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Review

Traumatic Brain Injury-Associated Coagulopathy

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Abstract

Traumatic injury is a common cause of coagulopathy, primarily due to blood loss and hemodilution secondary to fluid resuscitation. Traumatic injury-associated coagulopathy often follows a course of transition from hyper- to hypocoagulable state exemplified in disseminated intravascular coagulation. The incidence of coagulopathy is significantly higher in patients with traumatic brain injury (TBI), especially those with penetrating trauma compared to injury to the trunk and limbs. This occurs despite the fact that patients with isolated TBI bleed less and receive restricted volume load of fluids. TBI-associated coagulopathy is extensively documented to associate with poor clinical outcomes, but its pathophysiology remains poorly understood. Studies in the past have shown that brain tissue is highly enriched in key procoagulant molecules. This review focuses on the biochemical and cellular characteristics of these molecules and pathways that could make brain uniquely procoagulant and prone to coagulopathy. Understanding this unique procoagulant environment will help to identify new therapeutic targets that could reverse a state of coagulopathy with minimal impacts on hemostasis, a critical requirement for neurosurgical treatments of TBI.

Key words: coagulopathy; microparticles; tissue factor; traumatic brain injury

Introduction

Uncontrolled hemorrhage is a leading and preventable cause of death in patients with traumatic injury, accounting for 30–40% of all trauma fatalities.[1–3] It is primarily caused by direct injury to the vasculature and secondary coagulopathy. The cause of trauma-associated coagulopathy is multifactorial, including the consumption and/or dilution of coagulation factors and platelets, dysfunctions of platelet and coagulation system, and an increase in fibrinolysis.[4–6] A deteriorating physiology in severely injured patients, represented by a vicious cycle of coagulopathy, hypothermia, and acidosis, is often referred to as a lethal triad.[7,8]

Retrospective and observational studies have consistently shown that trauma-associated coagulopathy is more common in patients with traumatic brain injury (TBI),[9–11] but its prevalence varies considerably in the literature, ranging from 10 to 97.2%.[11] Many factors contribute to this variation: heterogeneity of the patients, types of laboratory tests used to define coagulopathy, the timing of performing these tests,[12–14] and last, but not the least, the lack of a clear consensus to define TBI-associated coagulopathy. TBI patients with coagulopathy are known to have poor outcomes[13–15] because they suffer more severe injury,[16,17] have delayed or progressive bleeding,[18–21] are prone to ischemic secondary injury,[22] and develop microvascular thrombosis.[18,19,21,23] Among injury types, coagulopathy is more common in patients with penetrating injury,[14] potentially due to more severe damage to cerebral tissue and disruption of the blood brain barrier. However, blunt TBI has increasingly been recognized for being capable of activating coagulation pathways.[10] In one report, the overall hospital mortality for isolated blunt TBI with coagulopathy was 50.4% as compared to 17.3% for patients without coagulopathy (an adjusted odds ratio of 2.97).[24] A recent study found that a low Glasgow Coma Scale ($GCS \leq 8$) or a high Injury Severity Score ($ISS \geq 16$), hypotension on admission, and the presence of cerebral edema, subarachnoid hemorrhage, and midline shift are independent risk factors for developing coagulopathy in patients with isolated TBI.[14] This risk association is supported by Cap and colleagues,[16] showing that GCS and ISS are independently associated with an increased risk for coagulopathy in U.S. combat casualties in Iraq and Afghanistan. These clinical epidemiological data are consistent with our observation at a busy urban level 1 trauma center for the four Northwestern states in the United States and the University General Hospital of Tianjin Medical University in China.

Despite extensive research in the past, mechanisms for the initiation and propagation of coagulopathy in patients with TBI remain poorly understood. Moreover, coagulopathy after TBI may follow a distinct mechanistic pathway as opposed to that arising after trauma to the trunk and limbs. For the latter patients, extensive blood loss (hemorrhagic shock) and fluid resuscitation (hemodilution and hypothermia) are major causes of coagulopathy.

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However, heavy blood loss is not common and the volume of fluid resuscitation is often restricted in patients with isolated TBI, suggesting that these factors may not be critical in TBI-associated coagulopathy. This review discusses the unique biochemical and cellular characteristics of the brain that make it uniquely procoagulant and prone to coagulopathy.

Platelets and Platelet-activating Factor

Hemostasis begins when platelets are captured to the subendothelial matrix exposed at sites of vascular injury to form a platelet-rich plug that is subsequently stabilized by fibrin crosslinking resulting from activating coagulation cascade and thrombin generation (Fig. 1). Platelet functions after TBI have not been systemically investigated, but circumstantial evidence, such as low platelet counts without heavy blood loss and spontaneous platelet aggregation,[25–27] suggests that TBI may result in platelet hyperactivity, a condition of platelets being partially activated or primed for activation. Although reported to be in normal range, platelet aggregation was often induced by agonists at maximal dosages that would not distinguish between normal and hyperactive platelets.[28,29] The presence of platelet hyperactivity is also supported by the finding of intravascular microthrombosis, now considered to be more common in TBI patients than clinically recognized.[9,23,30,31] Enhanced platelet aggregation and microthrombosis in the lungs and brain have been verified in animal models of TBI.[23,32,33] These brain microthrombi are mostly detected in the peri-contusion cortex[34] and contain platelets,[23,34] fibrin,[32] and von Willebrand factor (VWF).[34]

Multiple factors could make platelets hyperactive. Among them, platelet activating factor (PAF, 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine) may play a unique role. PAF was first identified as a basophil-derived molecule that induces aggregation and serotonin release from rabbit platelets.[35,36] It was subsequently found to be synthesized and secreted from many cell types through constitutive and induced pathways.[37] Cells synthesizing PAF are often also targeted by it, making PAF one of the classic autacoids. PAF acts on target cells through a G-protein-coupled seven transmembrane receptor.[37–39] Platelets have two types of PAF receptors: one with a high affinity (kDa, 37 ± 13 nM) and low abundance (1399 ± 498 sites/platelet) and the other with a low affinity and high abundance. Upon binding to the high-affinity receptor, PAF initiates intracellular signals to activate phospholipases C and A2 that hydrolyze phosphoinositide to release arachidonic acid, mobilize cytosolic calcium, and activate protein kinase C in platelets.[37] Leukocytes[39–41] and vascular endothelial cells[42,43] also express one or both receptors for PAF. The brain and spinal cord are a richer source of PAF than other tissues[44–46] and response to PAF through a G protein-coupled neuronal PAF receptor that is sensitive to pertussis toxin.[47–49] Neural cells release PAF during cerebral

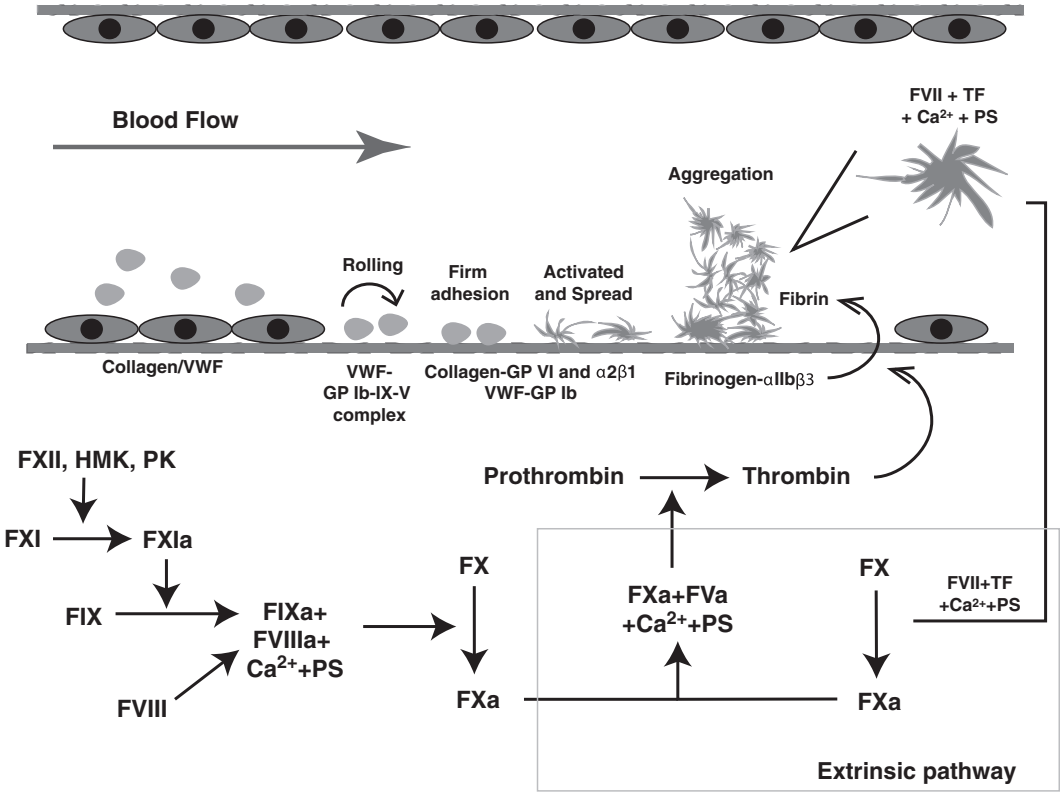

FIG. 1. Schematic of hemostasis and coagulation. Hemostasis is initiated by an interaction between von Willebrand factor (VWF) in the subendothelium and the GP Ib-IX-V complex on platelets that slows down the movement of platelets to engage other ligand-receptor interactions with slower on-rates. Platelets are rapidly activated and firmly adhere to the subendothelium through an interaction between collagen matrix and GP VI and the integrin $\alpha 2\beta 1$. Activated platelets aggregate by fibrinogen crosslinking the integrin $\alpha IIb\beta 3$. They also provide a phosphatidylserine (PS)-rich surface on which tissue factor forms a complex with coagulation factor VIIa to initiate the extrinsic pathway of coagulation. Thrombin generated through this pathway cleaves fibrinogen to fibrin that forms laterally associated fibrils to stabilize platelet aggregation. Several procoagulant molecules are enriched in the brain and key steps of hemostasis and coagulation are uniquely enhanced in TBI.

ischemia[50–52] and tissue hypoxia.[53] Lindsberg and colleagues[54] reported that the levels of PAF were increased by approximately 20-fold from baseline in rats with sustained ischemic and reperfusion injury to the spinal cord.

As an autacoid, extensive studies in the past have been focused on PAF's effects on neurological functions and inflammation.[55–58] A role of PAF in developing TBI-associated coagulopathy remains largely unknown, but should be expected because it is a potent platelet agonist. In addition, PAF also contributes to the hypoxia-induced breakdown of the blood brain barrier,[58] potentially resulting in the release of PAF and other brain-derived prothrombotic molecules to the systemic circulation. Several PAF antagonists are reported to attenuate ischemic edema, early post-ischemic hyperemia, and intravascular microthrombosis in animal models.[23,59,60] It is, however, not known whether these PAF antagonists improve clinical or experimental outcomes by reducing PAF-associated neurotoxicity or coagulopathy. Further, the pre-injury use of aspirin, which blocks platelet aggregation induced by arachidonic acid, a polyunsaturated omega-6 fatty acid released from platelets and neural membrane, and the platelet ADP P2Y12 receptor antagonist clopidogrel failed to improve clinical outcome of TBI.[61,62] These apparent conflicting data regarding the clinical efficacy of platelet antagonists beg for answers to a key question of whether globally inhibiting platelets improve clinical outcomes of TBI without aggravating the risk for intracranial bleeding. In this regard, specifically blocking PAF activity may prevent TBI-induced platelet hyperactivity without inhibiting systemic hemostasis.

Platelet-endothelial Interactions

Similar to platelet hyperactivity, a role played by major platelet ligands in TBI-associated coagulopathy is also not clear. For example, VWF is widely used as a marker for endothelial injury,[63,64] but its change in its adhesion activity and contribution to coagulopathy in TBI are not known. VWF mediates the initial tethering of platelets to perturbed endothelium or subendothelial matrix exposed at sites of vascular injury. By forming a high affinity complex, it also protects coagulation factor VIII from being inactivated by proteolytic degradation in the plasma. VWF is exclusively synthesized in endothelial cells and megakaryocytes/platelets (Fig. 2). VWF multimers are constitutively secreted and stored in the Weibel-Palade bodies of endothelial cells and α-granules of megakaryocytes/platelets.[65,66] The stored VWF multimers are enriched in ultra-large (UL) and prothrombotic forms[65,67] that are released upon stimulation to endothelial cells and platelets. These prothrombotic ULVWF multimers are rapidly and partially cleaved by the zinc metalloprotease ADAMTS-13 (a disintegrin and metalloprotease with thrombospondin type 1 repeats) into smaller multimers[68–71] that are active in hemostasis, but no longer prothrombotic. In the absence of this proteolytic activity, ULVWF multimers form rope-like long strings that tether platelets and leukocytes to endothelium,[71,72] leading to *in situ* thrombosis and thromboembolism in the downstream microvasculature.

There is no report on the contribution of VWF and ADAMTS-13 to TBI-associated coagulopathy, but several lines of evidence support this possibility. First, the secretion of ULVWF is significantly increased in traumatic injury, including TBI.[63,64] Second, ADAMTS-13 activity is reduced in traumatic and surgical injury. Third, intravascular thrombosis developed in the lesion boundary zone contained a substantial amount of VWF and platelets in a rat model of controlled cortical injury.[30,34] Fourth, endothelial cells in cerebral vascular beds, especially small vessels and microvasculature, synthesize significantly more VWF than vascular beds from other organs.[73] Fifth, VWF/FVIII infusion reduces regional blood flow after controlled ischemia in dogs.[74] The enhanced secretion of ULVWF multimers, coupled with a partial inhibition of their cleavage by ADAMTS-13, may allow accumulation of hyperactive ULVWF multimers on endothelium to promote leukocyte transmigration through the vessel wall and in plasma to agglutinate platelets, leading to thrombosis and tissue ischemia.

In addition to their role in forming initial clots, platelets also provide a phosphatidylserine (PS)-rich surface to initiate and propagate the extrinsic coagulation pathway by first forming a complex composed of tissue factor, coagulation factor VIIa, and

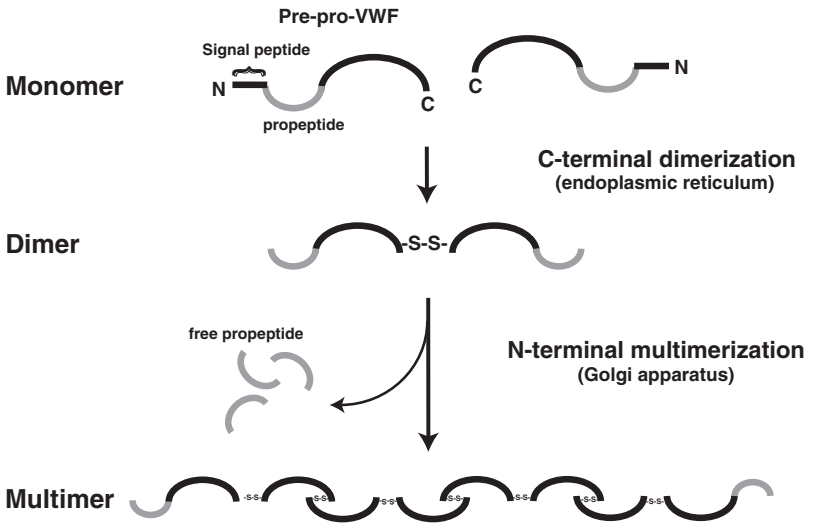

FIG. 2. Synthesis and multimerization of von Willebrand factor (VWF). VWF is initially synthesized as a monomeric glycoprotein that then forms a C-terminal disulfide-linked homodimer. VWF dimers subsequently form multimers through N-terminal disulfide linkages after proteolytic removal of a large 714 amino acid propeptide. Once secreted, ULVWF (ultra-large von Willebrand factor) multimers are subjected to cleavage at the A2 domain by ADAMTS-13. The cleaved VWF multimers have variable sizes, with the largest multimers being hemostatically most active.

calcium on a PS surface to activate factor IX and X (Fig. 1).[75] Two key molecules for the extrinsic coagulation pathway—tissue factor and phosphatidylserine—are richly expressed in cerebral tissue.

Tissue Factor and Tissue Factor–bearing Microparticles

Tissue factor is an integral membrane protein that is enriched in the central nerve system.[76–78] Human tissue factor is a lipoprotein of 263 amino acids containing an extracellular domain, a transmembrane domain, and a short cytoplasmic tail.[79,80] The extracellular domain contains two fibronectin type III domains with two potential disulfide bonds of Cys49-Cys59 and Cys186-Cys209[81] and can form a complex with factor VIIa, preferentially on a PS-rich membrane.[82] The amino acid Cys245 in the cytoplasmic tail is linked to a palmitate fatty acyl chain,[83] suggesting that tissue factor is selectively concentrated in lipid microdomains of the cell membrane.[84–86]

Tissue factor is not normally exposed to circulating blood because it is primarily expressed on fibroblasts and smooth muscle cells in the vessel wall and non-vascular cells such as astrocytes, epidermal cells, and renal glomeruli. Monocytes[87–89] and endothelial cells[90–92] express tissue factor, but only after induction by stimulating factors such as injury and inflammation. There is debatable presence of soluble tissue factor in blood at approximately 1–10 ng/mL,[93,94] but this blood-born tissue factor does not appear to trigger coagulation under physiological conditions because it may be either encrypted or in an insufficient amount. The latter can be overcome by concentrating tissue factor at a site of vascular injury or in a freshly formed thrombus.[95] A long-standing question is how tissue factor is integrated into the membrane surface of activated platelets, which do not express tissue factor or have an extremely low level,[96,97] to activate the extrinsic coagulation pathway. One potential mechanism is for tissue factor to be delivered by membrane microparticles.

In response to injury, cells such as platelets, erythrocytes, monocytes/macrophages, and endothelial cells shed membrane fragments through active microvesiculation[98,99] or apoptosis.[100–102] Intracellular signals that increase calcium influx activates the cysteine protease calpain, which then cleaves cytoskeletal proteins α-actinin, filamin, adducins, spectrin, talin, and actin[103] to disrupt membrane-cytoskeleton and produce microparticles.[104] Inhibiting calpain therefore blocks microparticle production.[104,105] Platelets generate microparticles during storage[106] in response to agonists and changes in hydrodynamic conditions.[107,108] These microparticles are primarily composed of membrane fragments with a limited amount of cytoplasm and measure 0.5–1 μM.[109] They are enriched in lipid microdomains (Fig. 3) where cholesterol and sphingolipids, adhesion receptors, and tissue factor are highly concentrated.[84,110] Adhesion receptors on these microparticles could bring tissue factor to cells expressing counter-receptors or ligands for these receptors. For example, P-selectin glycoprotein ligand 1 (PSGL-1) expressed on monocytes could bring tissue factor-bearing microparticles to activated platelets and endothelial cells that express P-selectin.[87,90,95] Immunoelectron microscopy has indeed detected tissue factor–positive membrane vesicles in large clusters near the surface of platelets.[95] Falati and colleagues[111] further showed that fluorescently labeled tissue factor-bearing microparticles derived from mouse monocytes accumulate in the leading edge of a developing thrombus when they were infused into mice prior to vascular injury. The accumulation was drastically reduced when these microparticles were infused into mice that were P-selectin deficient.

Brain tissue is a rich source of tissue factor.[76,78,112–114] In fact, brain homogenate was first used to induce blood coagulation as

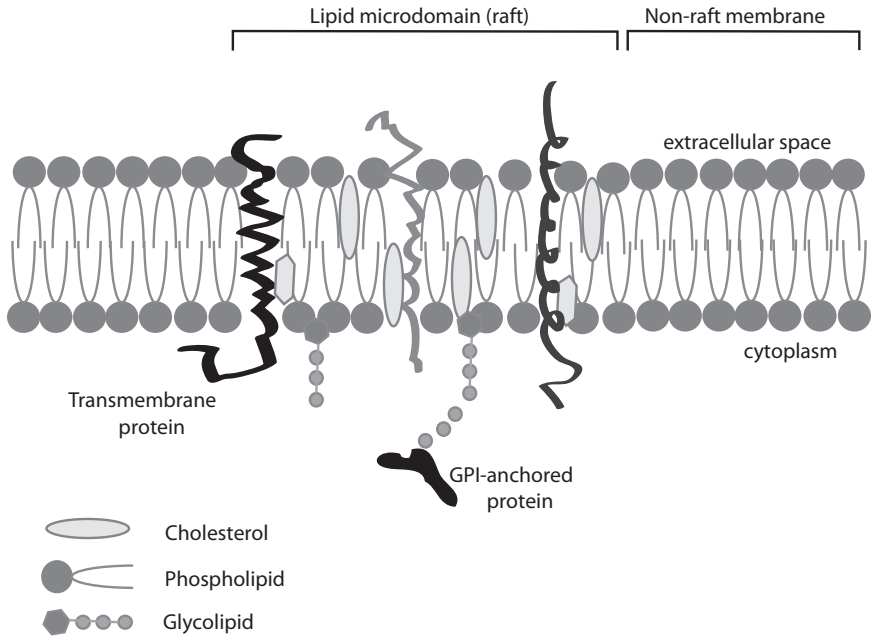

FIG. 3. Lipid microdomains in cell membrane. These microdomains (rafts) are cholesterol- and sphingolipid-rich compartmentalized regions of cell membrane where signaling molecules, receptors, and phosphatidylserine (PS) are selectively concentrated. These domains are orderly and tightly packed and can float freely in the membrane bilayer. Upon activation, platelets generate microparticles that are enriched in these lipid microdomains with concentrated adhesion receptors and PS. The latter could carry coagulation factors. Other cells may also produce microparticles that are PS-rich and contain signature molecules from the parental cells that deliver microparticles to target cells.

early as 1939. This rich source of tissue factor may account in part for a higher incidence of coagulopathy in patients with penetrating head injury as compared to those with blunt injury. Further, neural cells are highly sensitive to apoptosis to potentially produce tissue factor-bearing neural microparticles. Microglial cells have indeed been shown to produce microparticles upon ATP binding to the ligand-gated ATP receptor P2X7.[115–117] The microvesiculation is blocked by inhibiting acid sphingomyelinase and found to be extremely low in the culture of glial cells from acid sphingomyelinase null mice.[118] Platelet and endothelial cell microparticles were also detected in blood and cerebral-spinal fluid (CSF) samples from healthy subjects and TBI patients.[119] A high level of microparticles is associated with a low GCS score, a larger hematoma volume, and death in CSF.[120]

There are at least two reasons for determining the potential presence and procoagulant activity of neural cell-derived and tissue factor-bearing microparticles. First, these microparticles could deliver more tissue factor to platelets to enhance coagulation, a direct mechanism for enhancing coagulopathy. Second, they carry a different set of receptors and counter-receptors that may fuse with,[121] or be endocytosed by, other target cells.[122,123] As a result, the coagulation process may spread to cells that could not normally initiate coagulation, such as neurons. Fibrin deposition has indeed been detected in the brain.[124] The process of membrane fusion between microparticles and target cells could also allow otherwise difficult cell-cell interactions (such as between neurons and leukocytes or platelets) to occur.

Phosphatidylserine and Brain Phospholipids

Another group of molecules critical for initiating and propagating coagulation is anionic phospholipids. PS serves as a catalyst that links platelet activation to thrombin generation by providing a highly negatively charged surface and by binding to discrete and specific sites on prothrombin, factor Xa, and factor Va.[125] Platelet-derived microparticles are PS-enriched and have a 50- to 100-fold higher procoagulant activity than platelets.[126] PS is also abundantly detected on the surface of microparticles from ATP-stimulated microglial cells and injured neurons.[115]

Anionic phospholipids such as PS and phosphatidylethanolamine (PE) are normally present on the inner leaflet, whereas neutral phospholipids such as phosphotidylcoline (PC) and sphingomyelin are on the outer leaflet of a membrane bilayer. This asymmetrical distribution of membrane phospholipids is maintained by enzymatic transporters,[127,128] but is lost during apoptosis and/or cell activation, leading to the exposure of anionic PS on the outer membrane.[129]

The central nervous system has the highest lipid content next only to adipose tissue,[130] constituting more than half of the dry weight in a human brain.[131] The brain contains all three major categories of lipids: cholesterol, sphingolipids (sphingomyelin, cerebrosides, sulfatides, gangliosides), and phospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositols).[132–135] Phospholipids account for approximately 25% of the dry weight of an adult rat brain compared to about 10% in other tissues. Injury to cerebral tissue could therefore release a large quantity of phospholipids and PS-bearing neural microparticles. Both membrane-anchored and soluble PS promotes thrombin generation.[125,136–138] In addition to PS, the anionic PE has also been shown to bind coagulation factor Va with a high affinity ($\sim$10 nM kDa) and promote thrombin generation.[139]

Despite strong circumstantial clinical and laboratory evidence, the role of brain-derived phospholipids in the development of TBI-associated coagulopathy remains unknown. Phospholipids released from injured neural cells, are also highly susceptible to oxidation.[140,141] Whether oxidized PS is more or less active in initiating and propagating coagulation remains a virgin field, not only to TBI-coagulopathy but also to understanding the coagulation state under conditions of oxidative stress. This is the case despite the finding that the lipid peroxidation markers F2-isoprostane and malondialdehyde are increased in brain tissue, serum, and CSF after experimental and clinical TBI.[142–144] More importantly, this increase in lipid peroxidation is associated with worse clinical outcomes in patients with severe TBI.[145,146] Also unique is the fact that phospholipids in different regions of the brain differ in their fatty acid compositions and side chains,[132–134] but association among types of phospholipids, regions of brain injury, and rates of coagulopathy has not been examined. TBI may be the most suitable model system to study how different types of phospholipids affect the initiation and propagation of coagulation. Information thus learned could help developing treatment strategies to not only improve TBI-associated coagulopathy, but also help to understand coagulation deficiencies and thrombosis in other clinical settings.

Conclusion

In summary, TBI creates a coagulopathic state in part because cerebral tissue is a rich source of potent platelet activating and procoagulant molecules, but their contribution to the development of TBI-associated coagulopathy remains largely unknown. This lack of understanding has greatly slowed the pace of developing effective therapeutics targeting this potentially lethal pathological state. As a result, clinical trials on the efficacy of anti-thrombin III,[(147)] low-molecular weight heparin,[(148–150)] factor VIIa,[(151,152)] and prostacyclin[(153,154)] have yielded variable outcomes in the setting of TBI and associated pathologies. The transfusion of blood products, such as platelets, fresh frozen plasma, and cryoprecipitate, has been a standard treatment. However, they are not only time-consuming, but their efficacy remains to be investigated in large trials. Transfusion may also cause fluid volume overload to the patients with isolated TBI and transfusion-related complications.[155,156] Extensive clinical and basic science research is therefore needed to study the pathogenesis of TBI-associated coagulopathy, to understand its impact on clinical outcomes, and to develop effective therapies.

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Author Disclosure Statement

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