Activation of hemostasis in brain dead organ donors: an observational study

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Summary. Background: Brain death is associated with a systemic inflammatory response resulting in diminished organ function in individuals transplanted with organs from brain dead donors. As inflammation is accompanied by activation of coagulation, we hypothesized that activation of hemostasis occurs in brain dead organ donors. Objectives: To assess the hemostatic status in brain dead organ donors. Patients and methods: In this study, we systematically assessed the hemostatic system in samples taken from 30 brain dead donors. As controls, blood samples from 30 living kidney donors were included. Results and conclusions: Compared with the living donors, brain dead donors showed significant platelet activation (assessed by glycocalicin plasma levels), and a profound dysbalance in the von Willebrand factor/ADAMTS13 axis, which is key in platelet attachment to damaged vasculature. Furthermore, compared with the living donors, brain dead donors showed a significantly increased activation of secondary hemostasis with formation of fibrin (assessed by plasma levels of prothrombin fragment 1 + 2, fibrinopeptide A and D-dimer). Finally, brain dead donors showed profound hypofibrinolysis as assessed by a global clot lysis assay, which was attributed to substantially elevated plasma levels of plasminogen activator inhibitor type 1. Collectively, our results show activation of hemostasis and dysregulated fibrinolysis in brain dead organ donors. This prothrombotic state may contribute to formation of microthrombi in transplantable organs, which potentially contributes to deterioration of organ function.

Keywords: brain death, coagulation, fibrinolysis, inflammation, transplantation.

Introduction

Solid organ transplantation has become a routine procedure for treatment of patients with end-stage organ diseases. To date the majority of transplanted solid organs are obtained from brain-dead donors, although kidneys and partial liver grafts can be retrieved from living donors. Organs retrieved from brain dead organ donors are far from ideal and perform much worse in terms of early graft function as well as long-term graft survival compared with those derived from living donors [1,2]. The mechanisms by which donor brain death affects organ function are multifactorial and include major alterations in hemodynamics, hormonal changes, and a systemic inflammatory status. Activation of inflammation has been demonstrated in many transplantable organs, and it has been proposed that the inflammatory response in the end-organ (the graft-to-be) is key in brain death-mediated deterioration of organ function [3–5]. Indeed, both experimental models and clinical studies have shown that anti-inflammatory strategies will improve organ function after transplantation of organs procured from brain dead donors [6,7].

It has been well established that inflammatory responses are accompanied by activation of the hemostatic system. Inflammation-induced activation of hemostasis may range from subtle changes in sensitive makers of coagulation activation to fulminant disseminated intravascular coagulation, as observed in patients with severe sepsis. The molecular pathways linking inflammation and coagulation have been well established and include cytokines such as interleukin 6 (IL6) and tumor necrosis factor alpha (TNFα) [8]. This crosstalk acts in two directions: antagonism of IL6 diminishes inflammation-associated coagulation activation [9], while antihemostatic treatment by activated protein C is beneficial for the treatment of inflammatory diseases, such as bacterial sepsis [10].

As the inflammatory response related to brain death with elevation of cytokines such as IL6 and TNFα has been well established [11], we hypothesized that brain death can also result in activation of the hemostatic system. Systemic (low-grade) activation of the hemostatic system in brain dead organ donors may lead to clot formation in the microvasculature of transplantable organs. Recently, in both experimental and clinical studies the presence of platelet and fibrin deposition in kidneys and other transplantable organs, including liver and lungs, has been demonstrated [12,13].

In this study, we have systematically assessed the hemostatic status of brain dead organ donors compared with living kidney...
donors. We investigated the function of the primary hemostatic system, which results in the formation of a blood platelet plug, and the secondary hemostatic system, which leads to the deposition of fibrin. Furthermore, we have studied the fibrinolytic system, which is critical in removing physiological or pathological thrombi. Our analyses demonstrate activation of blood platelets, evidence for fibrin generation, and a decreased capacity to clear fibrin clots in brain dead organ donors. This prothrombotic state may contribute to formation of microthrombi in transplantable organs, which may compromise adequate preservation and contribute to a deterioration of organ viability and increased chance of non- or delayed graft function.

Materials and methods

Patient characteristics and sampling

Between 2009 and 2011, we included 30 brain dead individuals who underwent organ retrieval in hospitals in the Netherlands for transplantation purposes. Of these 30, seven became brain dead as a result of trauma, 15 had a hemorrhagic stroke or subarachnoid bleeding as a cause of brain death, and eight had other causes of brain death. All individuals were declared brain dead at an intensive care unit, and a citrated blood sample was taken short after declaration of brain death. A second blood sample was taken after the subjects underwent organ retrieval in hospitals in the Netherlands for transplantation purposes. Of these 30, seven received plasma from 20 healthy volunteers and was a generous gift from Dr Joost Meijers (AMC, Amsterdam, the Netherlands).

Assays

von Willebrand factor antígen (VWF:ag) plasma levels were determined using an in-house enzyme-linked immunosorbent assay (ELISA) using commercially available polyclonal antibodies (Dako, Glostrup, Denmark). VWF:ag levels were expressed as percentages relative to pooled normal plasma. VWF propeptide levels were determined with a commercially available kit according to the instructions of the manufacturer (Gen-Probe GTI Diagnostics Inc., Waukesha, WI, USA). A disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13 (ADAMTS13) antigen levels were determined with a commercially available kit (Bethyl laboratories, Montgomery, TX, USA) according to the instructions of the manufacturer. Plasma levels of glyecocalcin were determined using an in-house ELISA as described [14]. Plasma levels of prothrombin fragment 1 + 2 (F1 + 2) were determined using a commercially available ELISA kit (Siemens Healthcare, Marburg, Germany), as were levels of fibrinopeptide A (FPA) and D-dimer (both from American Diagnostica, Stamford, CT, USA) and plasmin-antiplasmin (PAP) complexes (Technoclone, Vienna, Austria). Plasma levels of tissue-type plasminogen activator (tPA) and plasminogen activator inhibitor 1 (PAI-1) were determined using in-house ELISAs using commercially available antibodies as described [15].

Overall plasma fibrinolytic potential was determined using an in-house assay as previously described [16]. Briefly, 50 µL plasma was pipetted into the wells of a 96-well microtitre plate. Subsequently, 50 µL of a mixture containing phospholipid vesicles (40% L-α-dioleoylphosphatidylcholine, 20% L-α-dioleoylphosphatidylserine and 40% L-α-dioleoylphosphatidylethanolamine, final concentration 10 µmol L⁻¹), t-PA (final concentration 56 ng mL⁻¹), tissue factor (final dilution 1/1000) and CaCl₂ (final concentration 17 mmol L⁻¹) diluted in HEPES buffer (25 mmol L⁻¹ HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), 137 mmol L⁻¹ NaCl, 3.5 mmol L⁻¹ KCl, 3 mmol L⁻¹ CaCl₂, 0.1% bovine serum albumin (BSA), pH 7.4), was added using a multichannel pipette. After thorough mixing, the plate was incubated at 37 °C in a Spectramax 340 kinetic microplate reader (Molecular Devices Corporation, Menlo Park, CA, USA), and the optical density (OD) at 405 nm was monitored every 20 s, resulting in a clot-lysis turbidity profile. The clot lysis time was derived from this clot-lysis profile and defined as the time from the midpoint of the clear to maximum turbid transition, representing clot formation, to the midpoint of the maximum turbid to clear transition, representing the lysis of the clot.

Statistical analyses

Data are shown as box-and-whisker plots. Comparisons were made using standard t-test or a Mann–Whitney U-test, as appropriate. All calculations were performed using the GraphPad InStat software package (GraphPad, San Diego, CA, USA). P-values < 0.05 were considered statistically significant.
Results

A dysbalanced VWF/ADAMTS13 axis and evidence for blood platelet activation in brain dead organ donors

Compared with living kidney donors, brain dead organ donors showed a substantial and significant increase in plasma levels of VWF (Fig. 1A). Surprisingly, no significant differences in VWF levels were present between donors who did or did not receive desmopressin [t = 0: no desmopressin 203 ± 117% (mean ± standard deviation), desmopressin 245 ± 102%, P = 0.31; t = 1: no desmopressin 187 ± 84%, desmopressin 200 ± 80%, P = 0.67]. Plasma levels of VWF propeptide were substantially elevated in brain dead donors at t = 0, but no differences between propeptide levels between brain dead and living organ donors were present at t = 1 (Fig. 1B). VWF propeptide levels in the living donors significantly increased between t = 0 and t = 1. Plasma levels of the VWF-cleaving protease ADAMTS13, which regulates VWF reactivity, were significantly and substantially lower in the brain dead donors compared with the living kidney donors (Fig. 1C). Profound differences between VWF and ADAMTS13 levels in brain dead compared with living donors were observed at both time points investigated. Furthermore, the ADAMTS13 levels dropped slightly but significantly between t = 0 and t = 1 in the living donors. The VWF/ADAMTS13 ratio was profoundly elevated in the brain dead donors as shown in Fig. 1(D). A dysbalance in the VWF/ADAMTS13 ratio may indicate an increased potential for unregulated platelet thrombus formation. Indeed, plasma levels of glycocalicin, a fragment of the platelet membrane glycoprotein Ibα that is shed upon platelet activation, were around 1.5 times higher in brain dead organ donors compared with living donors at t = 0 (Fig. 1E). In the brain dead donors, glycocalicin plasma levels dropped between t = 0 and t = 1, which either indicates attenuation of platelet activation, or dilution of the glycocalicin as a result of fluid administration.

Activation of coagulation and evidence for fibrin generation in brain dead organ donors

Compared with living donors, brain dead donors showed a significant elevation in plasma levels of prothrombin fragment 1 + 2 (F1 + 2) (Fig. 2A). Furthermore, an increase in F1 + 2 levels was observed between t = 0 and t = 1 in the living donors, which is likely to indicate activation of coagulation as a result of the surgical procedure. Plasma levels of FPA, which is released when thrombin converts fibrinogen to fibrin, increased significantly between t = 0 and t = 1 in the living donors (Fig. 2B).
Dysfunctional fibrinolysis in brain dead organ donors

Plasma levels of tPA, which initiates the fibrinolytic cascade, were not different between brain dead and living donors (Fig. 3A). In contrast, PAI-1, the physiological inhibitor of tPA, was substantially and significantly elevated in brain dead donors at \( t = 0 \), although at \( t = 1 \) levels were similar to those observed in the living donors (Fig. 3B). In line with this, overall plasma fibrinolytic potential was substantially diminished in brain dead donors at \( t = 0 \) (Fig. 3C). Despite a decreased fibrinolytic potential, plasma levels of PAP complexes were significantly elevated in the brain dead organ donor (Fig. 3D).

Discussion

This study demonstrates that brain death in organ donors is associated with substantial changes in the hemostatic system compared with living kidney donors. Specifically, a marked VWF/ADAMTS13 dysbalance and elevated plasma levels of glycoprocalcin are indicative of an increased activity of primary hemostasis, whereas elevated plasma levels of F1 + 2, FPA and D-dimer indicate ongoing activation of coagulation. Furthermore, a decreased fibrinolytic potential with a paradoxical increase in ongoing plasmin generation as assessed by PAP complex concentrations indicate a disturbed regulation of clot dissolution. The combined results of our investigations point to a systemic procoagulant status of the brain dead organ donor.

The procoagulant status in the brain dead organ donor is likely to be linked to the well-described proinflammatory status that develops after brain death [11]. Significantly elevated levels of IL6, which are known to link inflammation and activation of coagulation, have been found in brain dead individuals and in experimental animal models [5,17]. We hypothesize that the procoagulant status in the brain dead donor results in formation of microthrombi in transplantable organs that may affect organ quality. Previous analyses in both animal models and human biopsies have shown platelet and fibrin deposition in kidneys following brain death [12,13]. Clot formation in the organ microvasculature during the phase of donor management and prior to retrieval may diminish organ quality by ischemia or by direct toxic effects of these thrombi on cells [18,19]. Also, subsequent preservation may be hampered or inadequate as parenchymal tissues of the grafts-to-be are not well reached and preserved with the preservation solutions. In addition, it has been demonstrated that hemostatic activation increases organ damage in experimental models of ischemia and reperfusion injury [20–22]. Furthermore, in experimental sepsis models, antithrombotic therapy decreased the damage to liver, kidney and lungs, and is accompanied by a diminished platelet and/or fibrin deposition in these organs [23,24]. Our findings suggest that coagulation activation in brain dead organ donors results in organ damage, although this hypothesis requires further experimental testing.

The severely disturbed VWF/ADAMTS13 balance observed in the brain dead organ donor may also point to an increased capacity for systemic unregulated generation of microthrombi, as it occurs in patients with a complete deficiency of ADAMTS13 [25]. The situation in brain dead organ donors highly resembles that of patients with severe sepsis, in whom the levels of VWF/ADAMTS13 dysbalance correlate with severity of inflammation, degree of organ failure and mortality [26–28]. Whether these donors with the most unfavourable VWF/ADAMTS13 balance are in effect those whose organs function particularly poorly after transplantation does require further study. The increased VWF propeptide levels in the brain dead organ donors indicate that the increase in VWF levels was due to an acute activation of the endothelium [29]. Surprisingly, in
the living donors VWF propeptide, but not VWF itself, was increased at \( t = 1 \) compared with \( t = 0 \), which might indicate that during the surgical procedure acute VWF release with a concomitant consumption of VWF due to surgical hemostasis occurs. The drop in ADAMTS13 levels in the living donors between \( t = 0 \) and \( t = 1 \) is consistent with acute release of VWF, because ADAMTS13 has been shown to decrease after endothelial activation by desmopressin [30]. Previously, we have observed decreasing ADAMTS13 levels with constant VWF levels during liver transplant surgery, which we also attributed to a concomitant release of fresh VWF and VWF consumption due to surgical hemostasis [31].

The parameters used to assess activation of the coagulation system are clearly indicative of thrombin and fibrin generation in the brain dead donor. In particular, the substantially increased levels of D-dimer are consistent with a prothrombotic status. D-dimer levels are elevated in many prothrombotic states, and have a pivotal role in the diagnostic work-up of patients with a suspicion of venous thrombosis [32,33]. Also, in the living donor some activation of the coagulation system is observed. However, the increase in coagulation activation in the living organ donor can be attributed, at least in part, to vessel wall damage induced by surgery, which exposes initiators of coagulation to the bloodstream. In our study, the brain dead organ donor samples were taken before organ retrieval, so coagulation activation occurs in the absence of surgical dissection of vessels, and must be initiated by different triggers (e.g. inflammation).

Finally, we observed a hypofibrinolytic state, as indicated by elevated clot lysis times and elevated plasma levels of PAI-1, in the brain dead donor, which suggests that the clots that are generated are cleared less efficiently. A hypofibrinolytic status, as assessed by our plasma-based assay, has been associated with an elevated risk of venous and arterial thrombosis [15,34]. Nevertheless, plasma levels of D-dimer, which indicate ongoing fibrin clot lysis, suggest that in the brain dead donor, clots are actively being removed. This apparent paradox may be explained in several ways. In one scenario, clot lysis does occur in the brain dead donor, but is insufficient to clear all fibrin that is generated, which results in a net accumulation of fibrin. This theory is compatible with the observations of fibrin-rich clots in organs of brain dead donors [12,13]. In a second scenario, endogenous (tPA-mediated) fibrinolysis contributes little to the lysis of fibrin clots. Instead, it is mediated by other activators of plasminogen, which are generated in pathological situations. Notably, leukocyte elastase may be responsible for fibrin degradation in the brain dead donor, similar to the situation that has recently been described in patients with severe sepsis as well as after graft reperfusion in liver transplant recipients [35,36]. However, the increased PAP levels indicate active plasmin generation, and therefore the relative contribution of plasminogen-dependent and independent
mechanisms to in vivo clot lysis in brain dead donors remains to be investigated.

The brain dead donors investigated in this study became brain dead as a result of different underlying causes, some of which may have initiated coagulation activation by itself (e.g. in patients with major trauma). We did not, however, detect differences in any of the hemostatic markers assessed in the study between subjects with different causes of brain death (data not shown). Also, we were not able to find a correlation between any of the markers and the duration of brain death (data not shown). The lack of both correlations can be the result of the relatively small cohort studied.

In conclusion, in this study we have performed a systematic analysis of the hemostatic system in brain dead organ donors and observed a marked procoagulant status. We suggest that the inflammation-driven activation of coagulation during brain death and donor management leads to formation of microthrombi in transplantable organs. These microthrombi may then hamper adequate preservation of organs retrieved from brain dead donors and contribute to an increased chance of non- or delayed graft function after transplantation when compared with organs retrieved from living donors. Further study is now warranted to investigate whether anticoagulant therapy prior to organ retrieval can be beneficial and protect organ function in these donors, while enhancing preservation and early graft function after transplantation.

Disclosure of Conflict of Interests
The authors state that they have no conflict of interest.

References


