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Microparticles impact coagulation after traumatic brain injury

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ABSTRACT

Background: The pathophysiology that drives the subacute hypercoagulable state commonly seen after traumatic brain injury (TBI) is not well understood. Alterations caused by TBI in platelet and microparticle (MP) numbers and function have been suggested as possible causes; however, the contributions of platelets and MPs are currently unknown.

Materials and methods: A weight-drop technique of TBI using a murine model of moderate head injury was used. Blood was collected at intervals after injury. MP enumeration and characterization were performed using Nanoparticle Tracking Analysis, and platelet counts and coagulation parameters were determined using thromboelastometry. A MP procoagulant assay was used to compare activity between injured and sham mice.

Results: At 24 h after injury, there were no changes in circulating platelet numbers. However, there was a decrease in platelet contribution to clot formation. In contrast, there was a decline in circulating total MP numbers. When MPs from sham mice were added to the blood from head-injured animals, there was a normalization of platelet contribution to clot formation. Conversely, when MPs from TBI mice were added to sham blood, there was a significant decrease in platelet contribution to clot formation. Notably, there was an increase in MP procoagulant activity in head-injured mice.

Conclusions: MPs generated after TBI likely contribute to altered coagulation after head injury and may play a key role in the development of a posttraumatic hypercoagulable state in TBI patients.

1. Introduction

Traumatic brain injury (TBI) results in significant disability and mortality among trauma populations. Mortality rates have remained relatively stable over the past 10 years suggesting a need for better understanding and treatment of this disease process [1]. One of the major pathophysiologic disturbances shown to be predictive of increased morbidity and mortality after TBI is acute traumatic coagulopathy, with reported incidences ranges from 10%–97.5% [2–5]. After this immediate hypocoagulable state, a subacute hypercoagulable state develops with the incidence of deep vein thrombosis ranging

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from 1.8%–44% in major trauma patients [6–8]. More specifically, the presence of head injury is predictive of an increased risk for thrombotic events [9]. Although the mechanisms behind these changes are not fully understood [10–12], the commonly proposed causes include consumptive coagulopathy [13], tissue factor release [14], and platelet dysfunction [4,15–17].

More recent investigations have begun to explore the role of microparticles (MPs) in this process, as they have been identified as markers of and possible contributors to the hypercoagulable states seen in venous thromboembolism [18], cardiovascular disease [19], cancers [20], prothrombotic pregnancy complications [21], and posttraumatic states [22,23]. MPs are defined as 0.1–1 μm vesicles that bleb off cell membranes of activated or apoptotic cells and function in cell–cell communication [24]. Procoagulant MPs are often identified as those expressing tissue factor or phosphatidylserine, and previous studies have found them to be increased in the cerebrospinal fluid and blood of patients after intracranial hemorrhage [25] and head trauma [26–28]. However, the role of these particles in altering coagulation, and more specifically platelet function, has not been explored.

In the present study, we used a murine model to characterize alterations in coagulation after TBI to determine whether MPs contribute to these changes. Our hypotheses were that (1) TBI would result in a subacute hypercoagulable state, (2) increased circulating MPs would be seen after TBI, and (3) the changes in coagulation would be due to the altered MP populations.

2. Materials and methods

2.1. Materials

Male C57BL/6 mice between ages 6 and 8 wk and 20–28 g obtained from Taconic Labs (Hudson, NY) were used for all experiments. Only male mice were used as previous research has demonstrated that higher estrogen levels can impact immune status after trauma [29]. All murine experiments were performed between 8 AM and 10 AM and were performed under protocols approved by the Institutional Animal Care and Use Committee of the University of Cincinnati.

2.2. Traumatic brain injury

Mice were anesthetized with 2% inhaled isoflurane, and a traumatic closed-head injury was induced using an established weight-drop model [30]. In short, after anesthesia, mice were placed prone on a platform, and the posterior coronal suture of the mice was aligned below the weight. A 400 g weight was then dropped 1 cm to induce head injury. Sham mice were anesthetized and laid on the platform without head injury. Righting reflex time was measured immediately after TBI to ensure an injury of moderate severity.

2.3. Thromboelastometry

Rotational thromboelastometry (ROTEM; TEM Systems Inc, Durham, NC) analyses were performed to determine alterations in coagulation per manufacturer instructions. Whole blood collected via cardiac puncture from sham and TBI mice was anticoagulated with 10% citrate. All analyses were initiated within 10 min of whole blood collection. Overall coagulation was determined using NATEM, extrinsic pathway coagulation using EXTEM, and fibrin contribution to clot using the FIBTEM analysis. For EXTEM and FIBTEM analysis, 20 μL of thromboplastin and cytochalasin D were added to 300 μL of citrated blood. Clotting time (CT), clot formation time (CFT), clot lysis (LI30), α-angle (AA), and maximum clot firmness (MCF) were determined for each test. Treated samples had 30 μL of saline or MP preparations added. MPs were isolated as described in section 2.5. Percent of platelet contribution (%MCF-Platelet) was calculated by the equation: $(EXTEM_{MCF} - FIBTEM_{MCF})/EXTEM_{MCF}$, similar to the methods used by Kornblith *et al.* [31]. For NATEM analysis, sham mice ($n = 9$) and TBI mice at 10 min ($n = 7$), 24 h ($n = 10$), and 72 h ($n = 11$) were used. For EXTEM and FIBTEM analyses, the same treatment groups were used with 8–10 mice per group in both the untreated and MP-treated samples.

2.4. Platelet count determination

Whole blood collected via cardiac puncture from injured mice was anticoagulated with 10% citrate. Coulter AcT 10 Hematology Analyzer (Beckman Coulter, Brea, CA) was used to determine platelet counts.

2.5. MP isolation, enumeration, and characterization

The MP isolation protocol used was adapted from those previously published from our laboratory [32]. In short, whole blood collected via cardiac puncture from injured mice was anticoagulated with heparin. It was then centrifuged at 450*g* for 10 min; the supernatant was collected and centrifuged at 10,000*g* for 10 min to remove platelets. The platelet-free supernatant containing the MPs was then diluted at 1:1000 with Roswell Park Memorial Institute media and stained with 10 μL per sample CD41 antibody (Clone MWReg30; BD Pharmingen, San Jose, CA). Nanoparticle Tracking Analysis (NanoSight; Malvern Instruments Ltd, Worcestershire, United Kingdom) was then used to enumerate total and CD41+ MP concentrations. MP populations were characterized in sham mice ($n = 26$) and injured mice at 30 min ($n = 13$), 3 h ($n = 9$), 24 h ($n = 21$), and 72 h ($n = 25$) after TBI. For MPs used in ROTEM analyses, platelet-free plasma was collected per previously mentioned protocols and centrifuged at 25,000*g* for 30 min to pellet the MPs. MPs were then resuspended in sterile saline to a concentration of 3.0×10^8 MPs/mL and added to anticoagulated blood as stated in section 2.3. MPs isolated 24 h after injury from sham mouse blood will be referred to as sham MPs and those isolated from TBI mouse blood will be referred to as TBI MPs.

2.6. MP procoagulant activity

MP procoagulant activity was determined using a Zymuphen MP-Activity functional assay (Aniara, West Chester, OH). This assay first exposed the phospholipids on the surface of the MPs and then measured the activation of prothrombin into thrombin by these exposed phospholipids. Whole blood was collected via cardiac puncture from sham ($n = 8$) and TBI mice ($n = 7$) 24 h after injury and anticoagulated with 10% citrate.