Published in final edited form as:

J Trauma Acute Care Surg. 2019 April ; 86(4): 592–600. doi:10.1097/TA.0000000000002171.

MICROVESICLES GENERATED FOLLOWING TRAUMATIC BRAIN INJURY INDUCE PLATELET DYSFUNCTION VIA ADP RECEPTOR:

Platelet Dysfunction Following TBI

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Abstract

Introduction: Traumatic brain injury (TBI) can result in an acute coagulopathy including platelet dysfunction that can contribute to ongoing intracranial hemorrhage. Previous studies have shown ADP-induced platelet aggregation to be reduced after TBI. In addition, circulating microvesicles (MVs) are increased following TBI and have been shown to play a role in post-TBI coagulopathy and platelet function. We hypothesized that post-TBI MVs would affect platelet aggregation in a murine head injury model.

Methods: Moderate TBI was performed using a weight-drop method in male C57BL6 mice. Whole blood, plasma, MVs, and MV-poor plasma were isolated from blood collected 10 minutes following TBI and were mixed separately with whole blood from uninjured mice. Platelet aggregation was measured with Multiplate impedance platelet aggregometry in response to adenosine diphosphate (ADP). The ADP P2Y₁₂ receptor inhibitor, R-138727, was incubated with plasma and MVs from TBI mice, and platelet inhibition was again measured.

Results: Whole blood taken from 10 minute post-TBI mice demonstrated diminished ADP-induced platelet aggregation compared to sham mice. When mixed with normal donor blood, post-TBI plasma and MVs induced diminished ADP-induced platelet aggregation compared to sham plasma and sham MVs. By contrast, the addition of post-TBI MV-poor plasma to normal blood did not change ADP-induced platelet aggregation. The observed dysfunction in post-TBI ADP platelet aggregation was prevented by the pretreatment of post-TBI plasma with R-138727. Treatment of post-TBI MVs with R-138727 resulted in similar findings of improved ADP-induced platelet aggregation compared to non-treated post-TBI MVs.

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Author Contribution Statement: GEM and MDG contributed to the design of the study. GEM, AP, RM, RV and LAF contributed to the acquisition of data. GEM, TAP, ATM, CCC, and MDG contributed to data analysis. GEM drafted the manuscript. All authors contributed to critical review of the manuscript and have agreed to the final version of the manuscript.

This paper was presented as a podium presentation at the 77th Annual Meeting of the American Association for the Surgery of Trauma, 09-28-2018, San Diego, CA

Conflicts of Interest: Nothing to report.

Conclusion: ADP-induced platelet aggregation is inhibited acutely following TBI in a murine model. This platelet inhibition is reproduced in normal blood by the introduction of post-TBI plasma and MVs. Furthermore, observed platelet dysfunction is prevented when post-TBI plasma and MVs are treated with an inhibitor of the P2Y₁₂ ADP receptor. Clinically observed post-TBI platelet dysfunction may therefore be partially explained by the presence of the ADP P2Y₁₂ receptor within post-TBI MVs.

Level of Evidence: III

Keywords

Traumatic brain injury; microvesicles; platelet aggregability; P2Y₁₂ receptor; adenosine diphosphate

BACKGROUND

Traumatic brain injury (TBI) is a major public health problem resulting in at least 2.5 million emergency department visits annually and remains the leading cause of death in young adults in the United States.^{1,2} Coagulopathy following TBI is a serious consequence, resulting in significantly elevated rates of progression of hemorrhagic lesions and increased acute and long-term morbidity and mortality.^{3,4} Although studies estimate that nearly one third of head injured patients will develop clinically significant coagulopathy, the pathophysiology of this particular form of coagulopathy remains poorly understood.⁵

Patients with isolated TBI are equally as likely as patients with extracranial trauma to develop acute coagulopathy despite lacking many of the key causal factors, including massive tissue injury and blood loss, that are often responsible for extracranial traumatic coagulopathy.^{4,6,7} Given these and other physiologic differences between the two types of traumatic coagulopathy, a distinctive biologic pathway may be responsible for TBI-induced coagulopathy. Platelet dysfunction following head injury plays a role in TBI-induced coagulopathy and is defined by a diminished *in vitro* platelet response to individual agonists. Platelets may be stimulated by several different agonist pathways, including adenosine diphosphate (ADP), arachidonic acid (AA), collagen, and thrombin.^{8,9} In human studies, both ADP and AA platelet dysfunction have been observed, with ADP-related platelet dysfunction correlating with higher mortality rates in the isolated TBI population.^{10,11}

Microvesicles (MVs) are 100–1000 nm membrane-bound vesicles that are shed from plasma membranes during cell activation or cell death.¹² Elevated MV levels have been measured in the cerebrospinal fluid as well as the systemic circulation of patients with isolated acute TBI.^{13,14} Additionally, increasing numbers of TBI-induced brain-derived microvesicles resulted in progressively prolonged clotting times in a murine model of TBI.¹⁵ Microvesicles, especially those that are platelet-derived, are known to possess independent thrombotic activity, however the interaction of posttraumatic MVs, platelet function, and potential role in *ex vivo* platelet testing are unknown. More specifically, MV populations and their coagulability are affected by TBI and may therefore play a role in the assessment and treatment of post-injury coagulopathy and platelet dysfunction.

In this study we sought to better understand the role of MVs generated following TBI in platelet dysfunction that is observed after isolated head injury. We hypothesized that MVs released acutely after TBI may interfere with or inhibit the ADP and AA platelet activation pathways. If true, then recognizing and targeting the MV receptors could result in restoration of posttraumatic platelet aggregation and may provide a therapeutic target for patients with TBI-induced platelet dysfunction.

MATERIALS AND METHODS

Animals and Treatments

All animal experiments were examined and approved by the Institutional Animal Care and Use Committee of the University of Cincinnati. Male C57BL6/J mice between ages 8 and 10 weeks were purchased from Jackson Laboratory (Bar Harbor, ME). Female mice were excluded from this study as higher estrogen levels have been demonstrated to be independently neuroprotective in rodent TBI studies.^{16,17} Animals were housed in controlled conditions with a 12 hour light-dark cycle and were allowed to acclimate to the new environment for at least one week prior to experiments.

Moderate TBI Model

Our laboratory utilizes a single-impact weight drop device to achieve a concussive TBI of moderate severity to induce systemic and cerebral inflammation, motor and cognitive impairment, as well as a mortality rate of 10%.¹⁸ Mice were anesthetized with 2% inhaled isoflurane for 2 minutes in 100% oxygen at 1 L/min. While anesthetized, the animals were placed in a prone position with the head centered under a 400 g cylindrical weight. The weight was dropped from a height of 1.5 cm above the table surface, centered in both the rostral/caudal and lateral directions. Sham mice were anesthetized and positioned below the impactor weight but did not undergo TBI. Mice were sacrificed 10 minutes after TBI or sham injury for collection of blood, plasma, and microvesicles to evaluate the acute coagulation response to head injury. Sample sizes utilized were chosen to detect a 20% difference with a 10% standard deviation at 90% power, yielding a sample size of 5 for Multiplate and ROTEM analyses. (Figure 1)

Whole Blood, Plasma, and Microvesicle Isolation

Following TBI, mice were anesthetized with 0.1 mg/g of pentobarbital via intraperitoneal injection. Whole blood samples were collected via cardiac puncture and immediately anticoagulated with either 10% hirudin for aggregation studies or citrate for viscoelastic coagulation analyses. Anticoagulated blood samples from 5 mice were pooled to obtain adequate MV samples for protein and receptor analysis. Microvesicles were isolated by differential centrifugation using a modification of a protocol previously published.^{19,20} Fresh whole blood was centrifuged at 450g for 10 minutes at 4°C. The pellet, composed of red and white blood cells, was discarded. The platelet-rich plasma (PRP) supernatant was either utilized for experimentation or underwent another round of centrifugation at 10,000g for 5 minutes at 4°C. The resultant platelet pellet was utilized for platelet receptor studies and the platelet-poor plasma (PPP) supernatant was then centrifuged at 25,000g for 30 minutes at 4°C to pellet MVs. The resultant supernatant was MV-poor plasma. The remaining MV

pellet was washed with 0.9% saline and centrifuged again at 25,000g for 30 minutes. The total number of MVs was estimated using Nanoparticle Tracking Analysis (Nanosight, Malvern Instruments Ltd, Worcestershire, UK) and was defined as those particles between 0.1µm and 1µm in diameter. Microvesicles were resuspended in 0.9% saline to a concentration of 3.0×10^8 MVs/mL and added to anticoagulated blood as described below.²¹ Figure 1A gives a simplified depiction of this experimental design.

P2Y₁₂ ADP Receptor Inhibition

To inhibit the ADP P2Y₁₂ receptor, the prasugrel active metabolite R-138727 (50µM) was added to PPP and MVs. This concentration of R-138727 was based on a dose-response of R-138727 in normal blood and was the highest concentration of R-138727 that did not affect platelet aggregability of normal whole murine blood. (Supplemental Material 1) Samples of PPP were incubated with R-138727 for 30 minutes and then added to whole blood for additional analysis. Microvesicles inhibited with R-138727 also underwent a 30 minute incubation period; however, treated MVs underwent another round of centrifugation at 25,000g for 30 minutes to remove any remaining R-138727 and the MVs were again resuspended in 0.9% saline to a concentration of 3.0×10^8 MVs/mL.

Platelet Aggregation Analysis

Whole blood samples were collected and anticoagulated immediately with 10% hirudin. Samples of PRP, PPP, MV-poor plasma, and MVs were added to anticoagulated blood at a ratio of 1:5. Multiplate impedance aggregometry (Roche Diagnostics, Rotkreuz, Switzerland) was used to measure platelet aggregation. Platelet aggregation was induced using either 6.5 µM ADP or 0.5 mM AA as recommended for testing by manufacturer instructions. Results are reported as “Area under the curve” (AUC) which is affected by the platelet aggregation curve height and slope, as determined by the speed and final strength of aggregation.

Thromboelastometry

Whole blood samples were collected and immediately anticoagulated with 10% citrate. Samples of PRP, PPP, MV-poor plasma, and MVs were added to anticoagulated blood at a ratio of 1:5. Coagulation parameters were measured using rotational thromboelastometry (ROTEM, TEM Systems Inc., Durham, NC) analyses, as previously described.²² All analyses were performed within 10 minutes of blood collection. Both extrinsic pathway coagulation (EXTEM) and fibrin contribution to clot (FIBTEM) were measured for each blood sample in order to calculate the percent of platelet contribution to clot formation: %MCF-Platelet = $(\text{EXTEM}_{\text{MCF}} - \text{FIBTEM}_{\text{MCF}}) / \text{EXTEM}_{\text{MCF}}$.²¹ For EXTEM and FIBTEM testing, 20µL of thromboplastin was added to citrated blood to initiate clot formation. Cytochalasin D was added to FIBTEM samples to prevent platelet activation.

Microvesicle Western Blots

Fresh whole blood was collected 10 minutes following TBI (n=8) or sham (n=8) treatment and pooled to allow for adequate protein amounts to be analyzed. Half of the pooled samples of blood was used for platelet isolation and the other half for MV isolation. Platelets and

MVs were isolated from whole blood as described above. Figure 1B gives a simplified depiction of this experimental design. Following a washing with PBS, samples each underwent lysis with Modified RIPA buffer (50mM Tris HCl, pH 7.4 150mM NaCl, 1mM EDTA, 1% Triton X-100, 0.1% SDS, 1% Na⁺ Deoxycholate, 1mM PMSF, 100mM NaOV, 1µg/mL leupeptin, 1µg/mL aprotinin, 1µg/mL pepstatin, 10µg/mL soybean trypsin inhibitor) for 30 minutes at 4°C. Samples were centrifuged at 16,000g for 20 minutes at 4°C to remove cellular debris. Protein concentrations of each sample were determined using BCA protein assay kit (Pierce/ThermoFisher, Rockford, IL). Samples containing equal amounts of protein were resuspended in 5X sodium dodecyl sulfate sample buffer, separated in a denaturing 10% polyacrylamide gel, and transferred to a 0.2µm pore nitrocellulose blotting membrane (GE Healthcare Amersham^{TM}Protran^{TM}, ThermoFisher Scientific, Waltham, MA). Nonspecific binding sites were blocked with Tris-buffered saline (40mM Tris, pH 7.6, 300mM NaCl) with 0.1% Tween 20 containing 5% nonfat dry milk for 1 hour at room temperature. Membranes were then incubated with 2 µg/mL antibodies to murine P2Y₁₂ ADP receptor (AS-55043A; AnaSpec, Fremont, CA) in Tris-buffered saline with 0.1% Tween 20 overnight at 4°C. Membranes were washed and incubated with 1 µg/mL secondary antibodies conjugated to horseradish peroxidase. Immunoreactive proteins were detected by chemiluminescence (sc-2005, Santa Cruz Biotechnology, Dallas, TX).

Resulting films were imaged and analyzed using MyImageAnalysis v2.0 (Thermo Fisher Scientific, Waltham, MA). Results are reported as average intensity of each well.

Statistical analysis

Statistical analysis was performed utilizing the statistical software package Prism 7 (GraphPad, La Jolla, CA). Continuous data is reported as means with standard deviation. Two-tailed Student's t-tests were used to make comparisons between two groups, and Analysis of Variance with Tukey's post-test was used to make comparisons between three or more groups. Categorical and discrete data are reported as percentages and rates with comparisons performed using chi-square analysis. A p-value of less than 0.05 was considered significant.

RESULTS

Closed head injury model replicates platelet dysfunction via ADP pathway

When post-TBI blood was added to normal murine blood no differences were observed in the ability of AA to induce platelet aggregation between TBI (n=5) and sham (n=5) blood. However, blood from TBI mice (n=5) significantly impaired platelet aggregation when ADP was used as an agonist (Figure 2).

Post-TBI MVs induce platelet dysfunction via ADP pathway

Individual post-TBI components (n=5 for each component experiment) were added to normal murine blood and analyzed on the Multiplate. The PRP (Figure 3A) significantly impaired platelet aggregation (17.0 ± 4.6 units, TBI/PPP vs. 28.0 ± 3.5 units, sham/PPP, $p=0.003$). Neither PPP (Figure 3B) nor MV-poor plasma (Figure 3C) reduced ADP-induced platelet aggregation. However, when isolated MVs were added to donor murine blood

(Figure 3D), ADP-related platelet dysfunction was again demonstrated (14.6 ± 2.4 units, TBI/MVs vs. 24.0 ± 4.2 units, sham/MVs, $p=0.003$).

P2Y₁₂ ADP receptor isolated from microvesicles of post-TBI mice

Pooled samples (TBI $n=8$, sham $n=8$) of MVs and platelets underwent Western blot analysis, which confirmed the presence of the P2Y₁₂ receptor on both the platelets as well as MVs. (Figure 4A) With equal protein amounts being analyzed, there was no difference in the average intensity of the P2Y₁₂ receptor staining between sham MV and TBI MVs. (Figure 4B) Similarly, no significant difference was observed between sham and TBI platelet levels of P2Y₁₂. (Figure 4C)

MVs treated with P2Y₁₂ receptor inhibitor do not induce platelet dysfunction via ADP pathway

Treatment of post-TBI PPP ($n=5$) (Figure 5A) and post-TBI MVs ($n=5$) (Figure 5B) with R-138727 prevented the inhibition of ADP-induced platelet aggregation (42.8 ± 1.8 units, R-138727 TBI/PPP vs. 16.1 ± 2.3 units, TBI/PPP, $p<0.0001$) (44.0 ± 1.7 units, R-138727 TBI/MVs vs. 13.8 ± 3.2 units, TBI/MVs, $p<0.0001$).

Post-TBI microvesicles have no effect on platelet contribution to maximum clot firmness

Thromboelastometry was performed on the MV-blood mixture ($n=5$) and the percent of platelet contribution to maximum clot firmness was calculated. (Figure 6) No difference in the percent of platelet contribution to maximum clot firmness was observed between sham MV and TBI MV-treated blood.

DISCUSSION

Coagulopathy and platelet dysfunction can be a devastating result of moderate or severe TBI. Although relatively common, the biologic mechanisms behind TBI-induced coagulopathy and platelet dysfunction remain poorly understood. Utilizing our murine head injury model to induce a moderate TBI we were able to demonstrate a similar platelet dysfunction via the ADP pathway as is observed in human TBI. Furthermore, MVs from post-TBI mice were found to induce *in vitro* platelet dysfunction when added to normal murine blood. Treatment of post-TBI MVs with an inhibitor of the P2Y₁₂ ADP receptor resulted in improved platelet aggregability. These findings highlight a potential target for post-injury therapy while also providing an explanation as to why current therapies often prove ineffective.

Diminished ADP and AA platelet responses following isolated TBI have been demonstrated in both human and rodent studies.^{23,24} In this closed skull concussive model of moderate TBI we observed an acute platelet dysfunction via the ADP pathway without changes in the ability of AA to induce platelet aggregation. These findings are similar to previous studies in which acute ADP-related platelet dysfunction is detected up to 30 minutes following injury with subsequent resolution in platelet aggregation or, in some cases, rebound hyperaggregability.^{24,25} Although circulating MVs are increased following TBI, the impact these vesicles have on coagulation and platelet dysfunction remains uncertain. Given the

multitude of receptors, proteins, and lipids contained within MV membranes, these molecules may have the potential to either improve or reduce platelet function depending on the stimulus and timing. For example, while one study noted potent anticoagulant activity of platelet-derived microparticles another group demonstrated a procoagulant phenotype at 6 hours following TBI.^{21,26} Our results indicate that MVs released acutely following TBI may induce apparent platelet dysfunction by interfering with ADP-induced platelet aggregation, rather than by direct inhibition of platelet function.

Platelet dysfunction in the setting of TBI remains difficult to diagnose and treatment strategies often consist of platelet transfusion and/or supportive therapy. Although viscoelastic coagulation testing can help providers to more effectively diagnose post-traumatic coagulopathy and platelet dysfunction, many trauma centers continue to rely on platelet counts and the international normalized ratio (INR) to identify head injured patients who require blood products for reversal of anti-platelet agents or ongoing resuscitation. Platelet transfusion for thrombocytopenia in the isolated, non-anticoagulated TBI patient population produces unequivocal benefit while platelet transfusion for platelet dysfunction in this population remains understudied with inconsistent clinical results.²⁷ Currently, platelet transfusion is not recommended for isolated TBI patients without platelet dysfunction as no clear benefit has been elucidated.²⁸ Our results suggest that in isolated TBI, the aggregation effect of platelet transfusion may be minimal as posttraumatic MVs may interfere with the ability to reliably establish ADP-induced platelet hypoaggregability and its correction after transfusion. We recognize that other platelet agonists, such as AA and collagen, also contribute to platelet aggregation and caution should be taken in extrapolating individual platelet agonist studies *ex vivo* to overall *in vivo* platelet aggregability and function. Future studies are necessary to evaluate whether the inhibitory effect demonstrated by post-TBI MVs in this study are replicated when several agonists are utilized concurrently.

Several new modalities have been introduced to clinically test coagulation and platelet function. Viscoelastic tests of coagulation including thrombelastography (TEG) and ROTEM allow for evaluation of coagulation status based on the availability of clotting factors, clot strength, and lysis and therefore allow physicians to provide a more restrictive targeted resuscitation. Studies have shown that TEG platelet mapping predict platelet dysfunction; however, this dysfunction may not be correctable with platelet transfusion.²⁹ In addition, Stettler et al have shown that TEG platelet mapping results do not correlate with the need for massive transfusion.³⁰ This may be explained by our findings in that post-traumatic MVs produce an apparent *ex vivo* reduction in platelet aggregability. So, the question then remains: are the MVs inducing true platelet dysfunction or are they merely interfering in current *ex vivo* platelet assays due to their presence in plasma. Platelet function tests such as VerifyNow® use the agonists ADP and AA to detect platelet dysfunction but are designed to detect the presence of anti-platelet agents rather than intrinsic platelet dysfunction. Previous studies have demonstrated a lack of correlation between the TEG parameter for platelet contribution to clot (MA or MCF) and VerifyNow testing.³¹ Viscoelastic tests tend to utilize agonists other than ADP and AA, such as tissue factor and thromboplastin, and therefore compensate for isolated platelet dysfunction via other pathways. In this study, though a difference was observed in the ability of ADP to induce platelet aggregability when MVs

from TBI blood were added to normal blood, the percent of platelet contribution to maximum clot firmness was not impacted. This demonstrates that following TBI, platelets may not experience dysfunction, rather they may not have an adequate *ex vivo* response to the individual agonists utilized to induce activation and aggregation. These data may lend additional support in the TBI population to the idea that laboratory value based coagulopathy may be present more frequently but with lesser outcomes impact than clinical coagulopathy, as has been demonstrated recently in data from the Prehospital Resuscitation on Helicopter Study.³²

There are several limitations of our study. First, our TBI model does not perfectly reproduce human TBI in that it lacks rotational and angular elements of injury that can lead to axonal stretch injuries and are often the result of blunt TBIs occurring at high speeds. Second, no change in microvesicle P2Y₁₂ levels was observed between TBI and sham samples. Given our findings of microvesicle-induced platelet inhibition we expected to see a higher level of the P2Y₁₂ receptor in microvesicles isolated from TBI mice. Potential explanations may be that TBI mice have a larger proportion of the P2Y₁₂ receptor expressed on the membranes of MVs compared to sham mice or that these receptors are more active after TBI than without injury. The mechanism and expression of the P2Y₁₂ receptor on both platelets and MVs after injury will require further investigation. Additionally, although some human studies and rat TBI models have demonstrated platelet dysfunction via the AA and collagen pathways, porcine models of TBI with concomitant hemorrhage have identified similar posttraumatic effects on ADP-induced aggregation without affecting other markers of platelet performance including collagen- and AA-induced platelet aggregation as well as soluble P-selectin concentrations.³³ Finally, this model of isolated TBI employed young mice without concomitant injuries. Typically, the population most affected by isolated TBI are older patients, as younger patients will often have additional injuries that may complicate the mechanisms behind their coagulopathy. Future studies will be directed towards whether similar findings of platelet dysfunction related to the presence of the P2Y₁₂ receptor within MVs is present in older rodents and in polytrauma models.

In conclusion, coagulopathy and platelet dysfunction as a result of TBI is a poorly understood phenomenon. Here, we have demonstrated that MVs released following TBI contribute to ADP-related platelet dysfunction in a murine model. Selective inhibition of the P2Y₁₂ ADP receptor located on MVs resulted in restoration of ADP-induced platelet aggregation. This mechanism may help explain why platelet therapy remains ineffective for patients with severe isolated TBI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Funding: R01 GM124156-01A1 (MDG)

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Figure 1A:

Following TBI or sham treatment, whole blood samples were collected from 5 mice per treatment arm and were pooled to obtain adequate PRP, PPP, MV-poor, and MV samples for protein and receptor analysis via differential centrifugation. Whole blood samples were collected from five separate mice; these were not pooled. Samples of PRP, PPP, MV-poor plasma, and MVs were added to anticoagulated whole blood at a ratio of 1:5. Samples were then analyzed by either the Multiplate Impedance Aggregometer or the ROTEM.

1B

Figure 1B:

Following TBI or sham treatment, whole blood samples were collected from 8 mice per experimental arm and were pooled to obtain adequate platelet and MV samples for Western blot analysis. Half of the pooled samples of blood was used for platelet isolation and the other half for MV isolation. Protein was extracted from the platelet and MV samples and analyzed for P2Y₁₂ receptor presence using Western Blots.

Figure 2: Microvesicles from 10 minute post-TBI mice cause platelet hypoaggregation. Mice were subjected to TBI or sham treatment. Blood was collected after 10 minutes. Multiplate platelet aggregation analysis was performed with either ADP or AA as agonists. Sample sizes are n=5 per group. (* p=0.03 compared to Sham/ADP)

Figure 3: The presence of MVs in 10 minute post-TBI blood cause platelet hypoaggregation when ADP is used as an agonist.

Mice were subjected to sham or TBI. Blood from 5 animals was collected and pooled after 10 minutes. PRP, PPP, MV-poor plasma and MVs were isolated from each experimental group. PPP (Figure 2A), PRP (Figure 2B), MV-poor plasma (Figure 2C), and MVs (Figure 2D) were added to normal murine donor blood in a 1:5 ratio and Multiplate platelet aggregation analysis was performed with ADP as an agonist. Sample sizes are n=5–10 per group. (*p<0.05 compared to Sham)

Figure 4: Western blots demonstrate presence of ADP P2Y₁₂ receptor isolated from microvesicles.

Mice were subjected to TBI or sham treatment. Blood from 8 animals was collected and pooled after 10 minutes. Standardized amounts of protein from MVs and platelets (Figure 4A) or MVs underwent Western blotting and images of the resultant membranes were analyzed. The average intensity of P2Y₁₂ receptor staining from MVs (Figure 4B) and platelets (Figure 4C) is displayed. Sample sizes are n=4 per group.

Figure 5: Inhibition of P2Y₁₂ ADP receptor ameliorates post-TBI platelet hypoaggregation. Mice were subjected to sham or TBI. Blood from 5 animals was collected and pooled after 10 minutes. Platelet-poor plasma and MVs were isolated from each experimental group. The prasugrel metabolite R-138727 was added to PPP (Figure 3A) and MVs (Figure 3B) to induce P2Y₁₂ receptor inhibition. Inhibited PPP or MVs were then added to normal murine donor blood in a 1:5 ratio and Multiplate platelet aggregation analysis was performed with ADP as an agonist. Sample sizes are n=5–8 per group. (* p<0.05 compared to Sham) (^ p < 0.05 compared to TBI)

Figure 6: Microvesicles from TBI mice do not change platelet contribution to maximum clot firmness as measured by ROTEM.

Mice were subjected to TBI or sham treatment. Blood from 5 animals was collected and pooled after 10 minutes and MVs were isolated. Microvesicles were added to normal murine donor blood in a 1:5 ratio and thromboelastometry analysis was performed. The percent of platelet contribution to maximum clot firmness was calculated using results from EXTEM and FIBTEM analysis, neither of which utilize ADP as an agonist. Sample sizes are n=5 per group.