Chapter

Interplay between Platelet Dysfunction and Vascular Thrombosis in Traumatic Injury

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Abstract

Platelets halt bleeding accompanying traumatic injury by performing primary hemostasis to repair vascular leakage at injury sites. In trauma individuals, *ex vivo* platelet function tests often indicate impairment despite normal count. Moreover, incubation of platelets from normal non-traumatized individuals with plasma from trauma victims demonstrates impairment suggesting association with factors in circulation. Notably, not all trauma victims die from hemorrhage. Despite laboratory evidence of dysfunction, thrombotic vascular occlusions are persistent in trauma survivors as corroborated by postmortem findings from victims who die. The time course of platelet reactions post-traumatic injury, that is, the transition from states favoring bleeding to those that facilitate thrombosis is still unclear. Of the several terminologies describing platelet behavior with regards to injury, including hyporeactivity, anergy, exhaustion, and maladaptive states, few have focused on platelet-platelet interactions. It is increasingly becoming clear that platelet interaction with injured endothelium is a probable missing link in the mechanistic explanation of vascular thrombosis post-traumatic injury. This postulate is supported by evidence of increased adhesive protein, von Willebrand factor, and released from injured endothelium. In all, this potentially explains the suboptimal response to anticoagulants or antiplatelets post-trauma. This chapter will review current knowledge on platelet functions in relation to vascular thrombosis post-trauma, the time course, mechanistic hypothesis, and response to therapeutic interventions and clinical outcomes.

Keywords: traumatic injury, platelet dysfunction, vascular thrombosis, therapeutic interventions, interplay

1. Introduction

1.1 Clinical presentation of platelet dysfunction

Platelet dysfunction post-trauma presents as excessive and immediate bleeding in distinct patterns including scattered small ecchymoses at sites of minor trauma or venipuncture, spontaneous bleeding at various body surfaces such as mucosal (oropharyngeal, genitourinary, gastrointestinal, nasal), ecchymosis, petechiae

(mostly evident on lower limbs), purpura, epistaxis, and gingival bleeding. Bleeding into deep tissues, joints, or hematomas formation is rare [1].

Alternatively, hyperactive platelets contribute to occlusive vascular thrombus formation. Although thrombus formation on the venous system is generally rare, arterial thrombus on vascular territories associated with myocardial infarction and ischaemic stroke are common.

1.2 Platelet morphology and structure

Platelets are anucleate smallest blood cells with an average lifespan of 10 days. In circulation at rest they are biconvex, but *ex vivo* in EDTA they appear round on average 2.5 μm diameter and mean platelet volume (MPV) of 8–10 fL but could be as large as 4–5 μm. Their plasma membrane is made up of proteins and lipids (mainly phospholipids and cholesterol). Interspersed within the lipid bilayer are glycoproteins that serve as structural support, receptors for ligands, and components of platelet reactions. Additionally, the plasma membrane extends internally forming membrane-bound open canalicular structures (OCS) that are distinct from dense tubular system (DTS), which are remnants of rough endoplasmic reticulum parallel to the surface with blind endings [2].

Inside platelets are cytoskeletal structures and granular elements. The platelet cytoskeletons are made up of microtubules and microfilaments that are responsible for maintaining discoid shape and shape change, extension of pseudopods, and secretion of granule contents. The cytoplasm contains several granules named according to appearance on electron microscopy. Dense granules are electron-dense and contain simple molecules such as Ca++, Mg++, ATP, ADP, and serotonin. The less electron opaque alpha granules mostly contain proteins including vWF, fibrinogen, thrombospondin, coagulation factor V (FV), β-thromboglobulin (β-TG), P-selectin, glycoproteins (GP), HMWK, plasminogen, plasmin and inhibitors, and fibronectin [2]. Upon platelet activation, the granular contents are released into the canalicular system to participate in platelet reactions.

1.3 Platelet reactions at injury site

Under normal physiological conditions, circulating platelets are non-thrombogenic, neither adhering to endothelium nor aggregating to each other. This is because normal endothelial lining constantly releases suppressing factors such as nitric oxide (NO), prostacyclin (PI), and an ADPase (CD39) [3, 4]. Injury activates the endothelium to release ADP, thromboxane, and prostaglandins and promotes thrombin generation. Moreover, injury exposes subendothelial matrix composed of adhesive proteins collagen and vWF to circulating blood. Collectively, these factors lead to cascade of reactions on platelets including adhesion, shape change and spreading, granule secretion, thromboxane synthesis, and aggregation [4] (**Table 1**).

Following receptor binding, a series of intracellular events ensue that lead to activation of phospholipases and increase in second messengers, synthesis of eicosanoids that initiate further amplification of cellular activities such as shape change, granule secretion, GPIIbIIIa conformational change, membrane reorganization to exteriorize phosphatidylserine (PS), membrane blebbing, and microvesicle/microparticle formation [5, 6]. All these processes of platelet reactions are accompanied by increase in intracellular calcium [4].

Platelet reactions eventually end up in platelet plugs or pathological thrombus formation. Emerging evidence indicates that thrombus components and the signaling

Group	Ligand	Physiological Receptor	Family of receptor	Outcome of ligand-receptor interaction
Adhesive proteins	collagen	GPVI	Glycoproteins (GP) Integrins	Adhesion
	vWF	GPIb-v-IX (GPIbα)		Tethering
	Fibrinogen/fibrin	GPIIbIIIa (PAC-1)		Aggregation
	Immunoglobulins	FcRy& FcyRIIa	ITAM	Activation, aggregation
	Thrombospondin	GPIa-IIa	Glycoprotein	Spreading
Soluble agonists	Adenosine diphosphate(ADP)	P2Y12	G-protein coupled receptor (GPCR)	Activation
	Adrenaline	α2A		Activation
	Thromboxane	TP		Activation
	Arachidonic acid (AA)	PGE/PGI		Activation
	Thrombin	Protease-activated Receptors 1–4 (PAR 1–4)		Activation
Coagulation factors	Thrombinase	PS	Membrane lipid	Assembly of coagulation factors and amplification of coagulation reactions
Alarmins	DAMPS including NETs	Toll-like receptors (TLR)	Lipids-Pattern Recognition Receptors of Pathogen Associated Molecular Patterns(PAMP)	Activation

Table 1.
Platelet ligands in trauma, receptors, and outcome of their interaction.

pathways in platelet plugs are hierarchical. The outer layer of platelet aggregates is composed of loosely packed platelets poor in p-selectin and little and no fibrin. Thus, the decreasing density of fibrin from the core outward parallels thrombin levels, and reflects a hierarchy in the impact of agonists based on composition [7].

To limit thrombus growth and maintain vascular luminal patency, various intrinsic mechanisms that negatively regulate platelet activation come into play including immunoreceptor tyrosine-based inhibition motif (ITIM), endothelial cell-selective adhesion Molecule (ESAM), Wnt-β-catenin, and semaphoring 3A (Sema3A). Furthermore, integrin ectodomain receptors are shed by proteases such as thrombin and ADAM, microvesiculation, and internalization, resulting in loss of adhesive and aggregation features in thrombus formation [8]. These negative regulators limit the intracellular signaling, integrin activation, receptor desensitization, and response to secondary mediators [4]. Therefore, following trauma, platelets undergo changes from quiescence, activation, thrombus growth, and finally to self-regulation [4].

2. Assessment of platelet functions in trauma

Although multiple events occur concurrently in platelets during trauma, laboratory investigations only focus on one or two. Tests performed include (i) routine platelet count and bleeding time, (ii) flow cytometry assays of surface membrane receptor expression, (iii) perfusion analysis of adhesion to collagen or fibrinogen coated surfaces, (iv) analysis of agonist-induced platelet-platelet aggregation by light transmission aggregometry (LTA), impedance aggregometry or platelet function analyzer-100 (PFA-100), or (V) TEG-platelet mapping/ROTEM [9].

2.1 Bleeding time

This was the initial test for primary hemostasis for decoding platelet functions *in vivo*. This test reveals persistent longer bleeding time in traumatic brain injury patients compared to healthy controls patients [10]. However, this test has largely been abandoned due to the lack of specificity and sensitivity and not routinely performed.

2.2 Platelet count

Majority of patients arrive in emergency departments with normal or near-normal platelet counts [11]. In a cohort of trauma patients with mean injury severity score of 22, platelet count progressively dropped over a 72-hour observation period even though it did not reach the critical level associated with spontaneous bleeding [12, 13]. At admission, bleeding and requirements for transfusion occur at much higher platelet counts compared to other conditions [11] suggestive of dysfunction.

2.3 Adhesion

In trauma patients, platelet adhesion, as measured on collagen and fibrinogen by flow chambers, was decreased compared to normal healthy individuals [14].

2.4 Aggregometry

Upon activation, platelets undergo shape change and stick to each other through fibrin bridges. These biophysical changes can be evaluated to ascertain platelet function.

2.4.1 Light Transmission Aggregometry (LTA)

Considered the gold standard, LTA is a widely used *ex vivo* assay for platelet function [9, 15]. The method has been used to demonstrate a decrease in platelet function in response to ADP and TRAP agonists in trauma patients, findings that correlated with injury severity and level of consciousness but not measures of shock [16]. These results were consistent with those obtained using a modified LTA (optimal) [14]. Despite its centrality, no large-scale randomized studies of LTA in trauma have been conducted.

2.4.2 PFA-100/200™

This test estimates both platelet aggregation and adhesion and has replaced the bleeding time [9]. It has been used to demonstrate platelets dysfunction in a cohort

of trauma patients monitored over time. A nested control analysis revealed injured patients with trauma-induced coagulopathy (TIC) patients have longer PFA-100 Coll/Epi and coll/ADP closure time compared (CT) to their non-TIC injured counterparts [17].

In contrast, another study of a cohort of trauma patients showed a shorter PFA-100 closure time at admission compared to controls even though the CT progressively increased returning to normal baseline at 72 hours. Furthermore, closure times were longer in non-survivors compared to survivors [13]. PFA-100 closure time is influenced by a number of factors not specific to platelet functions that include platelet count, RBC, and vWF [18].

2.4.3 Multiple electrode aggregometry (MEA)/impedance aggregometry

This method uses whole blood instead and follows change in electrical resistance between two electrodes as platelets aggregate [19]. It has the advantage of not requiring centrifugation, uses small sample volumes, and is near physiological conditions since platelets are evaluated in the presence of red blood cells and leukocytes similar to *in vivo*.

Most trauma patients arrive at emergency departments with MEA below normal response to ADP, AA, thrombin, and TRAP agonists [20–22], with survivors having less impairment compared to non-survivors [23] and hyporeactivity persisting for 96 hours [24, 25]. Since MEA is sensitive to GP1b deficiency, reduction in total vWF, as well as activity that includes FVIII carrying capacity, the finding of decreased platelet ristocetin response in trauma patients [21, 26] strongly suggests impaired adhesive interaction with endothelium.

2.4.4 VerifyNow™

This is a turbidometric-based optical method detection system, specifically developed to detect sensitivity to antiplatelet therapy. It measures platelet binding to fibrinogen-coated polystyrene beads in whole blood following activation by a number of agonists acting on platelet GPIIbIIIa. Trauma patients not on aspirin have been shown to have greater VerifyNow aspirin reactivity units (ARU) and platelet reactivity units (PRU) compared to those on aspirin [27]. Platelet dysfunction results obtained using this system concurred with those obtained previously in trauma [28] and with those with intracranial hemorrhage [29]. However, a study of platelet function in traumatic injury found a high prevalence of poor platelet response that neither correlated with hemorrhagic outcome nor whole blood aggregometry [30].

3. Thromboelastography (TEG)/thromboelastometry (ROTEM)

A study that evaluated platelet functions using TEG platelet mapping in traumatic brain injury patients revealed decreased response of platelets to AA agonist, more pronounced in bleeders compared to non-bleeders, but no significant differences in ADP stimulation [10]. In another study undertaken in patients with blunt trauma, TEG MA remained unaltered, though MA-Platelet mapping (TEG-PM) AA and ADP were reduced [31]. The platelet inhibition evaluated with TEG occurs early (before 6 hours) post-trauma, worsened with severity of injury [31], hemorrhagic shock, and acidosis [32]. Similar to findings reported using ROTEM-fibtem [14].

A modification of TEG functional fibrinogen level (FLEV-TEG) uses GPIIbIIIa blockers to disentangle fibrin and platelet contributions to clot strength demonstrated that platelet contribution to clot strength at admission accounts for 80% but progressively decreases to 50% over 72 hours then stabilizes for the next 48 hours indicating platelet dysfunction [33], though there are differences in GPIIbIIIa blockers [34, 35].

3.1 Flow cytometry of platelet activation biomarkers

3.1.1 PAC-1 (GPIIbIIIa)

In severely injured patients with injury severity score of 22, admission PAC-1 was ten times higher than controls, progressively decreasing over 72 hours but remaining higher than controls at all the time points [13]. This is in contrast to another study where PAC-1 levels were on an upward trend for both TIC and non-TIC patients [17]. This was in contrast to Verni and coworkers [36] who reported decreased levels in response to ADP and CVX agonists. In this case, the response levels were dependent on calcium concentration.

3.1.2 P-selectin (CD62P)

P-selectins are stored in platelet alpha granules but are translocated to the membrane surface upon activation. There is a direct and linear relationship between increase in P-selectin and clot forming potential as represented by aggregation [37]. On admission, trauma patients have higher platelet P-selectin that reduces over 72 hours but remains above that of controls throughout [13, 17]. This finding contradicts that of Mathay and co-workers who reported low levels at admission [38]. Differences in response to various agonists have been noted: response to CRP-XL, and ADP is greater in healthy individuals than in trauma patients [14, 36]. There are also differences between survivors and non-survivors [17] indicating differences in signaling mechanisms.

Determination of platelet surface expression of P-selectin may not be an accurate measure of platelet prior exposure/activation *in vivo* as they get detached and released into plasma, while the degranulated platelet continues to circulate [39].

3.1.3 Phosphatidylserine (PS)

Trauma platelets showed decreased PS expression in response to ADP and convulxin [36].

3.1.4 CD 40

Traumatic injury is associated with increased expression of CD 40 receptors on platelets, and these interact with ligands on endothelium and leukocytes [40].

3.2 ELISA evaluation of activation dependent soluble plasma biomarkers

Platelet expressions of surface biomarkers are dynamic transient, and over time are shed off into circulating plasma [41]. These include soluble P-selectin

(sP-selectin), glycocalcin (soluble form of GPIbα), soluble GP VI (sGPVi), soluble CD40L (sCD40L), metabolic products such as thromboxane (TXA2), thromboglobulin (TBG), and platelet factor 4 (PF4) [42].

Trauma platelets GP VI and GP1b surface expression were less than healthy controls, but paradoxically the soluble plasma concentrations of sGPVI and sGP1b were higher than in controls [14] suggesting increased protease cleavage.

There is some confusion about the physiological dynamics response to most agonists such as thrombin, convulxin, and TRAP/CRP. Unlike GPIIbIIIa, *ex vivo* agonist stimulation leads to decreased surface expression and triggers internalization and vulnerability to proteolytic cleavage into the surrounding medium [43]. In trauma, the soluble form (sGPVI) is increased and correlates with soluble fibrin formation, D-dimers, and development of thrombosis [44].

Soluble CD40 was found elevated in trauma and correlated with endothelial and tissue damage, DAMPs, fibrinolysis, thrombin generation, acidosis, and sympathoadrenal hyperactivation [45]. Due to the relation with fibrinolysis (D-dimers) and thrombin generation (PF 1.2 and TAT), this could be a reflection of cleavage after surface expression.

4. Secretions

P-selectin is stored in platelet alpha granules and is translocated to the surface upon stimulation and its expression in response to agonist has been used as a marker of secretion. Although trauma patients platelet's p-selectin expression is higher compared to that of healthy controls, it is further elevated in response to agonists [14].

5. Microparticles

On admission, platelet microparticles in trauma patients are usually twice those of controls and remain unchanged for over 72 hours. Interestingly, non-survivors and head-injured patients have high initial microparticle counts but levelsdecrease in 24 hours to approximate that of survivors [13]. The high levels of circulating microparticles in trauma contribute to increased platelet activation [46]. Also, increased platelet microparticles in trauma patients that persist for over 72 hours are implicated in hypercoagulability [47]. On the other hand, low levels of platelet microparticles are associated with bleeding and mortality [48].

In laboratory animals, traumatic injury with shock is accompanied by increased elaboration of platelet microparticles, and these are associated with increased thrombin generation and DVT in mice [49].

6. Imaging/microscopy

A unique phenotype of platelets in trauma has been visualized characterized by transformation into balloon-like structures has been visualized [50]. Ballooning increases membrane surface area for PS exposure and procoagulant thrombus reactions, as well as microvesiculation [51, 52].

7. Spectrum of platelet dysfunction in trauma

7.1 Hyperreactivity

Few studies have reported findings of platelet hyperreactivity or increased response to stimulating agonists in trauma [13]. Platelets are more hyperreactive as demonstrated by amplified binding to fibrinogen in the presence of increased doses of ADP [53]. This phase is immediate within minutes to hours [54] and is often missed by most studies due to timing of blood sampling.

7.2 Hyporeactivity

Despite normal platelet count, below normal or decreased response to stimulating agonists *ex vivo* has been reported in 45% of patients for atleast for one agonist, 91% during intensive care stay [22, 25]. The independent predictors are injury severity, Glasgow Coma Scale and acidosis [24]. However, minor injury has been associated with platelet hypofunction [31]. Moreover, diminished platelet response has been linked to lowered calcium levels [36].

7.3 Platelet granule exhaustion

Despite normal platelet count at admission [11], there is a discordant reduction in aggregation response to stimulating agonists even with increased receptor expression [55]. Many studies of platelet function in trauma have referred to this phenomenon as –'platelet granule exhaustion' [32]' [31] in line with previous findings in other conditions [56–58] that share similarities with storage pool disorders [59]. However, this position lacks consistent support and has been refuted [60] since trauma platelets still retain response to P-selectin expression though with differences in agonists [14] indicating differences in signaling mechanisms rather than exhaustion. Moreover, there is overreliance of platelet aggregation studies which have been shown to be unreliable in storage pool disorders [61, 62].

Due to the inconsistency in terminology such as 'platelet exhaustion' or 'desensitized, stunned, inactive, post activated, dysfunctional, or degranulated' used to describe the platelet dysfunction in trauma. Perhaps better terminologies such as 'functional anergy' [63] and 'agonist refractoriness' [54] are more apt. It has been opined that what is called maladaptive or dysfunctional platelet in the acute phase of trauma is likely a misnomer, and perhaps it could be an adaptive natural selection mechanism for ensuring survival through possible microvascular thrombosis during the low flow states that kick in following hemorrhage in order to maintain organ perfusion [64].

7.4 Mechanisms for hypofunction

Empirical data have shown that the loss of platelet aggregation functions is plausibly due to: (i) loss of adhesive receptors through microvesiculation, downregulation/internalization [65], and ectodomain shedding of adhesive receptors GP1bα and GPVI [64], (ii) endothelial dysfunction (endotheliopathy) [66] in which glycocalyx release of mediators such as versacan been demonstrated to have impact on platelet dysfunction [67], (iii) reduction in calcium availability [38] and intracellular mobilization [36], (iv) shock acidosis [68], (v) reduced adhesive form of vWF due to hyperactive ADAMTs 13 proteolysis [69, 70], and (vi) low fibrinogen from consumption or defective activity [71].

8. Modifiers of platelet functions in trauma

8.1 Type and severity of injury

The extent of platelet dysfunction worsens with injury severity, acidosis [32], and brain injury [13]. However, platelets functions may be impaired even with minor injuries without acidosis or shock [31], or only correlated with extent of cerebral fatality but independent of injury severity [20].

8.2 Extent of endothelial injury and vWF-ADAMTS-13 axis

An imbalance of vWF: ADAMTS-13 ratio has been found in trauma patients soon after injury and was associated with increased thrombin generation [72]. Low plasma levels of ADAMTS-13 and high vWF are associated with mortality [73, 74], and persistently elevated levels in trauma patients were associated with development of ARDS predictive of survivors from non-survivors [75] indicating link with microvascular thrombosis. Although the increased vWF multimers would be expected to compensate for adhesion of low platelet count in trauma, however, the transient and decreased platelet adhesion and aggregation early in trauma could be multifactorial that include: abnormalities in vWF conformation [26], downregulation of platelet GP 1b receptors [76] from increased thrombin generated [43], and receptor loss through sheddases [8, 14].

8.3 Thrombin generation

There is increased thrombin generation in trauma [77, 78] linked to NETosis and glycocalyx syndecan-1 release [79]. The consequences of increased thrombin generation are platelet receptor activation and fibrin formation that promote aggregation.

8.4 Plasma calcium levels

A study that factored in calcium levels, it was found that platelet activation, aggregation, and membrane surface receptor expression were increased with increasing upward calcium titration [38] indicating importance of calcium-mediated processes.

8.5 Fibrinogen-fibrinolysis axis and role of plasmin

Despite increased expression of platelet GPIIbIIIa aggregation receptors post-trauma [13], most studies report paradoxical reduction in aggregation to most agonists [80]. The time course of reduced platelet aggregation and functional recovery parallels periods of fibrinolysis [81]. The role of plasmin on platelet function is controversial [82], depended on the methodology and testing conditions [83]. Although it has been reported that fibrin proteolytic products mediate platelet dysfunctions [84], it is plasmin that reduces platelet aggregation [85] without affecting GP receptor expression [86]. While the FDPs compete for fibrinogen binding sites on GPIIbIIIa [87] and association with PS- expressed on activated platelets [88], plasmin degrades fibrin/fibrinogen reducing its bridging function between adjacent platelet GPIIbIIIa [89]. Additionally, by cleaving vWF, plasmin reduces platelet adhesion to endothelium [90]. This explains the reduction in ristocetin agglutination in trauma platelets. However, this effect plays a minor role since plasmin also cleaves the regulatory enzyme ADAMTS 13 [91].

On the other hand, plasmin acts as a platelet activator of surface receptor expression and granule secretion under conditions likely found in trauma [92]. The platelet-activating effects of plasmin become evident during fibrinolysis shutdown since only free circulating plasmin are inhibited by plasma antiplasmin and α2-macrogolbulin, as well as PAI-1 on t-PA without affecting platelet bound plasminogen-plasmin. Perhaps the restoration of platelet aggregation by 72 hours post-trauma [93] may be explained by the fibrinolytic shutdown that also occurs at the same time period [94]. The transition from decreased aggregation to restoration and enhanced aggregation could be accounted for by the slow platelet release of PAI-1 [81, 95] that lags behind the plasmin activation but eventually shuts it down [94].

8.6 Damage associated molecular patterns (DAMPs)

Tissue injury, ischemia, and cell death trigger release into plasma damage-associated molecular patterns (DAMPs) including nucleic acids, csDNA, histones, high-mobility group box-1(HMGB-1), heat shock proteins (HSP), and S100 proteins among others [96]. DAMPS also termed alarmins [97] are elevated after trauma [96, 98] and are recognized by toll-like receptors (TLR) on platelets to trigger activation. The time course for plasma DAMPS parallels the duration of platelet hypofunction and recovery [99] strongly suggesting that they are potential drivers of platelet functional fluctuations in trauma in concert with cytokines and fibrinolytic system.

8.7 Neutrophil extracellular traps (NETosis)

Trauma increases platelet P-selectin expression and elevates the levels of neutrophils that release Neutrophil Extracellular Traps (NETs) [100]. NETs, composed of DNA, histones, and neutrophil elastase (NE) in turn promote platelet activation, aggregation, thrombin generation, and thrombosis [101]. In addition, histones promote platelet ballooning and microparticle formation [50] further escalating the risk of vascular thrombosis risks. Thus, the high NETs produced in association with trauma [79] could be considered sentinel markers of platelet activation and thrombosis.

8.8 Shock and acidosis

A study conducted with MEA revealed that injured patients with shock had decreased AUC irrespective of injury severity [68, 102]. During shock states, metabolites together with attendant acids are involved in fibrinolysis that decreases platelet aggregation [103].

Ex vivo, addition of lactic acid to canine platelets to create academia revealed reduced platelet aggregation on MEA [104], consistent with previous studies on LTA [105, 106]. The lowered platelet aggregation due to induced acidosis is linked to modification of intracellular store calcium traffic [107] and inhibition of GPIIbIIIa conformation (PAC-1) [108].

8.9 Inflammation

The intense inflammatory response immediately post-trauma [109, 110] has implications on platelet functions [111]. Interleukins, act directly as potent platelet activators through IL-6 [112, 113], and indirectly through thrombopoietin bone marrow megakaryopoiesis, vWF endothelial release, and sensitization to thrombin [114].

The effects of complements on platelet aggregation in normal and trauma patients are complex and controversial, perhaps reflecting differences in calcium fluxes [115].

8.10 Neurohumoral hormonal axis-sympathoadrenal activation

Trauma is associated with activation of sympathoadrenal system releasing adrenaline and noradrenaline into the circulation [116]. These catecholamines modulate platelet functions indirectly through endothelial damage termed 'endotheliopathy' [66] *via* vWF and exposure of subendothelial collagen, and directly on platelet receptors to cause activation, secretion, and aggregation [117]. Although *in vitro* catecholamines cause platelet hyper aggregation, at the circulating levels in trauma, platelets *ex vivo* response to stimulating agonists is paradoxical decreased aggregation despite increased expression of surface receptors such as GPIIbIIIa, PS, and P-selectin. This phenomenon has variably been referred to as 'granule exhaustion' or 'anergy'. But these references have not taken into account the various substances that are also released with tissue trauma [118].

8.11 Alcohol and toxins

Alcohol is known to have *in vivo* and *ex vivo* platelet aggregation inhibitory effects [119–121], related to alteration of blood osmolality, lactate, and acidosis [122]. Conversely, bidirectional effects termed rebound effects [121] composed of initial decreased aggregation followed by hyperaggregation have been shown [123]. The mechanism for increased aggregation appears to be erythrocyte release of ADP [124] and fibrinolytic shutdown [125, 126]. The bidirectional effects of alcohol may be to the early rise of acidic metabolites such as acetate in the initial phases promoting fibrinolysis, with the late inhibitory effects due to the slow release of PAI-1 that causes *in vivo* shutdown [127].

9. Time course of platelet function in trauma

Early in trauma, there follows a period of acute reduction in platelet aggregation that reaches a nadir after 4–12 hours [25, 36, 128] and gradually returns to normal by 48–96 hours [128]. While the changes in platelet functions are evident as early as one and a half minutes after injury [129], hypofunction may persists for 96 hours [24]. Platelet count also follows similar pattern albeit slowly [80] though onset of recovery occurs earlier than platelet functions [128] that correlates with changes in ADAMTS-13 levels [130]. The trend of initial hypofunction followed by restoration and rebound hyperfunction has been observed in diverse conditions such as head injury [93, 121, 131], critical care units [24], and spinal cord injury [132]. In an animal model of TBI, the changes paralleled increase in pro inflammatory cytokines such as IL-6, KC (keratinocyte chemoattractant), and soluble p-selectin. [133]. The observed restoration of platelet functions is consistent with the development of thrombosis [134].

10. Platelet functions and clinical outcomes in trauma

10.1 Bleeding

In the early period following trauma, bleeding is experienced despite normal platelet count [135], a concept referred to as trauma-induced coagulopathy (TIC) [67]. Also,

decline in post-traumatic platelet count is associated with intracranial hemorrhage [136]. It is still not clear whether the bleeding is a result of isolated platelet dysfunction, fibrinolysis, or combined effects.

10.2 Vascular thrombosis

Following traumatic injury has been associated with the development of venous thromboembolism in up to 58% of victims [137], although the incidence varies with time [138]. Despite platelet hyporeactivity in the initial phase, restoration of platelet function may trigger rebound hyperaggregation and hypercoagulation [133] potentially leading to development of DVT [22, 25, 139]. Although many trauma patients receive prophylactic anticoagulants, vascular thrombosis still occurs [35].

11. Therapeutic interventions

11.1 Platelet transfusion

Whilst circulating platelets are dysfunctional after trauma [140], platelet transfusion is not associated with restoration of function [54, 128, 141, 142]. The improvement in hemostasis reported in some studies [143] may be attributed to inhibition of fibrinolysis in bleeding trauma patients, but platelets functions remain unaltered compared to those who do not receive transfusion [144]. Notably, platelet transfusion reverses aspirin-induced hypofunction but not trauma-induced dysfunction assessed by MEA [145]. The critical time period when platelet transfusion may be useful has been identified as late in the phase (after 24 h) rather than early (prior to 12 hours) [80]. This time period coincides with decline in aggravating factors such as fibrinolysis, DAMPS, and acidosis allowing restoration of functions to normal levels.

11.2 Antiplatelets

Although some studies have indicated no difference in VTE incidence in surgical patients given antiplatelets [146], data on trauma patients are variable. The incidence of VTE in trauma patients on aspirin prior to injury is half that of matched controls with VTE but not on aspirin [147] suggestive of a protective role. The protective effect of aspirin is enhanced when combined with clopidogrel and systemic anticoagulants such as LMWH or heparin.

11.3 Novel therapeutic targets

In general, therapeutic targets are aimed at preventing bleeding in the initial phases by improving haemostatic functions and preventing thrombosis in the later resuscitation phases. Classic traditional interventions proven to target bleeding include DDVP/vasopressin, tranexamic acid, crystalloid minimization using plasma-based infusion, fibrinogen, and calcium. However, novel therapeutic targets are emerging that include nano-based semisynthetic platelets [148] but are still investigational.

12. Conclusion

Accidental or traumatic injuries are accompanied by changes in platelet function arising from endothelium and circulating factors alterations. Shortly after injury, platelets are dysfunctional characterized by increased expression of surface activation markers, decreased propensity to aggregate, and adherence to endothelial surfaces, which collectively increase the risk of bleeding. The hypofunction period that lasts for 72–96 hours is followed by restoration of function with attendant risk to thrombosis and vascular occlusion. The extent of platelet changes duration in each phase are dependent on modulating factors released during trauma and exogenously present either pre-trauma or added thereto. Unfortunately, *ex vivo* laboratory assays are neither predictive of functional performance nor designed to detect bleeding or thrombosis associated with trauma. A number of therapeutic interventions have been tried but lack clear equipoise.

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