho=0.58$; ($P<0.001$) and at T1 $\rho=0.63$ ($P<0.001$). GFAP levels did not correlate with the duration of brain death in the donor or length of hospital stay. The increased GFAP levels were not associated with other than head injury, as we showed in a subgroup analysis of DBD donors with only head injury, as shown in table 2.

Table 1 Donor Characteristics

Donor Charateristics	DBD Donors (N=30)	Control Group (N=20)[a]
Gender (M/F)	10/20	7/13
Age (years)	49 (42-57)	50 (44-58)
Cause of Death		N/A[b]
CVA	19	
Trauma	8	
Other	3	
Duration of brain death (min)[c]	679 (600-765)	N/A[b]
Hospital stay (h)	33 (24-58)	N/A[b]
Serum creatinine T0 (µmol/l)	60 (49-87)	64 (60-70)
Serum creatinine T1 (µmol/l)	57 (48-72)	64 (55-68)

[a]Control group consisted of living kidney donors.
[b]N/A, not applicable.
[c] Time between clinical declaration of brain death and perfusion)

Table 2 GFAP and IL-6 serum levels, including sub groups

Time Point	Donor Group	GFAP serum levels ng/ml	IL-6 serum levels pg/ml
T0	DBD donors (n=30)	1.31 (0.54 – 6.37)	111.2 (39.8 – 196.2)
	Head Injury Only (n=25)	1.31 (0.54 – 5.57) [a]	109.1 (39.4 – 173.3) [a]
	Controls	0 (0 – 0.03)[b]	1.1 (0.8 – 2.8)[b]
	DBD donors (n=30)	1.73 (0.62 – 3.56)	173.8 (46.1 – 490.8)
T1	Head Injury Only (n=25)	1.79 (0.75 – 3.43)[a]	190.0 (45.7 – 793.5)[a]
	Controls (n=20)	0.01 (0 – 0.04)[b]	19.2 (8.2 – 33.4)[b]

[a]Not statistically significant when compared to total DBD donor group

[b]$P<0.001$ when compared to DBD donors

Discussion

Our results show increased levels of GFAP in DBD donors compared to living donors at the declaration of brain death. During the brain death period, GFAP levels were markedly elevated in the majority of DBD donors, proving a distinct dysfunction of the blood-brain barrier. These increased GFAP levels are not associated with other than headl injury, as we showed in our subgroup analysis of DBD donors.

In this study, we investigated whether the inflammatory response in DBD donors could be associated with the leakage of pro-inflammatory proteins from the injured brain to the circulation. We have chosen healthy living donors as controls, as these donors do not have any cerebral injury, and therefore no GFAP release, but they may have some release of pro-inflammatory mediators resulting from the donor operation. To study the progression of GFAP during the brain death period, we compared levels of GFAP immediately after declaration of brain death and just prior to organ retrieval. In this way we could compare between these time points in both groups and estimate the effect of the donor operation.

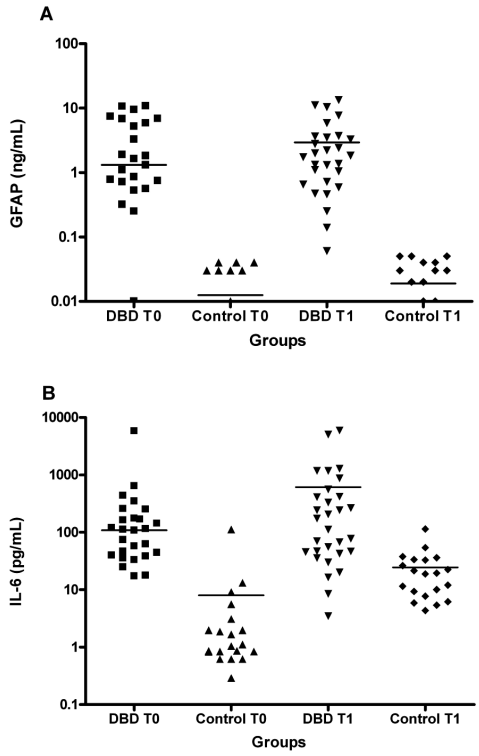

Figure 1 Graphical representation of GFAP and IL-6 serum levels.

Dot plots of GFAP (A) and interleukin-6 (IL-6) (B). Individual data points and the median are shown. Baseline samples were collected just after brain death diagnosis (T0) or at the beginning of the living donor nephrectomy in controls. A second sample (T1) was obtained during organ recovery just prior to wash-out and preservation.

The leakage of proteins from the blood brain barrier during brain death seems to contradict with a general explanation of the mechanisms of brain death. Brain death is generally explained by increased intracranial pressure, leading to progressive arrest of cerebral circulation and ultimately to brain herniation and brain stem death. Therefore, in most countries, negative tests for cerebral blood flow are accepted as a confirmatory examination for the diagnosis of brain death. Several authors have in fact stated that, because of this mechanism, a brain dead brain is not perfused at all, making the release of inflammatory substances from the brain impossible (16;17). However, there are other reports that cerebral blood flow may persist during brain death (18). In a series of 219 patients with suspected brain death, who were subjected to radionuclide angiography of the brain, some form of persistent cerebral blood flow was common (59.6%, mostly isolated venous

sinus visualisation), although arterial flow had an incidence of only 2.6% and normal flow was rare (19).

In the Netherlands, no cerebral angiography is required for the diagnosis brain death in adults. Cerebral angiography is only performed if an EEG or apnoea test is not feasible (20). In our series, no donor underwent cerebral angiography. In this study the incidence of GFAP rise during brain death in 56% of donors corresponds to Flowers's series as the amount of patients who showed some evidence of cerebral blood flow (19).

Further, we and others show that DBD donors have significantly increased IL-6 serum levels compared to living controls (21). A rise in IL-6 can be explained by tissue damage without involvement of the brain, as is seen in the differences between T0 and T1 IL-6 levels in our living kidney donors, where a rise of 10 pg/ml is seen due to injury related to the donor operation. However, in our subpopulation of DBD donors with only head injury, IL-6 levels were just as high as in DBD donors with additional injury, which may suggests a cerebral origin of circulating IL-6. To our knowledge, there are no reports supporting IL-6 predicting graft survival. However, Murugan et al showed that increased donor IL-6 level before procurement is associated with lower recipient six-month hospital-free survival (22).

Indeed, Kuecuek et al have shown that steroid treatment can decrease tissue and serum expression of pro-inflammatory cytokines in the DBD donor, although they did not report any data considering transplantation outcomes (23). Therefore, we think further study is needed to show whether treatment aimed at the reduction of inflammatory responses in the DBD donor will improve organ condition and thereby transplantation results.

In summary, GFAP levels are elevated in DBD donors. During the brain death period GFAP levels were markedly elevated in the majority of DBD donors, proving a distinct dysfunction of the blood-brain barrier, which might explain the pro-inflammatory responses in potential donor organs.

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