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Review

Platelet Physiology and Mechanics of Aggregation: A Review

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ABSTRACT

Platelets are anucleate blood cells that play a crucial role in haemostasis and thrombosis. They also participate in various inflammatory and immune processes, as well as in the pathogenesis of cardiovascular diseases. Platelet aggregation is the result of a complex interaction between platelet surface receptors, soluble agonists, and subendothelial components. Platelet aggregation can be modulated by antiplatelet drugs, which target different pathways of platelet activation and inhibit the formation of thrombi. Antiplatelet therapy is widely used for the prevention and treatment of arterial thromboembolic events, such as myocardial infarction, stroke, and peripheral vascular disease. However, antiplatelet therapy is not without limitations, such as bleeding risk, interindividual variability, and drug resistance. This review summarizes the current knowledge on platelet aggregation and antiplatelet drugs, with a focus on their mechanisms of action, clinical indications, and challenges.

KEYWORDS: Platelets, Thrombocytes, Aggregation, Haemostasis, Thrombolytic

INTRODUCTION - The Anucleate Cell

Giulio Bizzozero made the discovery of platelets in 1882[1], but for many years afterward, only biologists were interested in the dynamic and multifunctional properties of platelets. The tiniest blood particles are anucleate, discoid platelets, whose shape reveals their dynamic nature. They are mostly linked to the process of haemostasis, which starts blood coagulation. Despite their high level of activity, they often prefer to be dormant and only become active when a blood artery is compromised. However, platelets have several multifunctional purposes that monitor the body's homeostasis in addition to their primary role in haemostasis, or blood coagulation. One of the most accessible markers was finally determined by its great sensitivity to various illness states. It returns to its previous state as a significant inflammatory marker while maintaining connections with leukocytes and endothelial cells[2]. Certain physiologically active indicators, such as CD36, CD41, CD42a, CD42b, and CD61, are highly dependent on platelet reactivity for various disease aetiology. Several platelet secretory products and active surface receptors are among them. A vast area for studying illness development is made possible by platelets, which have a tendency to change the production and signalling of these markers in various disease diagnostic and prognostic situations.

Platelet activity is mostly linked to the start of coagulation cascades. When a blood artery is damaged, platelets primarily target the subendothelial surface, which maintains haemostasis. The activity of platelet adhesion to subendothelial surfaces is promoted by a variety of proaggregatory stimuli, commonly referred to as platelet agonists. A platelet undergoes a morphological change during this process, releases its granule contents, and

progressively adheres to other platelets to create aggregates[3]. Thus, reducing blood loss continues to be its major objective. But as was previously said, platelets are essential to the pathophysiology of many diseases and are not just involved in controlling thrombosis and haemostasis. For many years, the relationship between platelet interaction and the development of cardiovascular disease remains unclear[4]. Another intriguing topic to investigate is platelet hyper aggregation in diabetic individuals with CVD. In certain circumstances, platelet hyperactivity in a variety of conditions can have negative consequences, particularly in coronary artery disease, where hyper aggregation obstructs blood flow.

The linked processes of thrombosis and haemostasis are mediated by interactions between blood components and vascular walls. The physiological halt of bleeding at the site of vascular damage is known as haemostasis. The coordinated action of platelets, the vessel wall, and coagulation factors orchestrates it. The abnormal production of blood clots brought on by improper activation of haemostatic systems is referred to as thrombosis. The essential blood components in these processes are platelets, which do not interact with blood arteries in a physiological state. Nevertheless, they quickly attach themselves to the broken artery wall at the site of injury in reaction to vascular damage, which sets off a series of processes that include the recruitment of more platelets (aggregation), leukocytes, and the activation of blood coagulation[5]. While these processes facilitate the halting of bleeding and the healing of wounds during "physiologic" haemostasis, they also play a role in "pathologic" thrombosis, which is a complication of several human diseases, including atherosclerosis. While the outcomes of haemostasis and thrombosis are different, there is a significant commonality in the molecular processes behind both reactions.

STRUCTURE AND PHYSIOLOGY

Mammalian platelets are anucleated cells arising from cytoplasmic fragmentation of megakaryocytes in the bone marrow, and have a typical diameter of ~2–3 μm. Platelets circulate in a discoid form (***Figure 1***) and their average lifespan in humans is ~10 days[6]. However, following activation, they undergo dramatic changes in shape and ultrastructure; the membranes become ruffled with cytoplasmic projections and the granules are centralized and discharged[7,8]. Normal human platelet count is ~150,000–400,000/μl, though spontaneous bleeding resulting from reduced (but functionally normal) platelets is unusual at levels >10,000/μl[9].

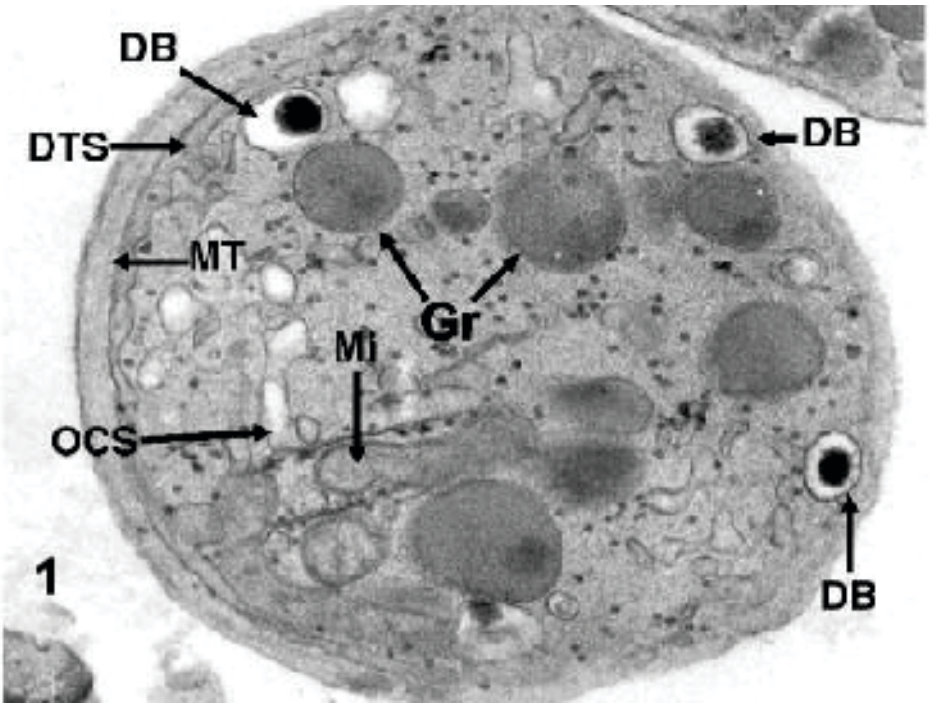

Figure 1: TEM image of Human Platelet, Granules (Gr), Dense Granules (DB), Open Canalicular System (OCS), and the Dense Tubular System (DTS), Glycogen (Gly), Mitochondria (Mi), Circumferential band of Microtubules (MT). (Re-distributed with permission from Reference 8)

Platelets participate actively in a wide range of physiological and pathologic processes despite not having a nucleus. Numerous mediators found in platelets control thrombosis and haemostasis in addition to performing a wide range of other tasks including chemotaxis, which is the attraction of other cells, vasomotor activity, cell proliferation, and inflammation. Both the cytoplasm and the cell membrane include components that are relevant to thrombosis, mostly in the form of platelet granules. The typical bilayer of phospholipids that makes up the platelet membrane also contains membrane glycoproteins that interact with different types of ligands, such as fixed ligands found on other cells or within the vessel wall that the platelets adhere to, or soluble ligands that activate the platelets. The network of many invaginations into the platelet interior, which is connected to the exterior via tiny holes known as the open canalicular system, is one of the platelet's distinctive characteristics (OCS)[10,11].

Platelet Markers

The phospholipid bilayer that makes up the platelet plasma membrane is where a variety of surface receptors and lipid rafts are expressed, aiding in intracellular trafficking and signalling. CD36, CD63, CD9, GPCR, IIbIIIa, and GLUT-3 are some of these markers. Additionally, these surface receptors cause the release of α granules, which are involved in coagulation, inflammation, atherosclerosis, host defence against microbes, angiogenesis, wound healing, and carcinogenesis[12]. GPCR is one of these surface receptors that has been shown to be essential for the secretion of ADP, the main secretory product, from dense granules. The inner layer of the plasma membrane contains asymmetrically ordered phospholipids (phosphatidylserine and phosphatidylinositol, for example) that stabilise the membrane's surface when it is not procoagulant[13].

Activation and Storage

In order to start coagulation cascades, the platelet surface progressively exposes amino phospholipids during activation (***Figure 2***) through ATP-dependent floppies and scramblases[14]. The "tunnel" system that runs the length of a platelet cell and is still attached to the plasma membrane is called the open canalicular system (OCS)[15]. The primary function of OCS is to allow external substances to enter platelets and to expel the granules inside. It not only serves as a significant location for the storage of glycoproteins in the plasma membrane but also aids in the development of filopodia during platelet activation[16]. The main function of the dense tubular system of platelets, which is a closed-channel network of remnant endoplasmic reticulum, is calcium sequestration through a series of events that activate the G protein-coupled receptor PAR-1[17,18]. Platelets' highly specialised cytoskeleton keeps the cell from shearing in the circulation and preserves its discoid features. The actin cytoskeleton, the marginal microtubule coil, and the Spectrin-based membrane skeleton make up its major parts.

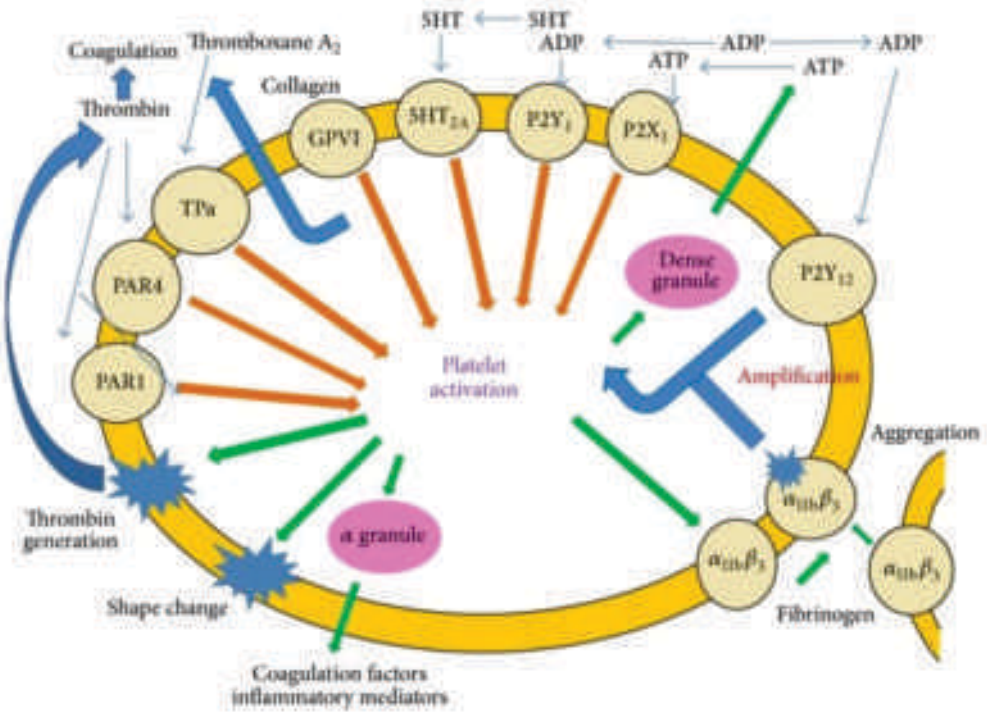

Figure 2: Following platelet activation, ADP is secreted in dense granule form, activating P2Y12 and amplifying procoagulant, proinflammatory, and aggregation responses. (Copyright Ghoshal K., Bhattacharyya M[19])

The two main storage granules found in platelets are called Dense and α granules, and they are responsible for storing physiologically active chemicals that are specifically engaged in the onset of coagulation and the recruitment of additional cells during inflammation[20]. The more common α granule has proteins (including fibrinogen, vWf, and GPIIbIIIa) that start the coagulation cascades. The α granule also contains a number of membrane proteins necessary for platelet function, including as CD36, P-selectin (CD62P), and GPIIbIIIa. The majority of cellular P-selectin is also present in the membrane of α granules. It has been revealed that P-selectin recruit's neutrophils through P-selectin glycoprotein ligand (PSGL1)[19,21]. Numerous hemostatically active substances, including as catecholamines, serotonin, calcium, adenosine 5′-diphosphate (ADP), and adenosine 5′-triphosphate, are released during platelet activation and are stored in dense granules (ATP). ADP is a mild platelet agonist that causes aggregation, granule release, and a change in platelet morphology[22].

Dense Granules

Because of their high calcium and phosphate content, platelet dense granules—the smallest granules—appear as dense masses under electron microscopy (***Figure 1***)[23]. Each platelet has three to eight thick granules. They also have elevated levels of serotonin and adenine nucleotides. Little GTP-binding proteins and related adhesion molecules, including as GPIb, GPIIb/IIIa, and P-selectin, which are typically found on other platelet compartments, have also been observed to be present in dense granules. Dense granule membrane proteins combine with the platelet plasma membrane during platelet activation, releasing the contents of the granules into the extracellular space. Both local vasoconstriction and the recruitment of more platelets (aggregation) are facilitated by the released components (e.g., serotonin). Dense granules' ADP is considered to come from the storage pool since it is mostly used in haemostasis and does not equilibrate with the metabolic pool of ADP. While the processes for releasing dense granules are similar to those found for α-granules, there have been reports of differences in the roles of certain SNARE proteins between the two granule populations[24]. Patients with a lack of these granules have bleeding disorders, demonstrating the critical role that dense granules play in proper haemostasis. Hermansky-Pudlak syndrome (HPS) and Chediak-Higashi syndrome are two uncommon human disorders linked to a susceptibility to bleeding. These illnesses have been linked to platelet dense granule deficiency[25].

α Granules

The most common, heterogeneous, and biggest platelet granules, measuring between 200 and 400 nm, are called alpha (α) granules[12]. Each platelet contains around 50–60 granules, which are what give the cytoplasm in peripheral blood smears its granular look (stained with Romanowsky stains). Most of the platelet factors involved in thrombosis and haemostasis are found in these granules. These comprise a number of coagulation-related factors as well as big polypeptides such thrombospondin, P-selectin, platelet factor 4, and beta thromboglobulins (Factors V, XI, XIII, fibrinogen, von Willebrand factor and high molecular weight kininogens). Numerous adhesion molecules, including vitronectin and fibronectin, that are involved in the contact between platelets and artery walls are also present in α-granules. Some proteins found on the membrane of α-granules are also expressed on the membrane of platelets, including P-selectin, GPVI, GP IIb/IIIa, and GPIb complex. Furthermore, proteins implicated in inflammation and wound healing, as well as a variety of chemokines and mitogenic growth factors such as transforming growth factor-β, vascular endothelial growth factor, and platelet-derived growth factor, are found in α-granules. Platelet α-granule components are being increasingly understood; for instance, a recent proteomic investigation of α-granules identified 284 non-redundant proteins, 44 of which had not been previously reported in these granules[26]. Moreover, the composition of α-granules varies, and different agonists may cause the release of distinct subpopulations of these granules[27]. Research is now being done to better understand the mechanisms underlying the differential release of α-granules and the wide spectrum of consequences that result from the release of their many elements.

The intricate process of α-granule secretion during platelet activation includes coalescence inside the platelet centre, granule fusion with the OCS and with one another, as well as fusion with the plasma membrane[12,20,28]. The intricate machinery required for granule release is present in platelets and includes membrane lipids, related proteins, and soluble NSF [N-ethylmaleimide-sensitive factor] attachment protein receptors (SNAREs)[28,29]. Certain components of α-granules, including P-selectin, perform their primary physiological function after being integrated into the platelet membrane[30]. After being released from granules, additional granule contents function and take part in platelet aggregation, thrombosis, platelet sticky contacts with leukocytes and other substrates, and the release of several growth factors that regulate cell proliferation. A rare genetic condition called Gray Platelet syndrome (GPS) is characterised by a deficiency of platelet α-granules, which leads to both quantitative and qualitative platelet dysfunction as well as a susceptibility to bleeding[31]. In GPS, aberrant α-granule development during megakaryocytic differentiation prevents proteins endogenously produced by megakaryocytes or endocytosed by platelets from entering the α-granules of platelets. Fibrosis ensues from the continuous release of α-granule contents, including cytokines and growth factors, into the bone marrow (myelofibrosis). In peripheral smears, the platelets have a grey morphology[31].

PLATELET RECEPTORS AND ADHESION MOLECULES

Selectin

The biggest adhesion molecule in the selectin family is P-selectin (CD62P), which has a molecular weight of 130 kDa. It is mostly seen on platelet α-granules, but it has also been reported on dense granules[32]. It is also found in endothelial

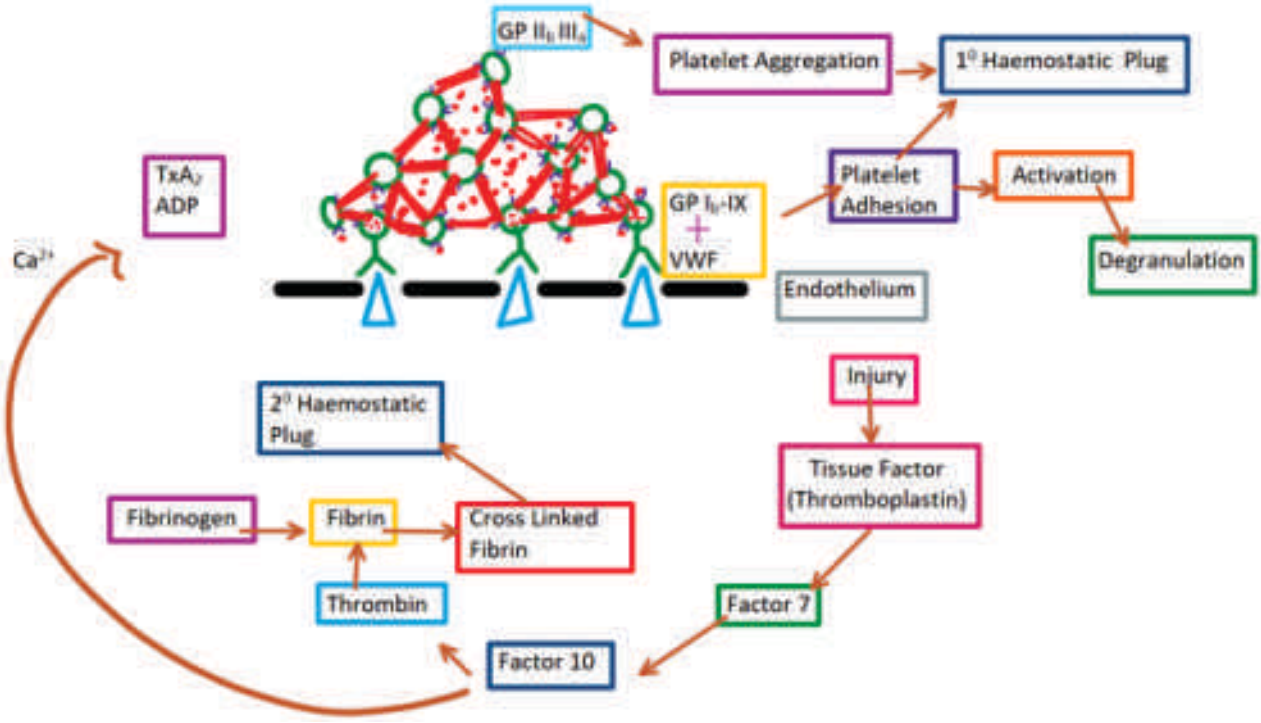

Figure 3: Platelet adhesion and Haemostatic Plug formation

cells' Weibel-Palade structures. There is not much P-selectin visible on the surface of platelets when they are at rest and not activated. On the other hand, P-selectin is rapidly expressed on the surface of activated platelets (or endothelial cells) due to the fusing of granule membranes with the cell membrane. It has been shown that the peak surface expression of P-selectin ranges from around 30 seconds to 10 minutes after stimulation, however the kinetics of this response depend on the agonist and dosage[33,34]. One of the most widely utilised indicators of platelet activation is P-selectin surface expression. 30 P-selectin glycoprotein ligand-1 (PSGL-1), expressed mostly on leukocytes[35], von Willebrand factor[36], glycoprotein Ibα[37], and sulfatides[38] are some of the ligands for P-selectin. In addition to its role in thrombosis and haemostasis, platelet P-selectin also plays a role in the interactions of endothelial cells, leukocytes, and platelets during inflammation[39]. Furthermore, thrombosis may be facilitated by a soluble version of P-selectin that is found in plasma[40].

GP (Glycoprotein) Ib/IV/IX

The primary platelet receptor for von Willebrand factor (vWF) is this big glycoprotein receptor complex, which is made up of four different components. These include the primary binding site for vWF, GPIbα (around 145 kDa), GPIbβ (around 20 kDa), GPIX (around 16 kDa), and GPV (around 82 kDa). Two GPIbβ subunits and GPIbα are connected by membrane-proximal disulfide linkages. GPIb, sometimes referred to as the α/β2 complex, has a non-covalent association with GPIX, whereas GVP has a more tenuous association with two GPIb-IX complexes[41]. When vWF binds to GPIb, signal transduction pathways are triggered, activating platelet integrin GPIIb/IIIa (αIIb/β3). This platelet integrin can then bind fibrinogen or vWF to drive platelet aggregation. Not only is GPIbα the primary vWF receptor on platelets, but it has also been shown to bind a wide range of ligands, such as kininogen[48], integrin Mac-1 on leukocytes[43], integrin Mac-1[42], thrombin[44], coagulation factors XI, XI, and VIIa[45-47], and thrombospondin[42]. On the other hand, GPIbα's interaction with vWF is more understood than its binding to these other ligands. GPIb's cytoplasmic C-terminal tails interact with filamin, calmodulin, 14-3-3, and the regulatory p85 subunit of phosphoinositide 3-kinase in addition to having many serine phosphorylation sites[41]. GPIb-IX-V is connected to the membrane cytoskeleton by interaction with filamin; effective production of the GPIb-IX complex on the transfected cell's plasma membrane requires the presence of all three subunits. The Bernard-Soulier Syndrome is a bleeding illness caused by deficiency or malfunction of the GPIb complex[49] and several mutations causing the disease have been linked to the genes encoding GPIbα, GPIbβ, and GPIX[50]. Although vWF is typically available in plasma and this receptor is constitutively present on the platelet plasma membrane, interaction of the receptor with its ligand requires a conformational change in one or both of these components. These modifications are brought about by variations in blood flow and the ensuing shear stress. In vitro evaluation of this interaction is conducted using the antibiotic Ristocetin, which causes these alterations in the absence of shear stress[51].

GP IIb/IIIa

Because platelet GP IIb/IIIa (αIIbβ3) is crucial for platelet aggregation, it has been the subject of the most research. It is a heterodimer, just like other integrins, with an alpha subunit (αIIb, about 136 kDa) and a beta subunit (β3, ~92 kDa). Unstimulated human platelets have around 80,000 copies of GP IIb/IIIa on their surface, and during platelet secretion, extra molecules from the membranes of platelet granules are translocated to the platelet surface[52]. When platelets are at rest, this molecule is constitutively produced on the plasma membrane in an inactive state; nevertheless, upon activation, it changes shape. This integrin is made up of flexible stalks that carry its transmembrane (TM) and cytoplasmic domains, as well as a large extracellular nodular head that contains its ligand-binding site[53,54]. The alpha subunit's nodular head is folded into a β-propeller shape, and the extracellular part of the αIIb stalk is made up of two "calf" and one "thigh" domain. The β3 head is composed of a hybrid domain with a fold like that of I-set immunoglobulin domains and a βA-domain with a metal ion-dependent adhesion site (MIDAS) motif. A PSI (plexin, semaphorin, integrin) domain, four tandem epidermal growth factor (EGF) repeats, and a distinct carboxyterminal βTD domain make up the β3 stalk. The head area is notably hunched over in a small "V" form while at rest . A structural alteration of the headpiece is brought about by activation, which causes the αIIb and β3 domains to move from their closed conformation with adjacent stalks to their open conformation with separated stalks. This reveals the ligand binding site, which is made up of a β3βA-domain and forms a “cap” with four loops on the upper surface of the αIIbβ-propeller domain. In addition to fibrinogen, platelet GPIIb/IIIa can bind to additional ligands such vWF, fibronectin, and vitronectin[55]. For individuals suffering from thrombotic diseases, the tTis molecule is a prominent target for targeted treatment[56].

Collagen Receptors

Primary haemostasis requires platelets to interact with subendothelial collagen. The two main collagen receptors, α2β1 integrin and glycoprotein VI (GP VI, approximately 65 kDa), are important for haemostasis. These receptors have varying degrees of affinity when binding to particular collagenous sequences[57]. Sequences containing the GER triplet (glycine-glutamic acid-arginine) are bound by α2β1, whereas sequences containing two or more GPO triplets (glycine-proline-hydroxyproline) are bound by GPVI[58,59]. Through the stimulation of the small GTPase Rap1b, which is dependent on phospholipase C (PLC), platelet adhesion facilitated by integrin α2β1 leads to the activation of platelet GPIIb/IIIa[60]. Platelet GPVI is expressed constitutively on both α-granules and the platelet plasma membrane[61]. After platelet activation, GPVI surface expression rises and intracellular expression falls, which is in line with their release from α-granules and plasma membrane integration. Comprising two C2 immunoglobulin-like domains and an arginine residue in the transmembrane region that forms a salt bridge with the aspartic acid residue of the Fc receptor γ (FcRγ)-chain, GPVI is a member of the immunoglobulin superfamily[62]. When collagen activates its immunoreceptor, its tyrosine-based activation motif (ITAM) is phosphorylated. This sets off a series of events involving many adaptor proteins, culminating in the phosphorylation and activation of PLCγ2[63,64]. Collagen type III, for example, is one of the collagen types to which GPVI primarily binds. A propensity for bleeding is linked to the lack of GPVI in humans, a crucial adhesion molecule involved in haemostasis and thrombosis[65].

Thrombin Molecules

Thrombin is a powerful platelet stimulant and an essential part of the blood coagulation cascade. Protease-activated receptors are responsible for mediating platelet responses to thrombin (PAR). Among G protein-coupled receptors, PAR are distinct in that thrombin's proteolytic cleavage of the receptor results in the unmasking of a particular ligand[66]. When thrombin attaches itself to the extracellular domain of PAR-1 and PAR-4, the receptor is activated and signalling is induced. The cleaved amino terminus has a tethered ligand attached to it. Activating the thrombin receptor potently without requiring receptor cleavage, synthetic peptides known as thrombin-receptor agonist peptides (TRAPs) imitate the new amino terminus and cause platelet activation, secretion, and aggregation. To stop platelet activation once thrombin binds to platelets, both PAR-1 and -4 activation must be blocked. In mouse platelets, the reaction to thrombin is mediated by PAR-3 and PAR-4, not PAR-1 and PAR-4[67,68]. Although PAR3 is expressed in human platelets as well, it does not seem to have a role in the platelet responses to thrombin, in contrast to mice[67,69]. Platelet activation, shape change, and granule release are all caused by thrombin signalling through either PAR1 or PAR4; PAR1-dependent reactions are seen at lower thrombin concentrations than those caused by PAR4[67]. Though both PAR-1 and PAR-4 bind to Gq and G12/13 G-proteins, activating phospholipase C, calcium mobilisation, and protein kinase C, the signalling pathways downstream of PAR-1 and PAR-4 in human platelets are not well understood[70]. Large-scale clinical studies are being conducted on thrombin receptor antagonists, which may offer a potential target for therapeutic antithrombotic treatment[71].

ATP and ADP

Although the reaction to ADP differs from that to thrombin, it has long been known to stimulate platelet adhesion and aggregation. Human platelets undergo an initial, reversible aggregation without granule release when exposed to modest amounts of ADP. Increased ADP concentrations cause prostaglandin production and granule release, which results in a distinctive biphasic response with irreversible aggregation.

The G-protein coupled P2Y receptor family of G protein-coupled, seven transmembrane domain receptors mediates the human platelets' response to ADP (P2Y1 and P2Y12). The P2Y1 receptor mediates platelet shape change and aggregation by coupling to Gq and releasing intracellular calcium ions. Antithrombotic drugs including ticlopidine, clopidogrel, and prasugrel target the P2Y12 receptor, which is linked to the suppression of adenylyl cyclase via Gi[72]. A lifelong bleeding disease is linked to mutations in the P2Y12 receptor[73].

Although ATP is not as strong as ADP, it nevertheless causes shape changes in platelets and increases their reactivity to other agonists like collagen. ATP-induced signalling is carried out by a ligand-gated ion channel (P2X1)[74].

Thromboxane A_2

A consequence of the metabolism of arachidonic acid is thromboxane A2 (TXA2). Two isoforms of thromboxane receptors, TPα and TPβ, have been discovered; TPα is expressed more often in human platelets than TPβ[74,75]. Moreover, TP is a member of the G protein-coupled receptor (GPCR) family. Studies have suggested that it might interact with Gq and G13 to activate phospholipase C and RhoGEF, respectively[76]. TXA2 is released by many cells, including platelets, and it exerts paracrine and autocrine actions. Through altering platelet shape, aggregation, degranulation, and amplifying reactivity to additional agonists, TXA2 raises platelet activation. Aspirin, a commonly used antiplatelet medication, works by inhibiting cyclooxygenase-1 to reduce the production of TXA2, and direct inhibitors of TP-α are being researched for potential therapeutic use[77,78]. Platelets express receptors for other prostanoids, including prostaglandin E2 and prostacyclin, which prevent platelet aggregation (which has a biphasic effect on platelets)[79].

PLATELET AGGREGATION

Physiology and Mechanics

After platelets first adhere to the site of damage, aggregation—which involves platelet-to-platelet adhesion—is required for efficient haemostasis. A variety of agonists, including collagen and adenosine diphosphate (ADP), which are present at the sites of vascular damage, activate platelets after adhesion. By attaching to certain receptors on the previously mentioned platelet surface, these agonists stimulate platelets. When these receptors are occupied, a chain of subsequent processes occurs that raise the intracytoplasmic concentration of calcium ions. Calcium influx via the plasma membrane[80] and release from intracellular reserves cause a rise in intracellular calcium in platelets. Phospholipase Cβ (PLCβ) is activated by receptors linked to G-proteins, such as those to thromboxane A2 (TXA2), thrombin, and ADP. On the other hand, phospholipase Cγ (PLCγ)[81] is preferentially activated by receptors acting through non-receptor tyrosine kinase pathways, such as collagen receptor GpVI. Two second messengers, inositol triphosphate and diacylglycerol (DAG), are produced when PLCβ or PLCγ is activated (IP3). While IP3 releases calcium from intracellular reserves, DAG facilitates calcium influx. Furthermore, certain agonists, such as ATP binding to the ligand-gated ion channel receptor P2X1[74], can directly trigger calcium influx.

Numerous structural and functional alterations in the platelet are brought about by an increase in the concentration of platelet-free calcium. The platelet undergoes a significant morphological transition from a disc to a spiny sphere (a process called shape change). The platelet's granules are centralised, and their contents are released into the open canalicular system's lumen before exiting the system altogether (the release reaction). Arachidonic acid is released from membrane phospholipids by membrane phospholipase A2, which is activated by an increase in platelet calcium. The enzyme cyclooxygenase 1 transforms arachidonic acid into prostaglandin H2 (PGH2), an intermediate product (COX-1). Thrombin synthase[82] proceeds to further convert PGH2 into TXA2. TXA2 is a powerful platelet activator. The shape-change response produces lengthy membrane projections that enable platelets to connect and form aggregates. The platelet cytoskeleton, which is made up of an ordered web of actin filaments, microtubules, and many related proteins connected to a range of platelet signalling molecules, is responsible for mediating shape change in platelets[83]. Actin polymerization, myosin light chain phosphorylation, and extensive cytoskeleton network remodelling are the outcomes of platelet shape change; these reactions vary depending on the time and stimuli[83,84].

Platelet aggregation is facilitated by the membrane protein GPIIb/IIIa complex, which is a major adhesion molecule. Platelets have a high density of GPIIb/IIIa integrin receptors on both the plasma membrane and α-granules[52]. In platelets that are at rest, it exists in an inactive state. Almost all agonists that activate platelets cause GPIIb/IIIa to undergo conformational changes, or "inside-out signalling," which makes them capable of binding soluble plasma fibrinogen. Thus, GPIIb/IIIa undergoes conformational changes that are directed toward the cytoplasm upon ligand attachment (a process known as "outside-in signalling"). It is still unclear exactly which set of circumstances led to these signalling events[53,86]. It is unclear how exactly receptor clustering, phosphorylation, and interactions with cytoskeletal and other cytoplasmic molecules cause conformational changes in GPIIb/IIIa. However, on neighbouring platelets[80], the receptor-bound fibrinogen serves as a link between two GPIIb/IIIa molecules. This completes the general process by which platelet chemical agonists cause platelet aggregation. When high shear circumstances are present, platelet aggregation is mediated by fibrinogen binding to GPIIb/IIIa[87]; however, in the case of platelet aggregation produced by low shear, vWF acts as a bridge molecule in place of fibrinogen.

Some molecules have lately been postulated as potential mediators of these reactions, even though GPIIb/IIIa is the most extensively investigated mediator of stabilising thrombi and connecting platelets to each other. These consist of CD40 ligand[88,89], SLAM (signalling lymphocyte activation molecule) family proteins, and junctional adhesion molecules (JAMs). It is yet unclear how these processes relate to one another in platelet aggregation.

Through many feedback amplification loops, activated platelets attract more platelets to the expanding haemostatic plug. They do this by releasing platelet agonists such ADP and serotonin that is stored in α-granules and by synthesising de novo proaggregatory TXA2. By encouraging the inclusion of more platelets in the haemostatic plug established at sites of

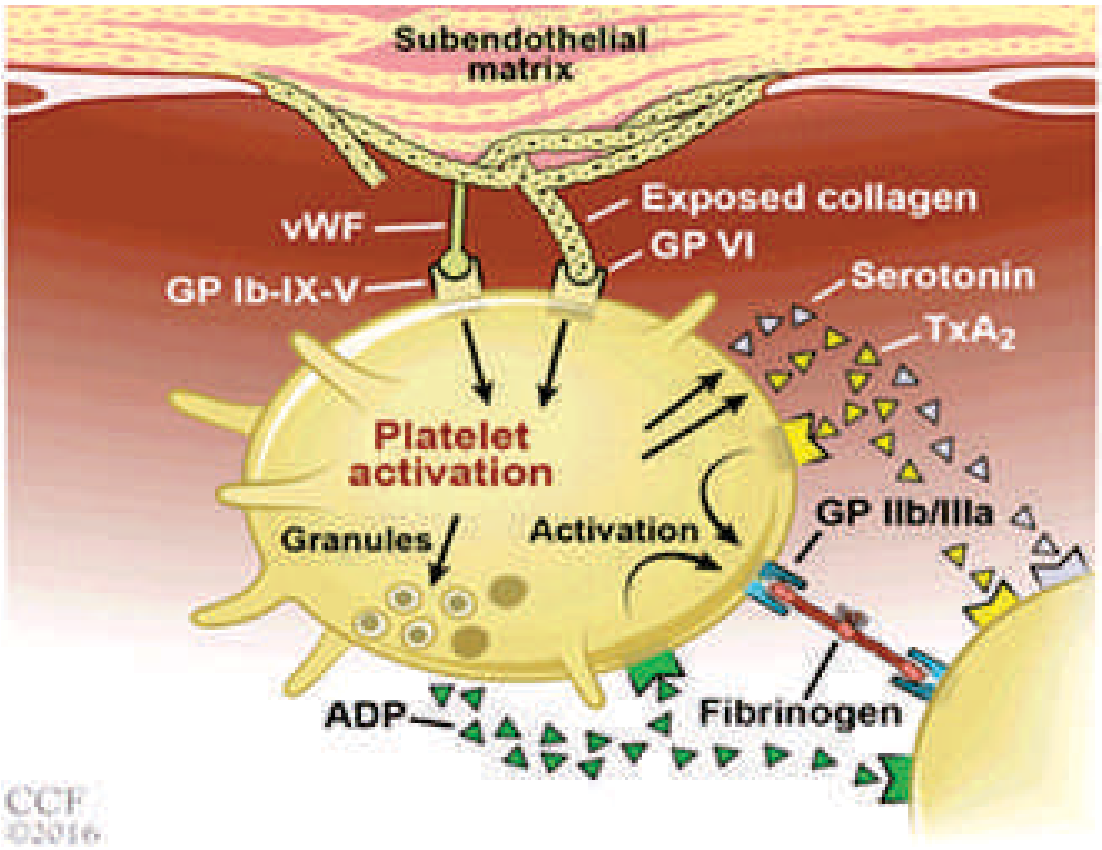

Figure 4: Activation of Platelet Aggregation Cascade

vascular damage, the release of ADP and TXA2 synthesis consolidates the original haemostatic plug. Last but not least, platelets also contribute significantly to secondary haemostasis by acting as a very potent catalytic surface to initiate the coagulation cascade. Negatively charged phospholipids go from the membrane bilayer's inner to outer leaflets when platelets are activated. Procoagulant vesicles rich in anionic phospholipids are known to bleb and release in response to the trans bilayer migration of these molecules. Microvesicles and active platelets both serve as binding sites for coagulation system cofactors and enzymes, which effectively produce thrombin, a powerful platelet agonist.

Pseudopods begin to grow on platelets when intracellular Ca2+ concentration rises over a certain threshold. Platelet-platelet aggregation is triggered by the exposure and activation of platelet fibrinogen receptors (GPIIb/IIIa) during shape change. This reversible process is often referred to as primary aggregation. Nevertheless, fibrinogen cannot be bound by platelets that are at rest. An essential route for platelet activation is the arachidonic acid thromboxane pathway. Acetylsalicylic acid, or aspirin, is a medication often used to treat cardiovascular disease (CVD). It works by permanently acetylating and inactivating COX, which stops TxA2 from being produced. This decreases platelet aggregation. Since anucleate platelets are unable to generate enzymes from scratch, mature normal human platelets exclusively express COX-1. Consequently, the effects of aspirin on them are cumulative and long-lasting. As a result, aspirin's cardioprotective action is achieved by permanently and irreversibly impairing thromboxane A2-dependent platelet activity, which lowers the risk of acute arterial thrombosis[90,91].

Another significant platelet activator is ADP. On the platelet membrane, P2Y12, an ADP-specific receptor, is linked to inhibitory G-proteins and mediates the release of Ca2+ produced by ADP. This process inhibits adenylate cyclase and activates the GPIIb/IIIa receptor, which causes platelet aggregation. By blocking the P2Y12 receptor, ticlopidine, clopidogrel, and other thienopyridines decrease platelet activation[92]. The active platelet releases chemicals including serotonin and thromboxane A2, which strengthen the platelet-rich clot and initiate irreversible secondary aggregation (Figure 5)[93]. These compounds also give crucial positive feedback.

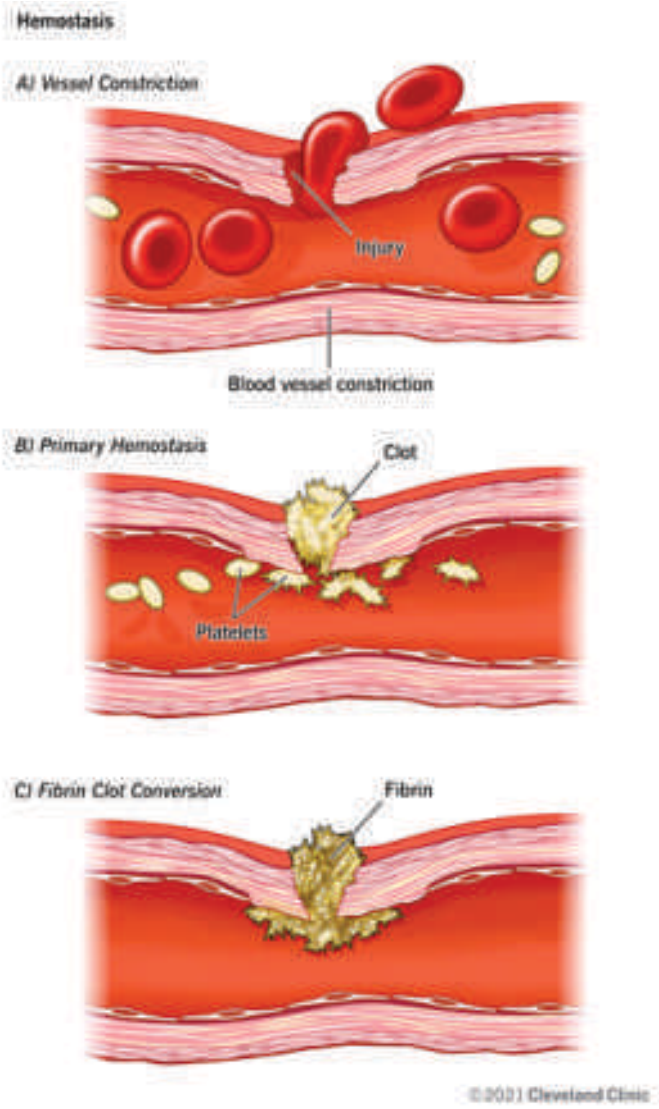

Figure 5: Haemostasis

Substances produced by platelet granules that attract other platelets and blood cells enhance the platelet response. In primary haemostasis, the platelet plug that first forms is rather fragile. The production of thrombin and fibrin, as well as the coagulation cascade, prolong secondary haemostasis. Platelet membrane phospholipids become negatively charged during platelet activation, which helps to activate coagulation (e.g., FV, FVIIIa, FIXa, and FX). This stage involves the prothrombinase complex (FXa, FVa, Ca2+, and prothrombin) binding to the platelet membrane. Thrombin production initiates further platelet activation. The blood clot is strengthened by the "red thrombus" that forms as a result of these cascades[94].

Assessment

The assessment of bleeding time (BT) using the Duke procedure95 marked the beginning of the history of platelet function testing (PFT). This was the initial test to evaluate platelets' ability to form a plug. It has long been the only screening test that can detect acquired or congenital platelet disorders[96]. Born's research suggest that the primary technique for diagnosing platelet function in the 1960s was light transmission aggregometry (LTA), a ground-breaking platelet aggregation test in platelet-rich plasma (PRP)[97]. This test measures the platelets' capacity to clump together in response to exogenous aggregating agents, or agonists, such collagen, adenosine-diphosphate (ADP), arachidonic acid (AA), and epinephrine (EPI)[98]. Other PFT techniques have become accessible since the late 1980s, including platelet aggregometry in whole blood (WB), activated platelets studied ex vivo by flow cytometry, the determination of certain chemicals generated by platelets, and the evaluation of platelet nucleotides[99,100].

But the growing number of antiplatelet medication users, who are more likely to bleed, particularly after trauma and surgery, has also made PFT a valuable tool for presurgical and perioperative settings, where it can be used to predict bleeding and track the effectiveness of various prohemostatic treatments. In this case, there are now greater opportunities to use PFT in general laboratories, various clinical settings, and specialised clinical or research laboratories due to the introduction of new, easier-to-use tools for evaluating platelet function at the point-of-care (POC) or bedside.[101]

1. Prostanoids Modalities

The primary by product of platelet metabolism of arachidonic acid is TXA2. At the location of vascular damage, it is produced and released from platelets, intensifying platelet activation[102]. Phospholipase A2 facilitates the extraction of arachidonic acid from membrane phospholipids (PLA2). It is converted to prostaglandin G2 and H2 by cyclooxigenase-1 (COX-1), and subsequently to TXA2, a strong vasoconstrictor, by thromboxane synthase. By hydrolysing TXA2, thromboxane B2 (TXB2)[103], a physiologically inactive and stable compound, is produced. TXB2 is converted into two main metabolites: 11-dehydro-TXB2 by dehydration and 2,3-dinor-TXB2 through β-oxidation. Urine contains 11-dehydro-TXB2, and the amount expelled indicates the total amount of platelets that may make TXA2[104]. The aspirin action on COX-1 lowers the urine 11-dehydro-TXB2[105,106] levels. These days, enzyme-linked immunoassays (ELISA)[107] are used instead of immunoradiometric assays (IRMA)[108] or radioimmunoassay (RIA).

2. Platelet Function Analysis

Siemens, Munich, Germany, manufactures the PFA-100 and PFA-200 platelet function analysers, which quantify in vitro the stopping of high-shear blood flow caused by the platelet plug. This point-of-care whole blood technique is straightforward, quick, and needs no sample preparation or small sample quantities. Its drawbacks include the need for pipetting and its reliance on haematocrit and von Willebrand factor levels. In the PFA-100 method, two distinct cartridges are applied: collagen plus ADP (CAPD) and collagen plus epinephrine (CEPI). Within the cartridges, citrated whole

blood runs via a capillary at a high shear rate, ending in a collagen-coated membrane with an opening of 147 μm that is filled with either EPI or ADP. Closure time is the amount of time until clot formation obstructs the opening (CT).[109]

The technique has been employed to track the results of acetylsalicylic acid treatment. In individuals on aspirin treatment, a brief CEPI CT scan may be suggestive of elevated residual platelet reactivity[110,111]. It is not advised to use PFA-100 to track the effects of thienopyridines[111]. Yet, the recently released INNOVANCE PFA P2Y cartridge demonstrated sensitivity to P2Y12 inhibition and was on par with other platelet function tests that are now accessible[113].

3. ROTEM System

One additional module that may be added to the ROTEM is the ROTEM Platelet System. The technique uses whole blood impedance aggregometry as its foundation. It offers details on the aggregation and function of platelets as well as how medications are impacted by platelet function. Unfortunately, there aren't much data on this relatively new method's applicability in clinical practice[114].

4. Thrombo-Elasto Graphy (TEG)

The viscoelastic alterations of the whole clotting process are measured by Thromboelastography (TEG) and thromboelastometry. There are several tests available for both the intrinsic and extrinsic pathways. The function of platelets in clot formation may be assessed by selectively activating the extrinsic route. More precisely, the platelet contribution to clot strength is measured by the thromboelastometry platelet test and the thromboelastogram platelet mapping system. The worldwide functional tasks of platelets in haemostasis-thrombin production, clotting, clot retraction, and fibrinolytic activation—are tested in both procedures[115].

Based on these principles, the most popular techniques include Sonoclot analysis, Thromboelastography (using the TEG Platelet Mapping System; Haemoscope, Braintree, MA, USA), and thromboelastometry (using the ROTEM; TEM Int, Munich, Germany) (Sonoclot Signature; Sienco, Arvada, CO, USA). A revolving mechanism with a pin held aloft by a torsion wire is a feature of TEG and ROTEM. A pin that is inserted into the Sonoclot device is pushed up and down at an ultrasonic pace. Whole blood samples are first subjected to the proper reagents to begin the test. All phases of the clot's development and resolution involve measurements and displays of changes in elasticity[116].

The original TEG has been modified to become the TEG platelet mapping system. It is a point-of-care technique suitable for keeping an eye on all kinds of antiplatelet treatments. Through four distinct whole blood tests, it offers information regarding platelets. A sample that has been activated with kaolin exhibits the potential for maximal clot strength by producing a robust thrombin response and cleaving all of the fibrinogen that is present. The strength of the clot resulting from fibrin is demonstrated by one aliquot that only contains Activator F, which blocks all thrombin. Additionally, all thrombin is blocked in the third and fourth experiments, which additionally activate platelets at the thromboxane A2 or ADP receptors. The patient's whole haemostatic potential is used as the baseline to determine the degree of inhibition[117].

5. Plateletworks

The foundation of Plateletworks (Helena Laboratories, Beaumont, TX, USA) is platelet aggregation that is dependent on GP IIb/IIIa. Samples with and without agonists are compared in terms of platelet count (ADP or AA). The procedure uses whole blood and needs very little sample preparation. The fact that samples must be measured shortly after blood is drawn is a drawback. This aspect restricts its use, and only a small number of studies have linked the approach to clinical outcomes[109].

6. IMPACT-R

Cone-and-plate technology is used by the Impact R analyser (DiaMed, Cressier, Switzerland) to measure shear-induced platelet aggregation. Under arterial flow conditions, the apparatus evaluates platelet adhesion and aggregation in anticoagulated whole blood. Platelet adhesion and aggregation occur as soon as plasma proteins from a blood sample are applied to the surface of a polystyrene well. Adherent platelets are quantified by an image analyser. The proportion of the well surface covered by aggregates is used as an index of adhesion, and the average size of the aggregates is used as an index of aggregation to represent the results. There is a clinical version of the device as well as a research version with an adjustable shear rate. The method's simplicity, lack of sample preparation, and small sample sizes are its advantages. Nevertheless, because pipetting is necessary, it is not a true point-of-care technique[109].

7. Global Thrombosis Test (GTT)

Using native, non-anticoagulated whole blood, the global thrombosis test (GTT) (Montrose Diagnostics Ltd., London, UK) is a new technique based on platelet activation owing to severe shear stress. This quick point-of-care test provides information on the patient's thrombotic condition. Its clinical utility is being assessed[118].

8. Verify Now

A point-of-care tool called VerifyNow (Accriva Diagnostics, San Diego, CA, USA) uses turbidimetric-based optical detection to assess platelet aggregation in anticoagulated whole blood. Beads coated with fibrinogen promote platelet aggregation; the amount of active GP IIB/IIIA receptors on the surface of the beads determines how many platelets aggregate there. The aspirin effect may be studied with the Aspirin Test, which uses AA as an agonist. The Platelet Reactivity Unit (PRU) Test assesses the clopidogrel impact by using PGE1 as a suppressor of intracellular free calcium and ADP as an agonist. As a baseline, a second channel studies platelet aggregation triggered by thrombin receptor activating peptide (TRAP-). The technique requires little sample volume and is quick and easy to use. Pipetting is not necessary[109].

9. Impedance Method (Aggregometry)

When an agonist causes platelet aggregation, impedance aggregometry analyses the change in electrical impedance between two electrodes. The method's premise is comparable to optical aggregometry, except it may be performed on whole blood, negating the requirement to prepare a platelet suspension. An increase in electrical impedance results from platelets aggregating to platelets that are adhered to the electrodes. The measurement of the increase in electrical impedance expressed in Ohms is used to evaluate platelet aggregation[99]. Patients with thrombocytopenic syndrome may also benefit from impedance aggregometry[119]. Since impedance aggregometry is done on whole blood, other blood components can affect platelet aggregation, allowing for a more physiological assessment of platelet function. It also occurs on a solid surface, mimicking the platelet adhesion and aggregation process in physiology.

Despite being based on impedance aggregometry, the Multiple Electrode Aggregometry (MEA) is a point-of-care tool[120]. Its five channels allow for the simultaneous monitoring of several agonists or substances. Two pairs of sensor electrodes are inserted into each cuvette as a means of integrated quality control. Each sensor unit is used independently to assess platelet aggregation concurrently in triplicate. The process of pipetting is mechanised. MEA can be used to identify increased platelet reactivity during therapy and to monitor antiplatelet treatments[121,122]. Finding patients with a bleeding diathesis is another appropriate use for it.

10. Flow Cytometric Methods

Numerous assays are used in flow cytometry (FC) study of platelets for a number of objectives, such as thrombopoiesis investigation, detection of illnesses related to platelet function, and antiplatelet medication monitoring[123].

Using antibodies coupled to fluorescent dyes, which may attach to certain proteins on cell membranes or inside cells and show their presence, flow cytometry quantifies the amount of these proteins. The fluorescent molecules of dyes linked to platelets are excited to a higher energy state by a light source. When the dyes go back to rest, they release light at various wavelengths. Double labelling is the process of coupling a particular secondary antibody to a fluorochrome that identifies the primary antibody[104].

The sorts of activation-dependent monoclonal antibodies that target granule membrane proteins or conformational changes of GPIIb/IIIa have been researched the most. The fibrinogen binding site revealed by a conformational shift in GPIIb/IIIa brought on by platelet activation is the target of the monoclonal antibody PAC-1. PAC-1 exclusively attaches to activated platelets as a result. A different surface marker that is frequently employed in relation to platelet activation targets platelet surface P selectin (CD62P). Monoclonal antibodies specific to P selectin exclusively bind to degranulated platelets because P selectin is expressed on the platelet surface membrane only after α-degranulation. Nevertheless, it was shown that circulating degranulated platelets quickly lose their P-selectin in vivo, which may restrict the use of this technique[109].

Activation-dependent platelet signalling is measured by the phosphorylation of the vasodilator-stimulated phosphoprotein (VASP). Its benefits include minimal sample quantities needed, the use of whole blood, stability (enabling samples to be sent to a distant lab), and reliance on the P2Y12 receptor, which is where clopidogrel acts. Its drawbacks include the need for skilled personnel and intricate sample preparation[124].

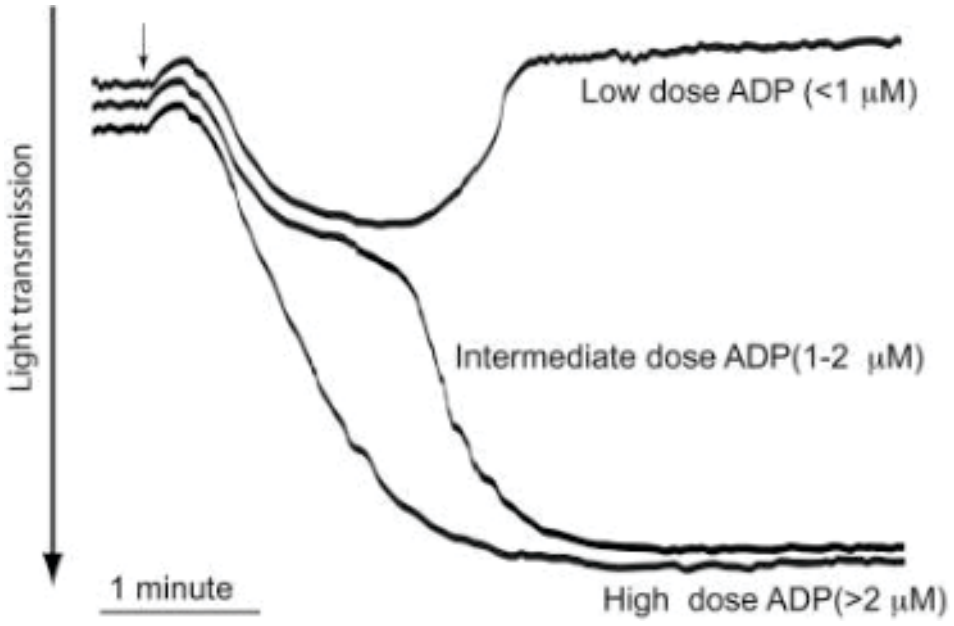

Figure 6: Human platelets responding to ADP are traced using platelet aggregometry.

11. *in vivo* Tests

Platelet aggregometry may not accurately capture the intricacies of platelet aggregation *in vivo*, even while it enables evaluation of platelet aggregation kinetics under meticulously regulated experimental circumstances (agonist dosage, platelet numbers, temperature, etc.). Numerous intravital video microscopy techniques have been employed to observe platelet aggregation in vivo during thrombus development; they are covered in more depth in another source[125].

These techniques typically involve an injury to microvascular walls, for example by micropuncture, electrical stimulation, laser, chemical, or photochemical injury[125,126]. The nature of vascular injury, as well as the vessel type may determine the molecular mechanisms responsible for platelet recruitment in the individual models. In some models (e.g., micropuncture, chemical stimulation) platelet adhesion occurs at sites of endothelial denudation and reflects the mechanisms of platelet adhesion to the subendothelial matrix outlined earlier. In others, platelet adhesion is evident without overt endothelial denudation[127]. Vascular injury results in platelet adhesion, which may progress to formation of an occlusive thrombus, as illustrated in ***Figure 7***. The kinetics of platelet adhesion and aggregation may be monitored in real-time with these techniques, and they have provided important novel observations of the mechanisms mediating platelet recruitment in vivo. These approaches also illustrate the redundancy of many mechanisms responsible for platelet recruitment. For example, mice lacking both vWF and fibrinogen are able to form occlusive thrombi following injury, albeit with marked delay in responses[128]. While intravital microscopy to visualize thrombus formation is not a new technique (e.g., Bizzozero used it in the late 19th century[129]), advances in image acquisition and processing techniques, molecular biology and genetic models of disease have expanded the recent interest in this approach to study interactions of platelets with vascular walls.

ANTI PLATELET DRUGS

When an atherosclerotic plaque suddenly ruptures or fissures, platelet adhesion, activation, and aggregation can be understood as a physiological repair response. However, if this process continues unchecked through a series of self-sustaining amplification loops, it can result in intraluminal thrombus formation, vascular occlusion, and subsequent ischemia or infarction. Antiplatelet medications already on the market disrupt one or more phases of platelet release and aggregation[130], resulting in a substantial decrease in thrombosis risk that is inextricably linked to an elevated risk of bleeding[131].

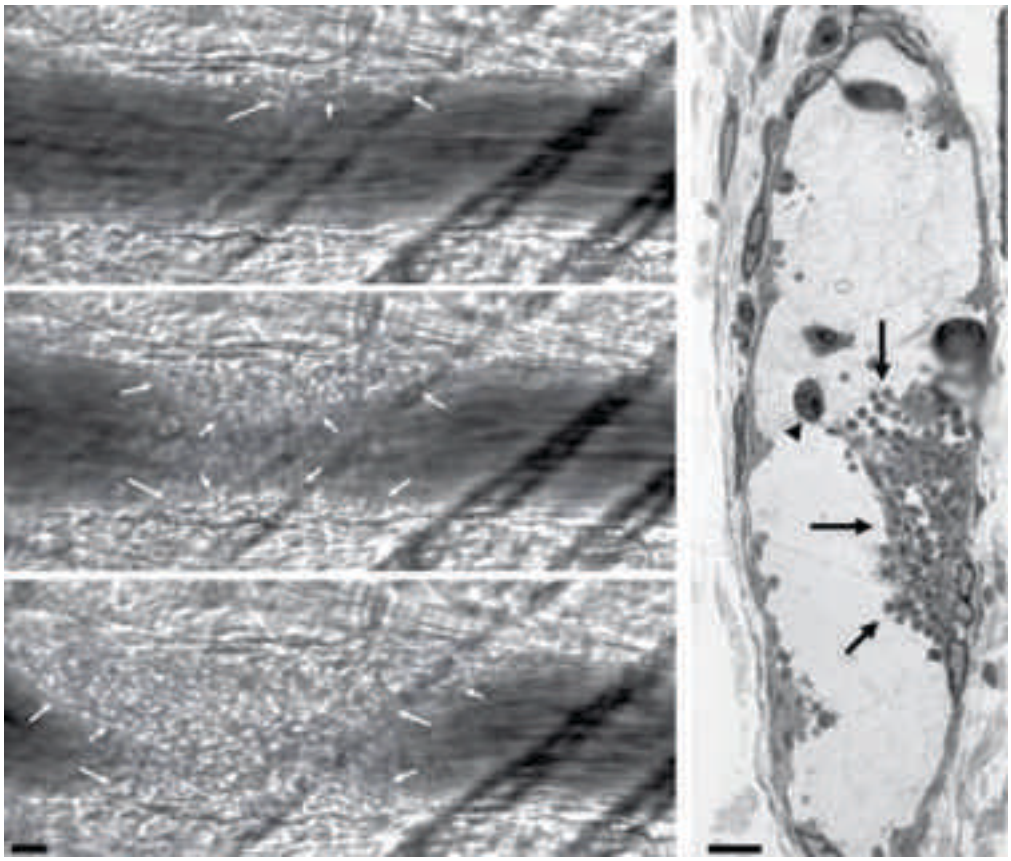

Figure 7: *in vivo* aggregation assessment in mouse model

ASPIRIN

The most researched antiplatelet medication is aspirin. Based on more than 100 randomised trials with high-risk patients, aspirin lowers the risk of vascular mortality by around 15% and nonfatal vascular events by approximately 30%[132].

Pharmacology and Mechanism of Action

The ability of aspirin to permanently inhibit prostaglandin H-synthase-1 and prostaglandin H-synthase-2 (also known as COX-1 and COX-2, respectively)[133] is the most well-characterized mechanism of action of the drug. The conversion of arachidonic acid to prostaglandin H2, the first committed step in the production of prostanoids, is catalysed by COX isozymes (PGH2). The direct antecedent of TXA2 and PGI2 is PGH2.

The acetylation of a strategically placed serine residue (Ser529 in COX-1, Ser516 in COX-2), which blocks substrate access to the catalytic region of the enzyme[134], is the molecular mechanism behind aspirin's persistent suppression of COX activity.

Low dosages of aspirin (75–150 mg) administered once day can result in total or almost complete suppression of platelet COX-1. However, because nucleated cells quickly resynthesize the enzyme, inhibiting COX-2-dependent pathophysiologic processes (such as inflammation and hyperalgesia) necessitates higher aspirin dosages and shorter treatment intervals.

Aspirin is rapidly absorbed in the stomach and upper intestine. Plasma levels peak 30 to 40 min after aspirin ingestion, and inhibition of platelet function is evident within 1 h. In contrast, it can take 3 to 4 h to reach peak plasma levels after administration of enteric-coated aspirin. Therefore, if a rapid effect is required and only enteric-coated tablets are available, the tablets should be chewed instead of swallowed intact. The oral bioavailability of regular aspirin tablets is ~40% to 50% over a wide range of doses. A considerably lower bioavailability has been reported for enteric-coated tablets and for sustained-release, microencapsulated preparations[135].

Aspirin is a useful antithrombotic drug when used long-term at levels between 50 and 100 mg/d; findings suggest that it may even be useful at doses as low as 30 mg/dl[132]. Placebo-controlled randomised trials have demonstrated this. A 75 mg/d dose of aspirin was demonstrated to (1) lower the risk of stroke or death in patients with transient cerebral ischemia[138], (2) lower the risk of acute myocardial infarction (MI) or death in patients with unstable angina [136] or chronic stable angina[137], and (3) lower the risk of stroke following carotid endarterectomy[139].

A meta-analysis of 14 studies on Aspirin efficacy was done. It shown that taking aspirin, as opposed to taking a placebo or receiving no therapy, was linked to a decreased risk of myocardial infarction (risk ratio [RR], 0.83, 95 percent confidence interval [CI]: 0.73–0.95, P = 0.005). Furthermore, aspirin usage was not linked to a decreased risk of cardiovascular or all-cause death when compared to the control groups. Regarding safety, using aspirin was linked to an increased risk of haemorrhagic stroke (RR, 1.30, 95 percent CI: 1.06–1.60, P = 0.011), severe bleeding (RR, 1.40, 95 percent CI: 1.25–1.57, P = 0.000), and gastrointestinal bleeding (RR, 1.58, 95 percent CI: 1.25–1.99, P = 0.000). Furthermore, the clinical features of the patients did not significantly alter the therapeutic outcome[140].

DIPYRIDAMOLE

A pyridopyrimidine derivative having antiplatelet and vasodilator characteristics is dipyridamole. There is debate over dipyridamole's antiplatelet agent's mode of action. It has been suggested that cyclic AMP, an inhibitor of platelet aggregation, can be produced by blocking cyclic nucleotide phosphodiesterase, which is the enzyme that breaks down cyclic adenosine monophosphate [AMP] to 5′-AMP. Another option is to block the uptake of adenosine, which binds to A2 receptors, stimulates platelet adenyl cyclase, and increases cyclic AMP. Furthermore, there have been reports of direct PGI2 synthesis stimulation and protection against its degradation; however, these actions need dipyridamole concentrations significantly higher than the low-micromolar plasma levels attained with oral administration of normal dosages (100-400 mg/d)[142].

Pharmacology

Conventional formulations of dipyridamole have very varied absorption rates, which might lead to a low level of systemic bioavailability. A combination tablet containing low-dose aspirin and a modified-release formulation of dipyridamole with enhanced bioavailability has been created. As a glucuronide conjugate, dipyridamole is excreted mostly via biliary excretion and is heavily protein bound to albumin. It is also prone to enterohepatic recirculation. Reports indicate a terminal half-life of ten hours. This aligns with the bid regimen that has been employed in the latest clinical research[143].

According to a meta-analysis of six randomised trials with 7,648 patients who had a history of TIA or stroke and for which stroke was reported as an outcome, aspirin plus dipyridamole (dose range: 50–1,300 mg/d) reduced stroke by 23% (RR: 0.77; 95 percent CI: 0.67–0.89) when compared to aspirin alone (dose range: 50–1,300 mg/d), with no statistically significant evidence of heterogeneity[144]. Trials using dipyridamole's immediate-release preparation (four trials) and those using its extended-release preparation yielded consistent estimates. The combination of aspirin plus dipyridamole is superior to aspirin alone for the prevention of vascular events in patients with a history of TIA or stroke, according to a Cochrane review of 29 randomised trials involving 23,019 patients. However, no evidence of a benefit of the combination was found in studies involving patients with a history of coronary or peripheral arterial disease, or in other high-risk patients[145].

CILOSTAZOL

A 2-oxoquinolone derivative called clostazol is said to have antiproliferative, antiplatelet, and vasodilatory qualities that lessen the proliferation of smooth muscle cells and the development of neointimal hyperplasia following endothelial

damage. Within the first two weeks of initiating therapy, headaches occur in up to 25% of patients, and GI side effects are frequently caused by ciprofloxacin. Because of the risk of inducing ventricular tachycardia in patients with heart failure, cilostazol should not be used. This effect has been linked to an increase in intracellular cyclic AMP, which is also probably responsible for the medication's vasodilatory effects[141].

Pharmacology

Significant variation exists in the absorption of cilostazol taken orally. The rate and amount of medication absorption are increased when food is administered concurrently. Due to its strong albumin binding and extensive metabolism by cytochrome P450 (CYP450) enzymes, Cilastazole is excreted in urine together with its metabolites. Its half-life is 11 hours, and in individuals with severe renal impairment, the half-life is extended[141].

Cilostazol (50 mg bid or 100 mg once daily) increases the maximal and pain-free walking distance in patients with intermittent claudication[146], prevents thrombotic events in patients with peripheral arterial disease, and prevents restenosis and target vessel revascularization in patients undergoing coronary or peripheral artery stenting, according to meta-analyses of mostly small, open-label, placebo- and active-controlled trials[147,148].

THIENOPYRIDINES GROUP

CLOPIDOGREL

When platelets transit through the liver, clopidogrel is quickly absorbed and converted into a highly labile active metabolite[149] that binds to the platelet P2Y12 receptor irreversibly[150.] With a half-life of about 8 hours, SR 26334, an inactive carboxylic acid derivative, is the primary systemic metabolite of clopidogrel.

The pharmacological pattern of clopidogrel's active metabolite is comparable to that of aspirin; modest dosages administered repeatedly daily result in cumulative inhibition of platelet activity. It takes seven to ten days following the final clopidogrel dosage for platelet function to recover to normal. Permanent inhibition of COX-1 and the P2Y12 receptor by the active moieties of aspirin and clopidogrel is consistent with both the cumulative nature of the inhibitory effects and the delayed rate of recovery of platelet thromboxane synthesis (aspirin) or ADP-induced platelet aggregation (clopidogrel) (active metabolite). This also explains why people with typical platelet turnover rates need take aspirin and clopidogrel once daily, even though their half-lives in the bloodstream are brief[141].

Because there were few phase 2 trials and only one big phase 3 study that compared the effectiveness and safety of clopidogrel (75 mg/d) with aspirin (325 mg/d), the clinical development of clopidogrel was unique.

In the Clopidogrel vs. Aspirin in Patients at risk for Ischemic Events (CAPRIE) trial, 6,400 patients were divided into three groups based on their increased risk of recurrent ischemic events: those who had recently suffered a stroke, those who had recently had a MI, and those who had symptomatic peripheral arterial disease. Clopidogrel decreased the absolute risk by 0.51 percent and the relative risk of MI, ischemic stroke, or vascular death by 8.7 percent (95 percent confidence interval [CI], 3 percent to 65 percent) in the whole CAPRIE trial group of 19,185 high-risk patients when compared to aspirin[151].

TICLOPIDINE

A single oral dosage of ticlopidine can be quickly absorbed up to 90% of the time[152]. One to three hours after a single oral dosage of 250 mg, plasma concentrations peak. Over 98% of the ticlopidine that is absorbed is reversibly linked to albumin and other plasma proteins. Ticlopidine is quickly and thoroughly metabolised. In humans, 13 distinct metabolites have been found. Only the ticlopidine 2-keto derivative has the ability to suppress ADP-induced platelet aggregation more effectively than the original molecule[152]. Ticlopidine's apparent elimination half-life is 24 to 36 hours following a single oral dosage and up to 96 hours following 14 days of consecutive administration[152]. Ticlopidine's recommended dosage is 250 mg bid.

Due to its documented toxicity, ticlopidine's place in the current treatment arsenal is questionable. In the majority of jurisdictions, clopidogrel has essentially taken its place.

PRASUGREL

Following oral consumption, prasugrel is quickly absorbed and transformed into its active metabolite, which reaches peak concentrations within 30 minutes of dosage. Food has little effect on absorption. The primary method of metabolite elimination is renal excretion, with the active metabolite having a half-life of approximately 4 hours[153]. S-methylation and cysteine conjugation transform the active metabolite of prasugrel into inactive metabolites.

According to preliminary pharmacological tests, prasugrel inhibits ADP-induced platelet aggregation more consistently and completely than clopidogrel, and its effects are seen more quickly in healthy persons[154], as well as in patients with stable coronary artery disease[154,155]. In comparison to clopidogrel, which goes through a two-step hepatic conversion process, prasugrel's more fast beginning of action may be partially explained by the CYP450 enzymes' single-step hepatic conversion to its active metabolite[156].

Prasugrel was approved by the FDA with a boxed warning about the possibility of severe or deadly bleeding. Patients who have a history of stroke or who are bleeding actively should not use this medication. The goal of the Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes (TRILOGY ACS) experiment is to see whether lowering the dosage of prasugrel[157] can result in a better benefit/risk ratio.

GP IIb / IIIa GROUP

ABCIXIMAB

A humanised form of a mouse antibody's Fab fragment that targets GpIIb-IIIa is called abciximab. When GpIIb-IIIa is blocked by abciximab, a phenotype resembling Glanzmann thrombasthenia[158] is produced. Each platelet has around 40,000 antibody molecules attached to its surface; however, since they interact in a bivalent manner, each platelet most likely has 80,000 GpIIb-IIIa receptors[159]. Antibody concentrations that reduce the number of accessible receptors to less than 50% of normal dramatically suppress platelet aggregation. About 80 percent receptor inhibition virtually eliminates platelet aggregation; nevertheless, bleeding time is only slightly impacted at this level of blocking. The bleeding period only noticeably prolongs when there is more than 90% receptor blockade[159].

About 1–2% of people receiving abciximab experience thrombocytopenia. Re-administration of abciximab appears to enhance the risk of thrombocytopenia[160,161]. The platelet count usually starts to decline within 24 hours after starting therapy, although it can start to decline as soon as 2 hours in. As a result, the package insert for abciximab stipulates that a platelet count must be taken two to four hours after starting treatment. It is thought that antibodies are the cause of the thrombocytopenia[162]. When the medication is stopped, the thrombocytopenia usually goes away. Platelet transfusions can be administered if need[163].

TIROFIBAN

Tirofiban is a derivative of nonpeptide tyrosine that binds specifically to GpIIb-IIIa[164,165]. Tirofiban has a 1.5–2 hour plasma half-life, and its excretion by the kidneys and liver helps to remove it; urine and faeces include unaltered tirofiban[166]. Patients with renal insufficiency need to have their doses adjusted, whereas those with hepatic illness do not.

A limited number of people using tirofiban have been documented to experience severe but reversible thrombocytopenia. It is thought that tirofiban binding causes a conformational shift in GpIIb-IIIa, and antibodies are produced against the recently exposed epitope[167], so inducing thrombocytopenia. Re-administration of tirofiban is not known to be safe, yet individuals who experienced thrombocytopenia following tirofiban exposure had elevated antibody titers[168].

EPTIFIBATIDE

Eptifibatide is a synthetic cyclic heptapeptide connected by a disulfide bond. It exhibits a strong selectivity for GpIIb-IIIa[169,170] and is designed after the KGD sequence present in the snake venom disintegrin (barbourin) isolated from Sistrurus miliarius barbouri. It is possible that the inhibitory effects of eptifibatide were overestimated due to the collection of blood samples into citrate, even though preliminary reports suggest that eptifibatide produces less prolongation of the bleeding time than other GpIIb-IIIa inhibitors at doses producing comparable inhibition of platelet aggregation[171].

Patients with renal impairment show extended suppression of platelet function after taking eptifibatide because the medication is excreted by the kidneys. It's unclear how much eptifibatide is appropriate for people with mild to severe renal insufficiency. The steady-state eptifibatide level is around 1,900 ng/mL at an infusion rate of 2 μg/kg per min, indicating that more than 50 eptifibatide molecules bind to each GpIIb-IIIa. As such, platelet transfusion might not counteract the medication's effects[172,173].

Efficacy and Current Scenario of GP IIb/IIIa Drugs

In patients with ACS without persistent ST-segment elevation, randomised trials have evaluated the safety and effectiveness of tirofiban, lamifiban (a nonpeptide GpIIb-IIIa blocker, whose development has been discontinued), eptifibatide, and abciximab in addition to conventional antithrombotic therapy[174,175,176]. According to these studies, the relative risk decrease in MI or mortality at 30 days ranged from 0% to 27%. The FDA has approved eptifibatide and tirofiban for the treatment of ACS, including patients who will be treated medically and those who are having PCI. Nevertheless, abciximab for 24 hours (0.25 mg/kg bolus followed by a 0.125 mg/kg per min infusion) or 48 hours was not helpful as first-line medical therapy in patients with ACS, according to the Global Utilization of Strategies to Open Occluded Arteries (GUSTO) IV-ACS study[175]. A 9 percent reduction in the probabilities of mortality or MI at 30 days is suggested by a meta-analysis of all significant randomised clinical studies with GpIIb-IIIa antagonists in patients with ACS who were not usually scheduled to receive early coronary revascularization[177].

Therefore, the benefit/risk profile of currently available GpIIb-IIIa antagonists is very problematic for patients with ACS who are not routinely planned for early revascularization. On the other hand, a good strategy for high-risk PCI patients to reduce the risk of thrombotic issues related to the procedure is to increase the dose of antiplatelet therapy and add an IV GpIIb-IIIa blocker.

Phase 2 studies using eptifibatide and abciximab in acute MI revealed that GpIIb-IIIa blockage may be beneficial when used in addition to thrombolysis. The GUSTO V trial evaluated 16,588 patients in the first six hours of developing ST-segment elevation MI to assess the safety and effectiveness of half-dose Reteplase and full-dose abciximab against standard-dose Reteplase. The two therapy groups' 30-day mortality rate was comparable as the major end point (5.6 percent vs 5.9 percent). Combination treatment consistently reduced the incidence of MI's subsequent consequences, such as reinfarction, albeit this was somewhat offset by a rise in extracranial haemorrhages. After a year, there was no mortality advantage from combination therapy; hence, combined therapy appeared to have little to no net benefit[178].

An updated meta-analysis[181] has critically re-evaluated the advantages and risks of this approach in light of the failure of several more recent randomised trials to show benefits of GpIIb-IIIa blockade among patients with ST-segment elevation MI treated with primary angioplasty and in patients treated with clopidogrel[179,180].

CONCLUSION

To summarise, platelets play an essential role in preserving the integrity of blood vessels and preventing the formation of blood clots. In order to put a stop to the bleeding, they clump together with the assistance of a number of different substances and receptors. In contrast, platelet hyperactivity may result in difficulties, particularly in cases of coronary artery disease. Antiplatelet drugs such as aspirin, clopidogrel, and GP IIb/IIIa inhibitors are able to reduce the likelihood of thrombosis by interfering with one or more phases of platelet release and aggregation. The utilisation of these, on the other hand, increases the likelihood of bleeding. Platelet activity may be measured in a variety of ways, including flow cytometry, platelet aggregometry, and global thrombosis tests, to name just 3 of the many possible methods.

CONFLICT OF INTEREST: None

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