A prospective study of platelet function in trauma patients

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BACKGROUND: Exsanguination associated with acute traumatic coagulopathy is a leading cause of death following injury. While platelets occupy a pivotal role in clot formation, clinical research has been scant because of complexities resulting from the need for rapid handling and complex testing of platelet functions. While the thrombin pathway has been proposed as a mediator of platelet dysfunction in trauma, it has not been systematically investigated. The purpose of this study was to evaluate the thrombin pathway in platelet dysfunction.

METHODS: Forty trauma patients and 20 noninjured controls were enrolled in the study at a Level I trauma center. Platelet aggregation was tested by light transmission aggregometry with two agonists, adenosine diphosphate (ADP) and thrombin receptor agonist peptide (TRAP). Mean fluorescence intensity and percent positivity of CD62 on ADP-activated platelets were evaluated using flow cytometry. Enzyme-linked immunosorbent assays were performed to evaluate the concentrations of D-dimer, thrombin-antithrombin complex (TAT), and prothrombin fragment 1 + 2 (PF 1 + 2) in each sample.

RESULTS: Compared with healthy controls, trauma patients had significantly decreased ADP- and TRAP-mediated platelet aggregation and ADP-mediated CD62 expression. In trauma patients, TRAP-mediated aggregation was inversely proportional to head Abbreviated Injury Scale (AIS) score. Glasgow Coma Scale (GCS) score was directly proportional to TRAP- and ADP-mediated aggregation. When compared with controls, significant differences of D-dimer, TAT, and PF 1 + 2 were found. Measures of shock, including admission blood pressure, pulse, base deficit, and lactate level, did not correlate with platelet dysfunction.

CONCLUSION: Trauma patients have significantly lower levels of platelet activation and aggregation compared with healthy controls. Severity of head injury was significantly correlated with platelet dysfunction in a stepwise fashion. Trauma patients also have significantly increased levels of D-dimer, TAT, and PF 1 + 2 when compared with healthy controls. Our data suggest that the thrombin receptor pathway plays an important role in platelet dysfunction in trauma. (*J Trauma Acute Care Surg.* 2016;80: 726–733. Copyright © 2016 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: Prognostic and epidemiologic study, level III.
KEY WORDS: Platelet function; acute traumatic coagulopathy; traumatic brain injury.

Exsanguination associated with acute traumatic coagulopathy (ATC) is a leading cause of death following severe injury.[1] Numerous studies have suggested that ATC is present in more than 25% of trauma patients admitted to emergency departments and is associated with increased morbidity and mortality.[2–4] The lethality and incidence of ATC have made the discovery of the underlying mechanisms a priority.

Several studies have evaluated and suggested enzymatic mechanisms of ATC, and current evidence suggests that activation of the protein C pathway, which results from tissue injury and shock, plays a large role in the pathophysiology of ATC.[5] Activated protein C has demonstrated antithrombotic, anti-inflammatory, and profibrinolytic properties.[6] Complexities related to the need for rapid handling and complex testing of platelets have limited the ability to study platelet dysfunction and its contribution to ATC.[7] Some work has suggested that traumatic platelet dysfunction, platelet adenosine diphosphate (ADP) receptor inhibition, has been noted to be a likely mechanism for platelet dysfunction in the trauma setting.[5,7] Other studies have focused on platelet activation by means of measuring surface biomarkers such as CD62 as a possible pathway for platelet dysfunction in ATC.[5]

Platelet dysfunction has also been associated with traumatic brain injury (TBI).[8–12] It has been proposed that platelet dysfunction is directly correlated with Glasgow Coma Scale (GCS) score in trauma patients.[10,11] In addition to the current evidence, preliminary work at our institution implicated the thrombin pathway as a mediator of platelet dysfunction in these patients; however, this pathway has not been systematically reviewed with regard to platelet function in this patient subset. The purpose of this study was to describe platelet function in trauma patients with a focus on ADP receptor inhibition and thrombin-mediated activation.

PATIENTS AND METHODS

Study Group

This translational research was conducted by means of a prospective, observational study. The study population consisted of a cohort of patients who were evaluated in the trauma resuscitation room at the Presley Memorial Trauma Center in Memphis, Tennessee, from July 2013 to September 2014, all of whom met criteria for Level I trauma activation. The patients were taken as a convenience sample because it was not possible to have a 24-hour coverage for screening of incoming patients by study personnel. A cohort of noninjured, healthy individuals, who were employees of either the hospital or the university, was used as a comparison with the trauma patients for baseline platelet characteristics. Exclusion criteria were age less than 18 years, pregnancy, or preadmission antiplatelet medication use. Demographics and injury data including GCS score; admission heart rate, systolic blood pressure, base excess, and lactate; blood product transfused; Injury Severity Score (ISS); as well as head and neck Abbreviated Injury Scale (HNAIS) score were obtained from the trauma registry. The requirement for informed consent was waived for the trauma patients because only excess blood was used and no procedures were performed for study purposes. Informed consent was obtained from the healthy volunteers, and The University of Tennessee Institutional Review Board approved the study as meeting all ethical requirements.

Sample Collection

With the use of excess blood from the initial collection in the resuscitation room, three 2.7-mL sodium citrate vacutainers (0.1 M sodium citrate) were filled with the patient's blood. The blood was adequately mixed with the sodium citrate to prevent clotting. This blood was then transported immediately to the

TABLE 1. Patient Characteristics

	Trauma	Control	*p*
Male, %	68	55	0.3436*
Age, y	40 (24 to 46)	24 (23–27)	0.0374**
ISS	10 (2 to 18)		
GCS score	15 (14 to 15)		
HNAIS score	3 (1 to 5)		
Admission lactate, mmol/L	2.2 (1.3 to 3.1)		
Admission systolic blood pressure, mm Hg	133 (119 to 149)		
Admission heart rate, beats per minute	94 (80 to 113)		
Admission base excess, mEq/L	−2.2 (−4.9 to −0.5)		

*χ^2 test.
**Wilcoxon rank-sum test.
All data reported as median (interquartile range).

Vascular Biology Program where light transmission aggregometry (LTA) was performed to determine the extent of platelet aggregation, and flow cytometry was performed to evaluate platelet activation by detection of CD62 (P-selectin) expression, a platelet surface activation biomarker. Platelet-poor plasma (PPP) from each sample was prepared, aliquoted, and stored in a −80°C freezer for multiple enzyme-linked immunosorbent assays (ELISAs) to be completed at a later date, including D-dimer, thrombin-antithrombin complex (TAT), and prothrombin fragment 1 + 2 (PF 1 + 2), biomarkers for fibrinolysis or thrombin generation.

Sample Preparation

In the laboratory, each sample was centrifuged to prepare platelet-rich plasma (PRP) by placing the vacuum-sealed tubes in an Eppendorf Centrifuge 5702 for 7 minutes at 135 G at room temperature. The PRP was transferred to a 15-mL polypropylene centrifuge tube using a disposable pipet. Platelet counts were determined using a Beckman Coulter. The residual blood samples were centrifuged for 15 additional minutes at 2,500 G to isolate PPP. The platelet count was determined upon adding 6.7 μL of PRP to a cuvette containing 20 mL of isotonic diluent. If platelet count was greater than 250 × 10^3/μL, autologous PPP was used to dilute sample to the desired platelet count of 250 × 10^3/μL. If the platelet count was less than 250 × 10^3/μL but greater than 100 × 10^3/μL, no adjustments were made to the sample. If platelet count was less than 100 × 10^3/μL, the sample was discarded and not used for the study. The remaining PPP was also centrifuged again in an Eppendorf Centrifuge 5702 for 15 minutes at 2,500 G to ensure generation of cell-free plasma. Once centrifuged, 500 μL of PPP was used to standardize the light transmission aggregometer representing no platelets in the plasma preparation and deemed equivalent to 100% aggregation. Adjusted count PRP represented 0% aggregation. The remainder of the PPP was aliquoted and stored in a −80°C freezer for later ELISA testing.

Light Transmission Aggregometry

LTA was used to study platelet function of each sample.[13] Two different agonists were used for the aggregations: 200 μM ADP and 150 μM thrombin receptor agonist peptide 6 (TRAP). The aggregometer was zeroed with 500-μL PPP. For each subject, two 450-μL samples of adjusted PRP were placed in two different cuvettes. The samples were allowed to equilibrate to 37°C for 2 minutes. At 2 minutes, 50 μL of ADP was added to one cuvette (final 20 μM), while 50 μL of TRAP (final 15 μM) was added to the other. The aggregometer recorded light

TABLE 2. Aggregometry, Flow Cytometry, and ELISA Results

	Trauma	Control	*p*
Platelet count (×1,000)*	792 (500–1,078)	619 (425–1,101)	0.5961
LTA			
% aggregation ADP-mediated	68.5 (54.5–74.5)	74.5 (70.0–78.5)	0.0024
% aggregation TRAP-mediated	45.0 (23.0–70.0)	75.5 (68.0–79.0)	<0.0001
Flow cytometry			
CD62 + ADP (percent positive)	79.5 (72.7–83.8)	85.5 (79.7–89.0)	0.0200
CD62 + ADP MFI (surface density)	50.0 (40.8–60.0)	59.5 (48.6–64.0)	0.0721
ELISA, μg/L			
D-dimer	1.11 (0.35–5.25)	0.20 (0.2–0.23)	<0.0001
TAT	37.1 (10.2–55.5)	4.05 (3.16–6.05)	<0.0001
PF 1 + 2	959 (577–1,025)	275 (182–720)	0.0002

*Obtained from PRP.
Results reported as median (interquartile range), Wilcoxon rank-sum test *p* value.

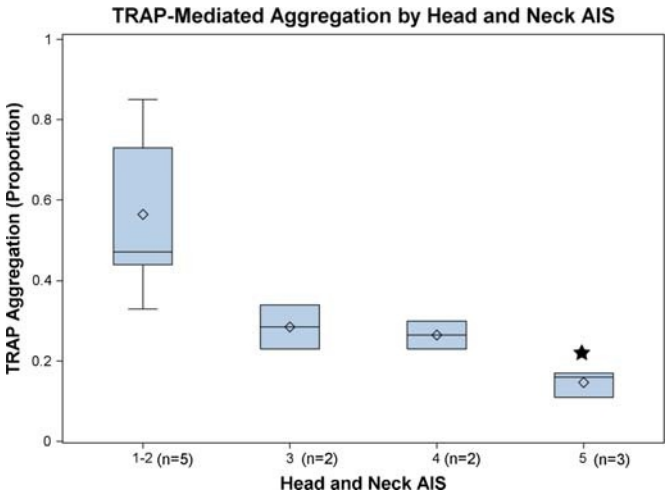

Figure 1. Aggregation of TRAP-activated platelets by head AIS score.

transmission for 6 minutes to 10 minutes or until maximal aggregation was achieved. The percent maximal aggregation (platelet function) was then manually calculated for each of the two agonists.

Flow Cytometry

After the adjusted PRP was prepared, three 1.5-mL polypropylene microfuge tubes were prepared containing PRP for flow cytometric analyses. In one tube, 90 μL of PRP and 10 μL of saline were added. In the second tube, 90 μL of PRP and 10 μL of 200 μM ADP were added. In the third tube, 100-μL PRP was added and used as the control. The three tubes were allowed to incubate at room temperature for 15 minutes. Then, the first two samples were incubated with CD62 antibody. The third sample was incubated with mouse IgG (MIgG) as a control. The three samples were allowed to incubate at room temperature for 15 minutes. Then, all three tubes were transferred to three separate tubes containing 4% paraformaldehyde for fixation, achieving a final concentration of 1% paraformaldehyde. The samples were then stored at 1°C to 2°C for a maximum time of 48 hours, until flow cytometry was performed. Flow cytometry was used to evaluate platelet activation.[14] Specifically, the percentage of the total platelet population positive for MIgG, CD62 + saline, and CD62 + ADP in each sample was recorded. The mean fluorescence intensity (MFI) of MIgG, CD62 + saline, and CD62 + ADP was also recorded.

Enzyme-Linked Immunosorbent Assays

The concentration of D-dimer was measured for each sample using a D-dimer ELISA (Diagnostica Stago Inc., Parsippany, NJ). The results were reported in microgram per milliliter with a reportable range of 0.2 μg/mL to 20 μg/mL and a normal range of 0.0 μg/mL to 0.5 μg/mL. In addition, the concentration of TAT was measured using an Enzygnost TAT micro kit (Siemens Healthcare Diagnostics, Deerfield, IL). These results were reported in microgram per liter, having a reportable range of 2.0 μg/L to 60.0 μg/L and a normal range of 2.4 μg/L to 6.1 μg/L. Lastly, the PF 1 + 2 concentration of each sample was measured using an Enzygnost F 1 + 2 monoclonal kit (Siemens Healthcare Diagnostics). The results were reported in picomole per liter, with a manufacturer's reportable range of 20.0 pmol/L to 1,200.0 pmol/L, and the accepted normal range for each sample was reported as 36.56 pmol/L to 240.94 pmol/L.

Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC). Normally distributed continuous variables were analyzed using Student's *t* test, and categorical data were analyzed with a χ^2 test. Nonparametric data were analyzed using a Wilcoxon rank-sum test or Kruskal-Wallis test. Spearman rank correlation analysis was performed to identify associations between LTA and flow cytometry results and clinical outcomes including amount of blood product transfused, intensive care unit (ICU) length of stay, overall length of stay, and mortality.

RESULTS

Forty study patients were enrolled in the study, with 20 healthy volunteers. Table 1 details the demographics and comparison of the two groups. Of the trauma patients, 72% experienced a blunt mechanism of injury. All of the patients with an HNAIS score of greater than 0 had a TBI seen on computed tomography of the head. Twelve (30%) of the patients were initially admitted to the ICU. Ultimately, two patients died in the

TABLE 3. Correlation Analysis

	LTA		Flow Cytometry		ELISA		
	% Aggregation ADP-Mediated	% Aggregation TRAP-Mediated	CD62 + ADP (Percent Positive)	CD62 + ADP MFI (Surface Density)	D-dimer	TAT	PF 1 + 2
Admission base deficit	0.338 (0.0544)	0.179 (0.3201)	−0.214 (0.2325)	−0.096 (0.5946)	−0.369 (0.0348)*	−0.230 (0.1985)	−0.202 (0.2601)
Systolic blood pressure	0.026 (0.8807)	−0.055 (0.7506)	−0.295 (0.0810)	−0.221 (0.1953)	−0.258 (0.1292)	−0.037 (0.8314)	−0.161 (0.3497)
Heart rate	−0.237 (0.1643)	−0.190 (0.2659)	0.045 (0.7935)	−0.055 (0.7504)	0.343 (0.0407)*	0.115 (0.5036)	0.268 (0.1146)
Admission lactate	−0.078 (0.6663)	−0.229 (0.1992)	0.087 (0.6322)	0.052 (0.7740)	0.190 (0.2905)	0.087 (0.6306)	0.100 (0.5812)
GCS score	0.435 (0.0091)*	0.381 (0.0239)*	−0.031 (0.8593)	0.067 (0.7018)	−0.542 (0.0008)*	−0.414 (0.0134)*	−0.206 (0.2356)
24-h blood transfusion	−0.268 (0.3992)	−0.243 (0.4468)	0.276 (0.3860)	0.098 (0.7621)	0.259 (0.4159)	0.253 (0.4279)	−0.169 (0.5998)
ICU days	−0.476 (0.1179)	−0.053 (0.8682)	−0.133 (0.6811)	−0.172 (0.5928)	0.365 (0.2438)	0.130 (0.6882)	0.063 (0.8461)
Length of stay	−0.261 (0.1241)	−0.083 (0.6285)	0.085 (0.6207)	−0.061 (0.7223)	0.429 (0.0089)*	0.440 (0.0072)*	0.6780 (<0.0001)*

Results reported as Spearman rank correlation coefficient (*p* value).

cohort. Both experienced severe blunt trauma and expired shortly after arrival to the trauma center.

Compared with the noninjured volunteers, trauma patients had significantly decreased median ADP-mediated (75% vs. 69%) and TRAP-mediated (76% vs. 45%) platelet aggregation response (Table 2). There were no significant differences in the baseline, inactivated platelet count, or CD62 expression between trauma patients and controls (20% vs. 14%, $p = 0.1007$); however, there was a significant difference in ADP-mediated CD62 expression in trauma patients versus controls (80% vs. 86%, $p = 0.02$). In addition, in patients with a known TBI, TRAP-mediated aggregation was found to be inversely proportional to HNAIS score (Fig. 1). Similarly, among the trauma patients, GCS score was found to be proportional to both TRAP-mediated ($p = 0.0263$) and ADP-mediated ($p = 0.0022$) aggregations. MIgG control values were similar between the two treatment groups (data not shown).

Results of the correlation analysis are shown in Table 3. GCS score and ADP- and TRAP-mediated platelet aggregation were positively correlated. Measures of shock including admission blood pressure, pulse, base deficit, and lactate level did not correlate with any of the measures of platelet dysfunction. In addition, there was no correlation between any of the measures of platelet function and length of stay, ICU days, or blood transfused.

ELISA testing showed elevated levels of D-dimer, TAT, and PF 1 + 2 in trauma patients compared with controls (Table 2). D-dimer was negatively correlated with base deficit and GCS score and positively correlated with both heart rate and length of stay. TAT was inversely correlated with GCS score and positively correlated with length of stay. PF 1 + 2 was also positively correlated with length of stay (Table 3).

DISCUSSION

Our study continues to support the concept that platelets have a role in the pathophysiology of ATC. Three phases occur in platelet contribution to hemostasis or thrombosis: adhesion, activation, and aggregation. Adhesion begins with damage to endothelial cells and exposure of subendothelial collagen, which facilitates platelet binding via GPIb-IX-V complex to collagen-bound von Willebrand factor.[15,16] This adhesion event leads to the activation of signal transduction cascades that cause shape change and platelet secretion or membrane surface expression of numerous molecular mediators from platelet granules, including CD62, and activation of platelet surface glycoproteins, specifically GPIIb-IIIa. The conformational changes to GPIIb-IIIa allow for platelet-platelet interactions via fibrinogen cross-linking and therefore aggregation. Substances released from platelet granules then continue to augment platelet-mediated vasculature reactivity. In addition, activated platelets provide a procoagulant surface for thrombin generation and propagation.[17–19]

Although ATC has been extensively investigated, few studies have evaluated the function and activation of platelets following trauma.[7,20] We pursued this by focusing on platelet aggregation (platelet function) and surface CD62 expression (platelet activation) by assessing ADP and thrombin-mediated pathways. LTA, although a relatively labor-intensive test, is the criterion standard for measuring platelet function.[10]

In a study conducted by Wohlauer et al.,[20] platelet map thromboelastography was used to evaluate platelet dysfunction in 51 trauma patients in response to two agonists, ADP and arachidonic acid. They found more than 80% median inhibition of ADP-mediated platelet function in trauma patients versus healthy controls. Kutcher et al.[7] used multiple electrode impedance aggregometry to assess platelet function in 101 trauma patients in response to ADP, TRAP, arachidonic acid, and collagen. They found that 46% of trauma patients had decreased platelet function in response to at least one agonist. These studies agree with our findings that trauma patients had significantly lower ADP- and TRAP-mediated platelet aggregation, as measured by LTA, when compared with healthy controls. Decreased ADP responsiveness has been postulated to be secondary to initial hyperactivation of platelets in the trauma setting, which decreases platelet response to further ADP exposure.[7] Bune et al.[21] have suggested that massive release of ADP caused by trauma and ischemia of tissues can, in turn, cause substantial platelet dysfunction. In addition to a significant decrease in ADP-mediated platelet aggregation, our added finding of impairment of TRAP-mediated aggregation that signals initially through the PAR-1 receptor signifies that the thrombin receptor pathway likely plays a role in platelet dysfunction. If the PAR-1 receptor is affected, then endothelial cells that also express the PAR-1 receptor may also contribute to the pathophysiology of ATC by causing endothelial cell imbalance in the affected vasculature.

Jacoby et al.[22] analyzed platelet function following trauma using a platelet function analyzer-100. They also studied platelet activation by performing flow cytometry to analyze platelet surface markers of activation, which included but was not limited to CD62. Their study found that platelet surface expression of CD62 was significantly increased in trauma patients when compared with controls. In contrast, we found that baseline CD62 expression did not significantly differ between trauma patients and controls. Our study indicates instead that the ADP-mediated platelet CD62 expression in response to ADP was decreased in trauma patients compared with controls. These data complement our aggregation testing results. In contrast, Jacoby et al. did not evaluate ADP-mediated CD62 expression. Our results suggest that impairment of platelet activation in trauma patients by means of exhausted platelet secretion mechanisms following activation by endogenous agonists such as ADP, however, needs further investigation.

Multiple studies have found an association between impaired platelet aggregation and mortality in patients with TBI.[23–25] Jacoby et al. demonstrated impaired platelet function (as presented by impaired collagen/epinephrine closure times using the platelet function analyzer-100) in numerous patients with substantial TBI (AIS score ≥ 4).[25] Kutcher et al. proposed that GCS score is an independent predictor of platelet dysfunction in trauma patients.[7] In addition, Davis et al. used platelet map thromboelastography to demonstrate that ADP inhibition was significantly higher in patients with TBI, as measured by GCS, and more pronounced in nonsurvivors with TBI than in survivors with TBI. That same study also showed that ADP inhibition correlated with the severity of the TBI.[24] Our results confirmed

that GCS score is proportional to TRAP- and ADP-mediated platelet aggregation. In addition, we observed significant inverse relationships between D-dimer and TAT levels with admission GCS score. Both biomarkers are related to clot lysis or thrombin generation, respectively, and thus lend significance to our findings. The clinical relevance of these findings is unclear at this time; however, it suggests that TBI plays a role in trauma-related platelet dysfunction.

The results of the ELISA tests support our hypothesis that thrombin generation is a significant component in the clinical course of these patients as the levels of TAT and PF 1 + 2 indicate that measureable thrombin has been generated. Elevated level of D-dimer, while previously used as a rule-out for venous thromboembolism, is gaining some adoption as a prognostic indicator for the presence of clot and subsequent clot lysis activity. D-dimer values in the trauma patients were more than twice the upper limit of normal range.

The changes in platelet function seen in this study are distinctly different from those seen in patients taking antiplatelet medications. Patients on clopidogrel therapy do have inhibition of ADP-induced platelet aggregation and have reduced CD62 expression when exposed to ADP.[26] Aspirin, which blocks the cyclo-oxygenase pathway and inhibits thromboxane A2 production, also has an effect on platelet secretion, and hence, platelet response to ADP as the source of endogenous ADP is the platelet dense granules. ADP and thromboxane A2 are considered secondary agonists that contribute to platelet reactivity in response to primary agonists such as collagen and thrombin. While both antiplatelet agents may contribute to the extent of platelet activation and overall stability of the platelet aggregate, when platelets are exposed to thrombin at sufficient concentrations, its activity can overcome the effects of aspirin and clopidogrel. In contrast to aspirin- and clopidogrel-sensitive pathways, thrombin plays a critical role in both coagulation and platelet reactivity. The only agents that have significant effect on thrombin-induced platelet aggregation are the GPIIb-IIIa antagonists and the new PAR-1 antagonist, vorapaxar. Endothelial cells also express PAR-1, and the role this receptor plays in hemostasis and thrombosis is unclear.

There are several distinct limitations in this study. The resources required, both in terms of personnel and cost, to analyze the samples that were collected limited the sample size of the trauma patients. Similarly, the number of agonists tested was limited by the amount of excess blood we could expect to obtain from each patient as well as the cost associated with each additional agonist. The age disparity between the healthy volunteers and the trauma patients may be a significant confounder in the analysis of baseline platelet function; however, again because of the small size of the study, we have opted not to attempt to control for this statistically. Finally, the preliminary nature of this study meant that the conclusions drawn are limited by the initial intent, which was to determine whether the thrombin pathway warrants further investigation as a mediator of platelet dysfunction.

TRAP-mediated platelet aggregation is relatively unstudied compared with ADP-mediated aggregation. One study showed a significant decrease in platelet function in TRAP-mediated aggregation in older individuals (≥55 years) versus younger patients (<55 years) (p = 0.030) and also showed that low TRAP responsiveness was an independent predictor of mortality.[7] Our study found a stepwise decline in TRAP-mediated platelet aggregation as HNAIS score increased. In addition, soluble biomarkers directly related to thrombin activity were also affected in ATC. This phenomenon indicates that the thrombin pathway, in addition to the ADP pathway, may play a role in ATC associated with head trauma.

CONCLUSION

Trauma patients have significantly lower levels of platelet function and activation compared with healthy control subjects. Severity of head injury, as measured by AIS, significantly correlates with platelet dysfunction in a stepwise fashion, specifically when TRAP, the thrombin agonist peptide that targets PAR-1, was used as a platelet aggregation agonist. In platelet aggregation studies, TRAP served as a surrogate thrombin agonist for the high-affinity thrombin receptor, PAR-1.[27] These data thus imply that perhaps thrombin-mediated activation of platelets via the PAR-1 receptor is blunted in trauma patients compared with healthy controls. This pathway should continue to be studied with the goal of eventually finding a mechanism of platelet-coagulation cross-talk that can be clinically altered to improve outcomes following trauma.

AUTHORSHIP

M.T.R., T.C.F., J.P.S., and L.K.J. contributed to the literature review and design of this study. M.T.R. and S.E.M. collected the data. M.T.R., T.C.F, C.P.S., and L.K.J. analyzed and interpreted the data. M.T.R., T.C.F., C.P.S., S.E.M., and L.K.J. wrote the manuscript. T.C.F., C.P.S., J.A.W., M.A.C., and L.K.J. performed the critical revision.

DISCLOSURE

The authors declare no conflicts of interest.

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DISCUSSION

Dr. Mitchell J. Cohen (San Francisco, California): This is a very well-conceived and well-done study by a prolific group and I want to applaud the authors, and particularly the presenter and lead investigator, on this study.

As was nicely presented, platelet dysfunction is common after trauma. I am happy that platelets are being recognized or perhaps re-recognized as being the primary drivers of outcome after injury. The Denver group and our group in San Francisco published several years ago widespread platelet dysfunction after trauma in 50% of patients after injury, despite normal platelet counts. Let me reiterate that. Half of our patients have platelet dysfunction immediately after trauma.

The balanced resuscitation literature critically shows a stronger effect of early platelet transfusions on bleeding and mortality than even plasma. This is, of course, meant to be taken with an air of caution, as late and unnecessary platelet transfusion is strongly associated with inflammatory complications such as multiple organ failure and ARDS.

There is very interesting biological plausibility for this in that there is early platelet dysfunction and a beautiful scientific literature showing platelet-associated inflammation with work, such as that from UCSF, showing platelet neutrophil sequestration as lungs as a mechanism for epithelial injury and lung injury. The work here expands on this evolving work to show platelet dysfunction and an interesting suggestion that this may be thrombin mediated. To this end I have several questions and comments.

The first is, why measure only ADP and TRAP? While there is clear plausibility for these agonists, the mechanism of the endogenous platelet function after trauma remains a very open question with nothing really to guide us and probably our assays should not be limited just to these two agonists.

Many have published that there is adequate thrombin production after trauma, and that thrombin doesn't seem to ever be the problem driving coagulopathy. Your data seem to show the same. These patients are making thrombin after injury. That said, there is an inverse correlation between GCS and thrombin production. Do you think that there is adequate thrombin production to drive platelet function or do you think this is below a threshold? What do you think is going on with this mechanism when you suggest that the thrombin pathway may be a prime mechanistic driver?

What are the data that show that thrombin is below a threshold to signal via PAR-1? While this is an interesting hypothesis that absolutely deserves exploration, I wonder whether there are any data to support this?

And, lastly, can you help me define your cohort? AIS has been questioned as a means of finding isolated TBI or even really being true to the severity of brain injury. What other injuries do these patients have? Was brain injury corroborated by imaging or some other adjudication of TBI? If not, I would really suggest direct evaluation of the CTs or professional adjudication by your neurosurgery colleagues, which in this size cohort is absolutely doable.

Lastly, tell me clinically what your data suggests. I know you are a student, and you should really be applauded for such outstanding mature work, but you are absolutely now one of us so tell us—what should we do? Should we give platelets empirically based on lab tests? Based on CT scanning? What should we do in 2015?

Overall there is much to do on this very important topic. I applaud the author and prime driver of this study and presenter and I look forward to great additional work from him. Thanks.

Dr. Mark Cipolle (Wilmington, Delaware): Enjoyed your talk very much and I think I have an extension of Dr. Cohen's last couple of questions. There is a pretty good signal in hemorrhagic stroke that there is a correlation between reduced platelet activity and change in the hemorrhage on the second CT scan.

Does your group have any data that suggests that? You have a very good model to do that. If you don't, I would suggest that you do.

Dr. Charles E. Lucas (Detroit, Michigan): That was a very nice presentation.

As you know, the primary and secondary platelet release reactions can be stimulated by both ATP and collagen. Did your agonist to ATP cause any problems with activation by collagen? And did you look at collagen stimulated aggregation?

Finally, did you do any studies on your patients regarding the bleeding time because that's probably very important as it relates to the injured patient.

Dr. Matthew T. Ramsey (Memphis, Tennessee): Thank you, again, Dr. Cohen, and members of the audience for the questions.

As far as ADP and TRAP are concerned, and why we only used those agonists, we saw in preliminary studies at our institution that TRAP showed some differences in platelet function in these trauma patients. ADP was used because it has historically been used to evaluate platelet function in patients.

While I agree we should not limit our agonists to only these two, I want to stress that this should be considered preliminary data and we are hoping it is something that is going to open up a gateway for additional research in the area.

Studies have shown that there is adequate or even possibly increased thrombin generation in trauma patients and therefore, I do not think it is a problem with thrombin generation, but rather, a threshold is not being reached for TRAP or for thrombin to activate these platelets and form a stable aggregate.

We do not have any additional evidence, per se, to support this other than the fact that in our study we found increased thrombin/antithrombin complex and decreased TRAP-mediated aggregation in these patients. That tells us that there is something going on in between those two processes, which could very well be blunting of the PAR-1 receptor.

I agree that AIS has its limitations. In our patients with traumatic brain injury we did evaluate it based on CT and clinical evidence but we did not break down the traumatic brain injury group any further because of the small size of the cohort.

As far as management for these patients, I want to stress, again, that this should be considered preliminary research. We cannot really say at this point that we need to change our current treatment algorithms for these patients, based on our single study, to help decrease morbidity and mortality.

Rather, we need to do more studies in that area in hopes of finding the specific mechanism causing the platelet dysfunction, so we can reverse and hopefully help treat these patients.

We did not evaluate hemorrhagic stroke. I believe that this would be a very interesting avenue that we could and should pursue in the future.

Other agonists such as collagen were mentioned. Again, we used the two agonists based on our clinical preliminary studies for TRAP and the historical use of ADP; therefore, we did not evaluate collagen.

Lastly, we did not evaluate bleeding time. That would also be something interesting to study in the future to give more clinical insight into this whole process.

Thank you, again, for the privilege of the podium.