# Platelet adenosine diphosphate inhibition in trauma patients by thromboelastography correlates with paradoxical increase in platelet dense granule content by flow cytometry

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***Background.*** *The mechanism of platelet dysfunction in acute traumatic coagulopathy is unknown. Traumatic brain injury is hypothesized as a cause, while some investigators presume platelets become "exhausted." We hypothesized that platelet hyperstimulation and consumption resulting from trauma leads to decreased platelet function secondary to depletion of platelet granules.*
***Methods.*** *Twenty-five trauma patients were divided into traumatic brain injury and no traumatic brain injury groups. Healthy volunteers served as controls. All had thromboelastography with platelet mapping and flow cytometric assays of mepacrine performed. Mepacrine uptake in unstimulated platelets was used for quantification of platelet content of dense granules.*
***Results.*** *Twelve patients with traumatic brain injury and 13 patients without traumatic brain injury were enrolled. Twenty-one trauma patients showed adenosine diphosphate inhibition (>30%) on thromboelastography with platelet mapping compared with the healthy volunteers who served as controls (*$P < .01$*). Mepacrine assay showed a difference in mean fluorescent intensity for all trauma patients of 4,259 ± 1,341 compared with controls of 3,143 ± 709 (*$P = .044$*), correlating with greater quantities of dense granules. Neither adenosine diphosphate inhibition nor average difference in mean fluorescent intensity between traumatic brain injury and no traumatic brain injury groups were significant (*$P = .2$*).*
***Conclusion.*** *Trauma patients maintain their dense granule, contradicting the theory of platelet granule exhaustion as the etiology for platelet dysfunction in traumatic brain injury. (Surgery 2016;160:954-9.)*

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In the setting of trauma, platelets serve a critical function in hemostasis. The process of platelet activation begins when a platelet encounters a break in the endothelium. When this occurs, the platelet encounters various molecules that trigger its activation. When a platelet is activated, there is exocytosis of its secretory granules; this process allows platelets to adhere to one another and to collagen, forming a platelet plug. If a platelet is not activated or if its granules are depleted, the platelet is not functional, and clot formation is impaired.

The recent adoption of thromboelastography with platelet mapping (TEG-PM) in trauma resuscitation has highlighted a disturbance of platelet function in patients with a traumatic brain injury (TBI).[1] The effects of antiplatelet agents are recognized on TEG-PM through prolonged inhibition in the arachidonic acid (AA) or adenosine diphosphate (ADP) pathways[2,3]; however, some trauma patients who do not take these agents have demonstrated similar platelet dysfunction on TEG-PM.[4]

With the goal of studying platelet dysfunction, we conducted a prospective cohort study of the platelet content of dense granules in severely

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injured trauma patients with and without TBI and compared them with healthy controls. We hypothesized that platelets from trauma patients demonstrating ADP inhibition on TEG-PM would exhibit a decreased content of dense granules compared with normal controls secondary to depletion of platelet granules, as suggested by Pareti et al.[3] We also hypothesized that there would be a difference in granule content in platelets between trauma and isolated TBI patients.

## METHODS

This study was approved by the University of Missouri Institutional Review Board (200325 HS). Informed consent was waived for the trauma patients but all individuals in the control group were consented. Our study population included severely injured adult trauma patients—consecutively activated level 1 traumas—and healthy controls. A thorough medication reconciliation was obtained by the pharmacy for the trauma patients. Patients and controls known to be taking anticoagulant or antiplatelet agents were excluded. The trauma patients were divided into an isolated TBI group and a no TBI group (N-TBI). We did not screen by injury severity score, because this could not be calculated until after the initial 24- to 48-h workup. TBI was defined by the presence of traumatic intracranial hemorrhage identified on head computed tomography (CT). All study patients had TEG-PM performed to determine functional AA and ADP platelet inhibition, in addition to a flow cytometric assay of mepacrine (quinacrine) content; mepacrine uptake in unstimulated platelets (10,000 events) was used for quantification of platelet content of dense granules using mean fluorescent intensity (MFI) analysis.

**Thromboelastography with platelet mapping.** Thromboelastography with platelet mapping (TEG-PM; Haeomoscope Corporation, Niles, IL) offers both numeric and graphic representations of the ex vivo rate and strength of clot formation. The addition of platelet mapping provides specific information regarding the reactivity of platelets to the activators AA and ADP. With TEG-PM, one can then assess the percentage of inhibition in both AA and ADP pathways from antiplatelet agents or other hindering factors; significant inhibition is defined as <30%.

We focused on the ADP inhibition component of the TEG-PM due to its reported contribution to acute traumatic coagulopathy in the literature. In addition, ADP inhibition contributes more to coagulopathy than AA inhibition. This has been demonstrated in the cardiac surgery literature, with preoperative inhibition of ADP predicting the development of microvascular bleeding in patients on clopidogrel.[5] The thought is that AA inhibition is a minor contributor to bleeding, and ADP is the larger component of platelet inhibition.

**Mepacrine assay.** Platelets contain granules (dense granules, lambda granules, and alpha granules) that break down to release fibrinogen, von Willebrand factor, platelet-derived growth factor, ADP, calcium, and 5-hydroxytryptamine- serotonin. Activated platelets secrete the contents of these granules through their canalicular systems to the exterior. Flow cytometric methods have been developed to study platelet activation, aggregation, and protein release from alpha granules. In this study, we used flow cytometry for analysis of the uptake and release of platelet dense granules using mepacrine as a fluorescent marker.

Mepacrine (quinacrine) is taken up and localized rapidly in dense granules of platelets ex vivo. In both human and mouse platelets, mepacrine uptake is proportional to platelet size. Therefore, both platelet uptake and release of mepacrine can be detected readily by flow cytometry. Flow cytometry provides an attractive alternative to aggregation and radioactive serotonin as methods to study defects in the function of platelet dense granules.[6-8] Results are reported as mean MFI.

**Statistics.** Patient data, including MFI, were compared between the three groups using Student *t* test for continuous variables and Pearson $\chi^2$ test for categorical variables. All statistical analysis was performed using Excel 2013 (Microsoft Corp, Redmond, WA) and SPSS for Windows (version 23, IBM, Chicago, IL).

## RESULTS

Twenty-five severely injured adult trauma patients were enrolled; 12 in the TBI group and 13 in the N-TBI group. Eight volunteers were enrolled as healthy controls. Table I compares admission variables between groups. The sex of the healthy control group was not well matched with the trauma groups, although the difference was not significant by the $\chi^2$. The Glasgow Coma Scale (GCS) between TBI and N-TBI groups was significantly different ($P < .05$). In the TBI group, 10 patients had blunt trauma and 2 had penetrating wounds. In the N-TBI group, there were 8 blunt traumas and 5 penetrating traumas. The average injury severity score for all trauma patients was 19.9. There were 3 deaths among the 25 trauma patients.

**TEG-PM.** Figure 1 compares the percent ADP inhibition measured by TEG-PM results of all