Early Platelet Dysfunction in a Rodent Model of Blunt Traumatic Brain Injury Reflects the Acute Traumatic Coagulopathy Found in Humans

Deborah L. Donahue,¹ Julia Beck,^{1,2} Braxton Fritz,³ Patrick Davis,¹ Mayra J. Sandoval-Cooper,¹ Scott G. Thomas,³ Robert A. Yount,³ Mark Walsh,³ Victoria A. Ploplis,^{1,2} and Francis J. Castellino^{1,2}

Abstract

Acute coagulopathy is a serious complication of traumatic brain injury (TBI) and is of uncertain etiology because of the complex nature of TBI. However, recent work has shown a correlation between mortality and abnormal hemostasis resulting from early platelet dysfunction. The aim of the current study was to develop and characterize a rodent model of TBI that mimics the human coagulopathic condition so that mechanisms of the early acute coagulopathy in TBI can be more readily assessed. Studies utilizing a highly reproducible constrained blunt-force brain injury in rats demonstrate a strong correlation with important postinjury pathological changes that are observed in human TBI patients, namely, diminished platelet responses to agonists, especially adenosine diphosphate (ADP), and subarachnoid bleeding. Additionally, administration of a direct thrombin inhibitor, preinjury, recovers platelet functionality to ADP stimulation, indicating a direct role for excess thrombin production in TBI-induced early platelet dysfunction.

Key words: brain injury; coagulopathy; platelets; thromboelastography; trauma

Introduction

TRAUMATIC BRAIN INJURY (TBI) is a major cause of death and morbidity among trauma patients,^{1–3} with approximately 1.7 million cases of TBI in the United States diagnosed annually.⁴ Among these patients, ~50,000 die, ~230,000 are hospitalized and survive, and ~85,000 suffer long-term disability. A potential cause for the high mortality and morbidity rates in TBI patients is development of a coagulopathy.^{5–8} In cases of acute traumatic coagulopathy, debates have occurred on the relationships between disseminated intravascular coagulation and acute endogenous coagulopathy, facilitated by excess activated protein C.⁹ In addition, it has recently been shown that platelet dysfunction may play a role in early development of coagulopathy in these cases.^{9–11}

Trauma-induced platelet dysfunction takes place when platelets do not respond to agonists. Platelets can be activated by different pathways, two of which involve stimulation by adenosine diphosphate (ADP) and arachidonic acid (AA).^{12,13} ADP activates platelets by directly binding to its receptors, P2Y₁, P2Y₁₂, and/or P2X₁, on the platelet membrane. AA indirectly stimulates platelets after its conversion to thromboxane A₂ (TxA₂) by the cyclooxygenase pathway. TxA₂ then activates platelets by binding to its receptor on platelet membranes. Once these agonists bind to their

respective receptors, a subsequent increase in Ca²⁺ influx occurs and changes the platelet conformation from a nonadhesive biconvex disc to a more irregular spherical adhesive form with numerous pseudopodia. These transformations allow for a higher platelet surface area that increases interactions between platelets and the vessel wall. A recent study demonstrated that a decrease in the ability of ADP to activate platelets correlated with a lower survival rate in TBI patients.¹¹

To date, no animal model exists that exhibits the characteristics of the platelet dysfunction induced by TBI that is observed in humans. The current coagulopathy models employ hypothermia, hemorrhage, acidosis, or trauma inflicted on areas other than the brain to induce coagulopathy.¹⁴ A recent study described platelet dysfunction in a TBI and hemorrhage model in swine. However, the results do not completely correspond to the human condition. In that study, only a small change in ADP response was observed, and the model did not account for the changes in responses to AA and collagen that have been described in humans.¹⁵ Another common technique for inducing TBI in animal models is fluid percussion. This model has been applied to a wide range of species and has been used to address TBI issues related to pathology, physiology, and pharmacology.¹⁶ Other methods have also been developed, including a constrained concussion model.¹⁶ These

¹W.M. Keck Center for Transgene Research, University of Notre Dame, Notre Dame, Indiana.

²Department of Chemistry and Biochemistry, University of Notre Dame, Notre Dame, Indiana.

³Memorial Hospital of South Bend, South Bend, Indiana.

methods, however, do not properly reflect the nature of the acute coagulopathy of human TBI.

The aim of this study was to develop and characterize a rodent model that mimics platelet dysfunction after blunt TBI in humans, using a controlled constrained concussion model on adult male Sprague-Dawley rats. In this model, the head is fixed to attenuate rebound injuries. Then, the brain is damaged by a nonpenetrating impact on the skull. This leads to both physiological and pathological changes that are also observed in humans. The results of this study are summarized herein.

Methods

Injury model

Male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA), weighing 245–285 g, were used in this study. Rats were anesthetized with isoflurane throughout the induction of injury. A constrained nonpenetrating injury was utilized after stabilizing the head with a stereotaxic device. The TBI 0310 (Precision Systems and Instrumentation, Fairfax, VA), which we modified with a rubber tip 4.55 mm in diameter, was placed over the metal impactor and set to hit the exposed skull between the bregma and lambda fissures at 6 m/sec, with a dwell time of 50 msec and a depth of 0. After impact, the skull and the dura of rats were intact, demonstrating a blunt head injury. Next, the incision site was glued together and the rats were removed from anesthesia. Animals awakened postinjury at ~6–10 min, as compared with 1–2 min after isoflurane induction without injury, indicating that the impact caused an attenuated consciousness. For some studies, the direct thrombin inhibitor, Refludan ([Leu1/Thr2]-63-desulfohirudin; Lepirudin; Berlex, Mortville, NJ), at 4 µg/g, was injected into the retro-orbital sinus with a 30-gauge needle just before injury and rats were sacrificed 15 min after injury, or in the case of the control, the injection was given 15 min before sacrificing.

All animal experiments were performed after approval from the University of Notre Dame (Notre Dame, IN) Institutional Animal Care and Use Committee.

Histology

At 0, 15, 30, and 60 min postinjury, rats were perfused with saline followed by 4% paraformaldehyde fixative. Brains were removed and 2-mm coronal sections (rostral to caudal) were sliced in a matrix. Individual 2-mm sections were tissue processed and embedded in paraffin. Sections (4 µm) were stained with hematoxylin and eosin for assessment of bleeding subsequent to injury. Scanned images (40×) were accessed by Aperio Spectrum Library (version 11.2.0.780; $N=9$ [control], $N=8$ [15 min], $N=10$ [30 min], and $N=11$ [1 h]).

Blood collection

Rats were sacrificed at 0, 15, 30, or 60 min after the injury. Whole blood was collected from the vena cava in 3.2% sodium citrate Vacutainer® (BD Biosciences, Franklin Lakes, NJ) or 50 U/mL of a heparin sodium solution (Sagent Pharmaceuticals, Schaumburg, IL) using a 20-gauge needle. Complete blood counts (CBCs) were acquired from ethylenediaminetetraacetic acid-treated blood using a Hemavet 950FS (Drew Scientific, Waterbury, CT). Platelet-poor plasma was collected from citrated whole blood by centrifugation (10 min at 3200g). Fibrinogen levels, prothrombin (PT), and activated partial thromboplastin time (aPTT) values were determined utilizing a Diagnostica Stago STA4® Hemostasis Analyzer (Diagnostica Stago, Parsippany, NJ) and Fibrinprest® Automate, Neoplastine® CL, and PTT Automate (Diagnostica Stago), respectively.

Thromboelastography

Thromboelastograms were obtained using a TEG 5000 Thromboelastograph® Hemostasis Analyzer System (Haemoscope; Haemonetics Corporation, Braintree, MA) and analyzed using Haemoscope TAS software (version 4.3). Representative curves of the temporal features of thrombus formation allowed determination of the following parameters: R, time until the first evidence of clot formation, reflective of the activity of pathways generating thrombin; K, the time required for the clot strength to reach 20 mm starting from the end of R, representing the rate of clot formation; A, the angle in degrees, also representing the rate of clot formation; and MA_{max}, the maximum amplitude (mm) attained, representing the total clot strength (fibrin and platelets).

Recalcified whole blood was obtained after the addition of 20 µL of 0.2 M of CaCl₂ to 340 µL of citrated fresh blood. For RapidTEG™ curves, 340 µL of citrated blood was added to 20 µL of 0.2 M of CaCl₂ and 10 µL of RapidTEG solution (recombinant tissue factor; Haemoscope Corporation, Niles, IL). For thrombin curves, 340 µL of citrated blood was added to 1 unit of thrombin and 20 µL of CaCl₂.

Platelet mapping

The TEG® platelet mapping kit (Haemonetics Corporation) was used to assess platelet function. Total clot potential (thrombin contribution; MA_{Thrombin}) to the viscoelasticity of the clot was performed by adding 1 mL of citrated blood to a vial of kaolin (Haemonetics Corporation). Next, 360 µL of citrated-kaolin blood was added to the cup, along with 20 µL of 0.2 M of CaCl₂. Heparinized blood was used for the remaining assays. To determine the fibrin contribution to the clot viscoelasticity of the clot (MA_{Fibrin}), 360 µL of blood and 10 µL of Activator-F (reptilase and human factor XIIIa; Haemonetics Corporation) were mixed. The MA_{Analyte}, namely, the effectiveness of the analyte toward platelet responses, was determined by adding 10 µL of AA (final concentration, 1 mM), 20 µL of ADP (final concentration, 50 mM), or 12 µL of protease-activated receptor 4 (PAR-4) agonist (final concentration, 397 µM; PAR-4 test; H-AYPGKF-OH; Verum Diagnostica GmbH, Munich, Germany) and 360 µL of heparinized blood. Each platelet stimulator assay was run separately. Analyte-specific stimulations (%) were calculated using the following equation: $(MA_{Analyte} - MA_{Fibrin}) / (MA_{Thrombin} - MA_{Fibrin}) \times 100$.

Platelet aggregometry

Platelet aggregation measurements were evaluated using the Multiplate Impedance Aggregometer (Verum Diagnostica). Each test cell contained 300 µL of saline and 300 µL of heparinized blood. After a 3-min incubation, 20 µL of ADP (final concentration, 0.2 mM), Aspirin® (final concentration, 15 mM), collagen (final concentration, 100 µg/mL), or PAR-4 agonist (final concentration, 645 µM) was added to individual test cells. Aggregation response was recorded for 6 min and plotted as arbitrary aggregation units (AU) versus time.

Statistical analysis

Injured rats were compared to control rats for each test using unpaired *t*-testing. For studies using Refludan, injured rats were compared to injured rats pretreated with Refludan using unpaired *t*-testing. Data are expressed as the mean ± standard error of the mean.

Results

Evidence that this model generates clinically relevant head injury is supported by the observation of bleeding in the subarachnoid space, exemplified by the 1-h postinjury slide in Figure 1. Plasma

FIG. 1. Subarachnoid blood pools 1 h postinjury. Representative 4- μ m coronal sections of control (A and C) and 1-h postinjury (B and D) rat brains. Arrows indicate bleeding in the subarachnoid space of the injured brain. Images were scanned at 40 $\times$ magnification and zoom magnified at 1.2 $\times$ for (A and B) and 4.5 $\times$ for (C and D).

hemostasis parameters and CBCs were also performed on injured and noninjured rats. In both cases, platelet counts were normal (Table 1). Fibrinogen levels for the noninjured and 1-h postinjury time points were within the normal range; however, both the 15- and 30-min postinjury fibrinogen levels were slightly depleted, but this did not lead to a functional difference in the fibrinogen contribution to clot strength. The aPTTs of the injured rats were in the normal range of noninjured rats, indicating that the factors involved in the intrinsic pathway of coagulation were still functional. At all injury time points, PT values were comparable to noninjured PTs, suggesting that no dysfunctions of the extrinsic pathway of coagulation were present.

Platelet aggregation, based on impedance, for ADP, AA, collagen, and the PAR-4 agonist were assessed for both injured and noninjured rats. No significant differences were found for the AA

and PAR-4 assays in this assay, and only a slight, albeit a significant, difference was observed at 30 min and 1 h for the ADP assay. Collagen showed the weakest platelet stimulation for the injured rats at the 30-min injury time. Rats at 15 min and 1 h postinjury also displayed a decrease in platelet aggregation, compared to the noninjured rats (Table 2).

Another platelet function test was employed through platelet mapping using thromboelastography (TEG). Representative TEG traces are displayed in Figure 2. The extent that ADP and AA stimulated a platelet response (%) was determined for both injured and noninjured rats (Fig. 3). Significant changes were observed for ADP stimulation throughout all time points, in comparison to noninjured rats. Platelet stimulation by ADP was significantly diminished at the 15-min time point and was slightly improved at 30 min. At 1 h postinjury, the percent platelet response to ADP

TABLE 1. PLASMA HEMOSTASIS PARAMETERS AND CBCs AFTER TBI IN RATS

Rat	Fibrinogen (mg/dL)	PT (sec)	aPTT (sec)	Platelets ($\times 10^3/\mu$ L)	Mean platelet volume (fL)
Control	352 $\pm$ 21	16.9 $\pm$ 0.4	12.5 $\pm$ 0.8	1084 $\pm$ 34	5.5 $\pm$ 0.1
15 min	317 $\pm$ 17*	16.6 $\pm$ 0.3	13.9 $\pm$ 0.8	1043 $\pm$ 28	5.3 $\pm$ 0.1*
30 min	327 $\pm$ 10*	16.8 $\pm$ 0.3*	15.0 $\pm$ 0.8	1037 $\pm$ 43	5.3 $\pm$ 0.1
1 h	369 $\pm$ 16	16.3 $\pm$ 0.2	14.4 $\pm$ 0.8	1102 $\pm$ 30	5.4 $\pm$ 0.1

Values are expressed as the mean $\pm$ standard error of the mean ($N \geq 12$). Statistical analyses were performed using an unpaired *t*-test by comparing a specific post-injury time to the control.

* $p < 0.034$.

CBCs, complete blood counts; TBI, traumatic brain injury; PT, prothrombin; aPTT, activated partial thromboplastin time.

TABLE 2. PLATELET IMPEDANCE-BASED AGGREGATION (ARBITRARY UNITS) AFTER TBI IN RATS

Rat	ADP (AU)	Aspirin (AU)	Collagen (AU)	PAR-4 agonist (AU)
Control	82.9 ± 2.7	75.1 ± 2.1	77.3 ± 3.7	73.5 ± 1.5
15 min	77.6 ± 1.2	73.6 ± 2.5	59.0 ± 6.1 [†]	71.1 ± 2.4
30 min	69.2 ± 4.8*	71.8 ± 6.9	54.4 ± 5.4 [‡]	70.8 ± 6.8
1 h	75.0 ± 2.7*	67.1 ± 2.5	59.2 ± 6.3 [†]	61.5 ± 6.0

Values are expressed as the mean ± standard error of the mean ($N \geq 13$). Statistical analyses were performed using an unpaired *t*-test comparing a specific postinjury time point to the control.

* $p < 0.05$; [†] $p < 0.006$; [‡] $p < 0.0005$.

TBI, traumatic brain injury; ADP, adenosine diphosphate; AU, arbitrary units; PAR-4, protease-activated receptor 4.

stimulation continued to rise. AA showed significant differences from normal only at the 15- and 30-min postinjury time points. At 30 min, AA least stimulated a platelet response, indicating that there was a delayed response for the AA pathway of stimulation. There was no significant difference found for PAR-4 agonist stimulation at all time points (Fig. 3).

Whole-blood assays were performed to measure clot strength and kinetics of clot formation for Ca^{2+} , Ca^{2+} /thrombin, and Rapid TEG. No essential differences were observed in any of the measured parameters, when injured rats were compared to the non-injured rats, for all whole-blood assays (Table 3).

In this study, administration of the thrombin inhibitor, Refludan, just before injury, resulted in a protective effect on platelets in their ability to be stimulated by ADP and AA 15 min postinjury, thus demonstrating potential mechanism(s) for dysfunctional platelets after TBI (Table 4). The effectiveness of our dosage of Refludan in inhibiting thrombin in the rat was noted by the prolongation to the

same extent of PT and aPTT in Refludan-treated injured and non-injured mice, as compared to nontreated controls.

Discussion

Previous studies have demonstrated that the brain contains a large reserve of tissue factor (TF), a cofactor for factor VIIa in the extrinsic blood coagulation system.^{17,18} After TBI, it has been hypothesized that there is a burst of TF release from the brain.^{8,19,20} This burst of thrombin activity could potentially overstimulate and consume platelets, rendering them desensitized to agonists for a time. Alternatively, thrombin degrades cyclooxygenase,²¹ an enzyme required for conversion of AA to TxA_2 . This could lead to platelets being nonresponsive to AA stimulation.

Identifying mechanisms associated with platelet dysfunction after TBI has been hampered by a lack of appropriate animal models that reflect the human condition. The results from this study describe and characterize a rodent TBI model that closely mirrors phenotypes of platelet dysfunction observed in TBI in humans. Initial observations indicated that factors involved in both the plasma extrinsic and intrinsic coagulation pathways remained fully functional and that fibrinogen levels were still within a normal preinjury range. This implies that decreases in clot strength were the result of platelet dysfunction, to the exclusion of significant changes in soluble plasma coagulation events. Upon assessing platelet responses, it was found that, in the case of ADP, there was significantly diminished platelet response at both 15 and 30 min postinjury. However, ADP responses in platelets recovered after 1 h. Changes in AA responses were delayed, such that platelet dysfunction was most prominent at 30 min postinjury. Similar to ADP, AA responses recovered after 1 h. A decrease in platelet responses to collagen and, to some extent, ADP at later time points, was found using platelet impedance measurements, but this does not suggest that platelets were functional in stabilizing the clot and

FIG. 2. Representative TEG traces in control and injured rodents. Representative TEG traces were assembled for (A) the control and (B) 15 min postinjury. Representative traces were determined by calculating the mean of the MA for each blood assay. The TEG trace includes the citrated-kaolin, ADP stimulated, AA stimulated, and Activator-F assays. The trace is plotted as pin oscillation (amplitude, mm) versus time (min). TEG, thromboelastography; ADP, adenosine diphosphate; AA, arachidonic acid.

FIG. 3. Platelet mapping expressed as percent platelet response induced by (A) ADP, (B) AA, (C) PAR-4 agonist, and (D) summarized values. Percent responses were calculated using the following equation: $(MA_{\text{Analyte}} - MA_{\text{Fibrin}}) / (MA_{\text{Thrombin}} - MA_{\text{Fibrin}}) \times 100$, where MA_{Analyte} was determined from ADP, AA, or PAR-4 agonist stimulations. MA_{Thrombin} was obtained from citrated-kaolin, and MA_{Fibrin} was acquired from Activator-F. The subsequent platelet aggregation for each analyte was plotted for the controls and each time point. (D) Quantitation of dot plots. Statistical analysis was performed using an unpaired *t*-test by comparing a specific postinjury time to the control. For ADP stimulation: **p* = 0.01; ****p* < 0.0001. For AA stimulation: ***p* = 0.003; ****p* < 0.0001. *N* ≥ 22 rodents for each agonist. ADP, adenosine diphosphate; AA, arachidonic acid; PAR-4, protease-activated receptor 4.

provides more limited knowledge than TEG analyses. Thus, our overall findings demonstrated that after TBI, platelet responses to ADP, AA, and collagen were diminished.

Recent work has revealed that TBI patients have diminished ADP and AA platelet responses.^{9,11} In one of these studies, a significant correlation was found between mortality and ADP

responses, but not AA responses.¹¹ Another difference in agonist-induced platelet responses observed in this current study was a decrease in platelet responses to collagen. This has also been observed in human TBI patients.²² The events leading to the development of platelet dysfunction in trauma are largely unknown. In patients with a number of diverse medical conditions, for example, thrombotic thrombocytopenic purpura, hemolytic uremic syndrome, and systemic lupus erythematosus, exhausted platelet syndrome has been described. This could be the result of exposure in these diseases of the procoagulant subendothelium surface, thrombin generation, and/or the presence of immune complexes. As a result, platelets, *in vivo*, become desensitized to agonist stimulation, resulting in prolonged activation defects.^{22,23}

One hypothesis, with experimental support, proposes that a release of TF into the systemic circulation occurs post-TBI. Exposure of TF after head trauma could be the result of injury-induced blood-brain barrier disruption and exposure of the TF-rich subendothelium, release of cell membrane fragments containing TF, and/or expression of TF by circulating monocytes. One study demonstrated that trauma-associated head injury results in a transient expression of TF on monocytes and concomitant platelet/monocyte interactions.²⁴ In this study, TF exposure occurred early and diminished to baseline levels within 24 h, unlike other trauma-associated injuries, where exposure of TF occurred for at least 72 h. Additional studies have demonstrated that whereas platelet activation (active conformation of GPIIb/IIIa, P-Selectin exposure, or platelet-derived microparticles) is increased in TBI patients, platelet function (sensitivity to collagen or ADP) is decreased.²⁵ However, platelet function returned to normal within 24–48 h, whereas platelet activation returned to normal more slowly (~72 h). In this case, initial time (*t* = 0) is recorded as time of

TABLE 3. WHOLE-BLOOD TEG PARAMETERS AFTER TBI IN RATS

Rat	<i>R</i> (min)	<i>K</i> ₂₀ (min)	<i>A</i> (degrees)	<i>MA</i> _{max} (mm)
Control				
Ca ²⁺	5.2 ± 0.5	1.8 ± 0.3	68.4 ± 2.8	78.2 ± 0.9
Ca ²⁺ /thrombin	0.7 ± 0.1	0.8 ± 0.0	83.4 ± 0.5	80.7 ± 0.7
RapidTEG	0.7 ± 0.0	0.8 ± 0.0	84.4 ± 0.2	77.4 ± 0.8
15 min				
Ca ²⁺	5.4 ± 0.3	1.5 ± 0.1	70.0 ± 1.5	78.6 ± 0.6
Ca ²⁺ /thrombin	1.0 ± 0.3	0.8 ± 0.0	82.5 ± 1.1	78.8 ± 0.6
RapidTEG	0.8 ± 0.1	0.8 ± 0.0	84.0 ± 0.3	76.1 ± 0.6
30 min				
Ca ²⁺	4.7 ± 0.4	1.4 ± 0.2	71.4 ± 2.1	79.2 ± 0.7
Ca ²⁺ /thrombin	0.6 ± 0.0	0.9 ± 0.1	82.4 ± 0.9	73.5 ± 3.3
RapidTEG	0.6 ± 0.0	0.8 ± 0.0	84.2 ± 0.2	76.6 ± 0.9
1 h				
Ca ²⁺	4.8 ± 0.4	1.3 ± 0.1	71.9 ± 1.6	76.2 ± 0.8
Ca ²⁺ /thrombin	0.9 ± 0.1	0.8 ± 0.0	83.9 ± 0.2	79.1 ± 1.1
RapidTEG	0.6 ± 0.0	0.8 ± 0.0	84.6 ± 0.2	76.2 ± 0.9

Values are reported as mean ± standard error of the mean. Statistical analysis was performed using an unpaired *t*-test by comparing a specific postinjury time point to the control (*N* ≥ 17).

TEG, thromboelastography; TBI, traumatic brain injury.

TABLE 4. EFFECTS OF PREINJURY ADMINISTRATION OF REFLUDAN ON PLATELET RESPONSES TO AGONISTS

	ADP (%)	AA (%)	PT (sec)	aPTT (sec)	PLT ($\times 10^3/\mu\text{L}$)
Noninjured control without Refludan	65.1 $\pm$ 3.4	72.5 $\pm$ 2.4	16.9 $\pm$ 0.4	12.5 $\pm$ 0.8	1084 $\pm$ 34
Noninjured control with Refludan	73.5 $\pm$ 6.6	75.0 $\pm$ 9.8	25.4 $\pm$ 1.1 [†]	35.9 $\pm$ 9.5 [†]	1005 $\pm$ 62
15-min injury without Refludan	24.8 $\pm$ 6.2	57.5 $\pm$ 4.6	16.6 $\pm$ 0.3	13.9 $\pm$ 0.8	1043 $\pm$ 28
15-min injury with Refludan	65.9 $\pm$ 10.8*	91.4 $\pm$ 2.6*	27.8 $\pm$ 1.1 [†]	43.4 $\pm$ 9.6 [†]	1089 $\pm$ 83

ADP (*) compared 15-min time points; AA (*) compared 15-min time points; PT and aPTT (†) compared control versus control with Refludan, 15-min time points $\pm$ Refludan. PLT: no significant differences ($N=3$). Control with Refludan: $N=5$, 15-min injury with Refludan: $N=30$ for control and 15-min injury without Refludan.

* $p < 0.009$; † $p < 0.0005$.

ADP, adenosine diphosphate; AA, arachidonic acid; PT, prothrombin; aPTT, activated partial thromboplastin time; PLT, platelets.

arrival in the emergency room. This would correlate with our findings, in which administration of a thrombin inhibitor, Refludan, before TBI, protected the platelet response (platelet function) for both ADP and AA, thus implicating thrombin as an early participant in regulating platelet function in blunt TBI patients, but not inducing a systemic hypercoagulable state. Additionally, thrombin activation of platelets at the site of injury could lead to platelet degranulation and release of the platelet agonists, ADP and TxA₂, resulting in a cascading event of platelet hyperstimulation.²⁶ That PT and aPTT did not change during the proposed thrombin burst suggests that this burst is small and the plasma-based PT and aPTT is insensitive to this small change in thrombin. However, platelets are sensitive to this altered thrombin level and assemble the thrombin on platelet receptors.

In summary, our model closely resembles the TBI-induced platelet dysfunction observed in humans. In humans, it is reported that there is no change in PT, aPTT, or platelet counts, but there is a large decrease in platelet stimulation toward ADP, AA, and collagen. Our rat model mimics these effects. Therefore, the results indicate that this model accurately reflects, at the least, the nature of the platelet dysfunction found in TBI patients and can be used to understand the mechanism of platelet dysfunction as well as to discover improved treatments for TBI patients.

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Author Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Francis J. Castellino, PhD
W. M. Keck Center for Transgene Research
230 Raclin-Carmichael Hall
University of Notre Dame
Notre Dame, IN 46556

E-mail: fcastell@nd.edu