REVIEW

Should aspirin be replaced with ADP blockers and anti-GPVI to manage thrombosis?

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Abstract

Platelets have a pivotal role in maintaining cardiovascular homeostasis. They are kept docile by endothelial-derived mediators. Aberration in haemostatic balance predisposes an individual to an elevated risk of a prothrombotic environment. Anti-platelet therapy has been a key component to reduce this risk. However, understanding how these medications affect the balance between the activation and inhibition of platelets is critical. There is no evidence that a key anti-platelet therapy – aspirin, may not be the most efficacious medicine of choice, as it can compromise both platelet inhibition and activation pathways. In this review, the rationale of aspirin as an anti-thrombotic drug has been critically discussed. This review looks at how recently published trials are raising key questions about the efficacy and safety of aspirin in countering cardiovascular diseases. There is an increasing portfolio of evidence that identifies that although aspirin is a very cheap and accessible drug, it may be used in a manner that is not always beneficial to a patient, and a more nuanced and targeted use of aspirin may increase its clinical benefit and maximize patient response. The questions about the use of aspirin raise the potential for changes in its clinical use for dual anti-platelet therapy. This highlights the need to ensure that treatment is targeted in the most effective manner and that other anti-platelet therapies may well be more efficacious and beneficial for CVD patients in their standard and personalized approaches.

Keywords

- platelets
- endothelial dysfunction
- thrombosis
- aspirin
- clopidogrel
- prasugrel
- ticagrelor
- prostacyclin and thromboxane

Introduction

Cardiovascular disorders (CVD) have a significant impact on global health that results in CVD being the cause of 32% of worldwide mortality (1). It encompasses conditions that affect the heart and blood vessels of the body, such as coronary heart disease, cerebrovascular disease, peripheral arterial disease, congenital heart disease, arrhythmias, deep venous thrombosis and pulmonary embolism. Among them, coronary heart disease and cerebrovascular disease are a major cause of CVD mortality (1). A key component in the development of CVD is the platelet. The platelet is a blood cell that has an inherent propensity to respond to any damage to the blood vessel and to generate a thrombus that prevents bleeding. However, in patients suffering from CVD, these thrombi can become large, and block the blood vessel, potentially leading to events such as heart attacks and strokes (1).

Management of CVD disorders relies on the patient following a 'healthy lifestyle'. This involves eating a

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balanced diet while maintaining an age-appropriate weight, maintaining a physically active life and avoiding smoking or using any other tobacco-related product. It also encompasses managing the level of stress in life, maintaining adequate sleep, keeping a check on the levels of high and low-density cholesterols and maintaining tightly regulated blood sugar levels (2). It is estimated that maintaining a healthy lifestyle helps to prevent 80% of CVD cases (2).

The current pharmacological approach to manage CVD includes a list of anti-thrombotic medications that are either prescribed as a mono and/or combination therapy. The anti-platelets are the mainstay in the preventive management of CVD implications by preventing platelets from clumping into a clot, while anti-coagulants are effective in slowing down clot formation with appended active monitoring requirement (3).

This review will shed light on the current understanding of thrombus and platelet activity. It will then expand on the current use of a key anti-platelet therapy, aspirin along with the controversy that surrounds it. In addition, it will discuss additional anti-platelet therapies such as the P2Y1 and P2Y12 antagonists that are currently used and potential new therapies under consideration. Finally, the review will consider the need for a more personalized approach to CVD medications.

Functions of 'endothelium' and 'platelet' in the vascular system

Critical to a well-functioning cardiovascular system are two key players – an intact healthy vascular endothelial layer and healthy platelets. The vascular endothelium is a monolayer of cells that lines the vascular luminal surface, preventing the exposure of the thrombogenic collagen that forms the extracellular matrix. Importantly, the endothelial layer produces multiple compounds which help to maintain haemostasis. This is possible, as platelets are found circulating in the blood in close proximity to the endothelial lining and therefore can come into contact with these compounds, such as nitric oxide (NO) and prostacyclin (PGI_2) that help to inhibit their function (4, 5). PGI_2 inhibits platelet function by binding to the IP receptor and activating adenylyl cyclase, while NO diffuses into the platelet and activates soluble guanylyl cyclase. This causes an increase in either the intracellular cAMP or cGMP levels, respectively. These cyclic nucleotides negatively regulate platelet activation by modulating cytoskeletal reorganization, inhibiting calcium release, restricting degranulation and limiting expression of platelet receptors required for further activation and thrombus propagation.

In addition to PGI_2 and NO, there are a raft of other compounds which are aimed to maintain haemostasis and prevent unwanted thrombus formation (Fig. 1). On the surface of the vascular endothelium is a glycocalyx that is designed to keep platelets and other cellular elements at bay to avoid getting unwanted activation by prothrombotic collagen of the sub-endothelial matrix (6, 7). In addition, the endothelial cells contain tissue factor and von Willebrand factor (vWF) in vesicles beneath the cell membrane which are only expressed upon vascular damage. Finally, vascular endothelial cells produce tissue plasminogen activator (tPA) that triggers fibrinolysis by enabling dissolution of unwanted clots and also expresses on their luminal surface the thrombin–thrombomodulin complex (8). This complex activates protein C in the circulation, which, in the presence of protein S, inactivates the coagulation factors V and VIII, thereby limiting the activation of the coagulation cascade (9). Therefore, the endothelial layer plays a key role in both maintaining inactivated platelets and preventing activation of the

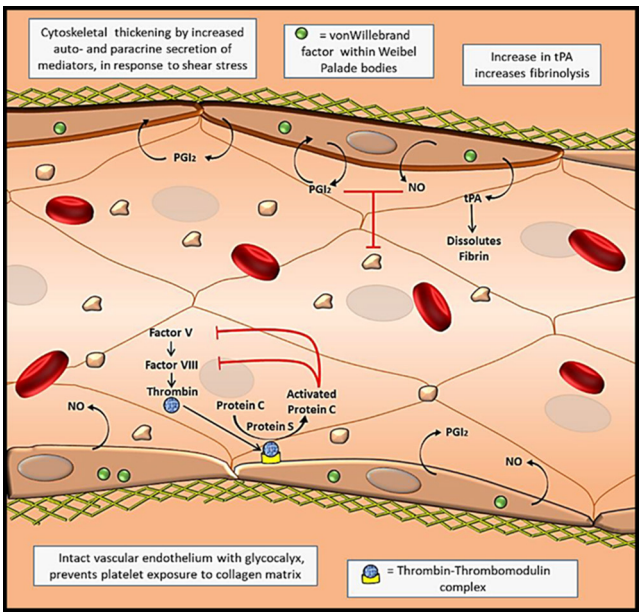

Figure 1
Endothelial and platelet perspective to haemostasis. The diagram shows the endothelial and platelet factors engaged in maintaining haemostasis. The intact endothelial lining with an overlying glycocalyx (not shown) deters direct interaction of platelets to pro-coagulant sub-endothelial matrix. The endothelium stores the tissue factor and von Willebrand factor; which on release, promotes formation of a clot. The intact endothelium activates thrombin and adhering-circulating platelets to exposed matrix, respectively. Endothelial mediators, such as NO and PGI_2, act as vessel vasodilators and platelet inhibitors and thickens the sub-endothelial cytoskeleton.

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coagulation system. Damage to the vascular endothelial layer removes these inhibitory systems, reveals the prothrombotic extracellular matrix proteins and so drives thrombus formation.

Thrombus architecture

There are several mechanisms by which platelets are maintained in their quiescent state. Damage to the vascular endothelium or exposure to agonists overcomes the inhibitory signal and platelet activation ensues. Platelets respond to the multitude of receptors and several signalling pathways that enable them to respond to these activatory stimuli is illustrated in Fig. 2.

The platelets tether by binding to endothelial-released vWF that is immobilized on collagen, via GPIb-IX-V, that results in platelet rolling and promotes glycoprotein VI (GPVI) interaction with collagen. Increased platelet activity promotes integrin expression on the platelet surface and their engagement with fibrinogen and vWF. To reinforce this interaction, the platelet actin cytoskeleton is reorganized, and this simultaneously facilitates the release of platelet granule contents (ADP, thromboxane (TxA_2) and fibrinogen). These released mediators potentiate further platelet activation and hence provide additional stability in the formation of the clot.

During thrombus formation, some platelets generate phosphatidylserine (PS) on their surface and engage the coagulation system for the generation of thrombin-led fibrin meshwork. Changes in this meshwork is a critical factor to ensure that a clot can effectively form and resist the shear forces of the flowing blood (10).

Research has identified a distinct hierarchical architecture within the thrombus with graded platelet activation, shown in Fig. 2 (11). This structural organization of the thrombus has a distinct central core. As you move away from the core of the thrombus, there is a transition zone and an outer shell (11). Platelets forming the core of the clot are under direct influence of thrombin which is marked by their greater activity and increased packing density, which prevents easy access of compounds into the thrombus core. These platelets fully secrete their granules and so help activate platelets within the periphery. However, the platelets in the periphery (the shell region) are less affected by thrombin and so rely more on the action of ADP and TxA_2 to mediate their activation. This means they are less activated, and they achieve reduced packing density, thereby enabling this area of the thrombus to be leakier. This is important as PGI_2 and NO can therefore access the thrombus shell region but cannot effectively access the thrombus core. This makes the shell region more susceptible to the reversal of platelet activation by PGI_2 and NO, inducing thrombus instability, leading to embolization and preventing excessive thrombus growth (12, 13). Importantly in the presence of a prothrombotic environment, especially one with oxidized LDL (oxLDL), thrombus formation is excessive as the oxLDL helps to prevent the inhibition mediated by PGI_2 and NO, and therefore, the thrombus can grow more effectively (14, 15). The balance between the activatory and inhibitory signalling is key to the establishment of the core and shell regions of the thrombus and as such, ensuring that this balance is maintained effectively is key to attaining a graded thrombus organization (11).

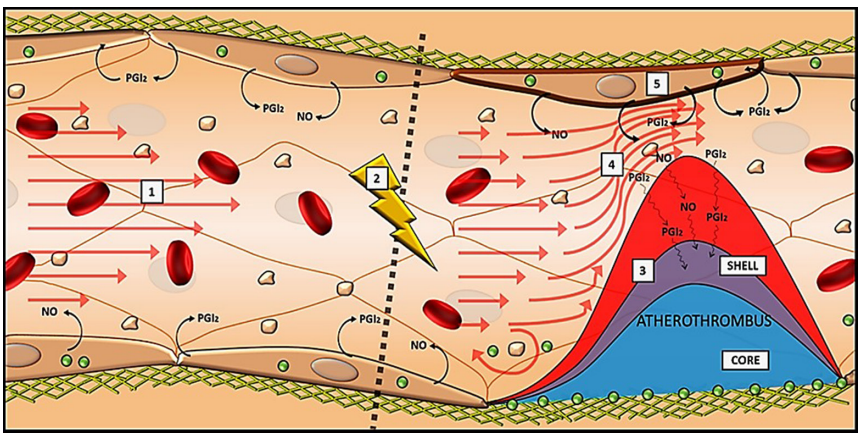

Figure 2
Schematic of thrombus architecture and blood flow dynamics: Blood flows in a (1) laminar approach during normal circulation. (2) Endothelial damage or accumulation of prothrombotic factors predisposes an individual to atheroma or thrombus formation via platelet activation. This damage exposes the underlying matrix along with the release of vWF from the endothelium to bind platelets. Activation of further platelets results in the formation of (3) clot with a graded architecture having a defined core (blue) and a shell (mauve). Increase in the size of the clot shifts the blood flow to (4) non-linear dynamics and increases shear on the endothelial lining. The endothelium responds by generating greater amounts of mediators such as PGI_2. The produced (5) PGI_2 responds by inhibiting platelet activation and thickening the endothelial cytoskeletal framework; providing a reduction in the size of the thrombus and endothelial capacity to withstand shear force of the flowing blood.

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Aspirin – a key anti-platelet therapy

As the platelet plays such a key role in CVD, effective targeting of the platelet can therefore have a profound effect on thrombus formation within CVD patients. Historically, a drug therapy commonly prescribed for preventive and emergency protocols for managing CVDs is aspirin.

Aspirin is classified by World Health Organization (WHO) as 'one of the essential drugs, inevitable for any basic health system' (16). Importantly, the WHO categorized low- and middle-income countries as having greater than three-quarters of all global CVD mortalities (1). As such, aspirin plays a key role in these low- and middle-income countries to treat CVD, especially as these countries may lack the economic capability to prescribe more expensive therapies for CVD.

Impact of aspirin on platelets and endothelium

Aspirin is an irreversible inhibitor of the cyclooxygenase (COX) enzyme. Physiologically, the COX enzyme converts arachidonic acid to produce prostaglandin H_2 (PGH_2). The PGH_2 is then processed in a cell-specific manner with platelets converting it for the production of TxA_2, while endothelial cells produce PGI_2. Interestingly, both prostanoids affect platelets in an opposing manner, TxA_2 activates platelets, whereas PGI_2 inhibits them.

The fact that aspirin causes irreversible inhibition of the COX enzyme is key to its clinical use. Platelets on treatment with aspirin are not able to produce new COX enzyme, as they lack the genetic machinery to do so, and so will no longer be able to produce TxA_2. In contrast, although aspirin will also inhibit the COX enzyme in the vascular endothelial cells, these cells have the relevant genetic machinery, to replace the inhibited COX enzyme. Therefore, this helps aspirin's effect to be permanent on the platelet but transient on endothelial cell (17). This permanent inhibition of TxA_2 reduces the ability of platelet to activate and form a thrombus.

This reduction in PGI_2 and TxA_2 production is critical to fully understand the effect of aspirin on platelets and thrombus formation. Importantly, it needs to be remembered that the inhibition mediated on platelet by PGI_2 and NO is highly effective as it works in a synergistic manner. Therefore, the action of aspirin in reducing PGI_2 levels could be more profound due to the reduction in the synergistic inhibition of platelet by PGI_2 and NO. Interestingly, Taubert *et al.* indicated an increase in the circulating levels of NO after aspirin use. This may in part compensate for the reduction in PGI_2 levels and so identify why aspirin is anti-thrombotic (18).

Aspirin has also been identified to reduce the inflammation associated with thrombus formation. This is attributed to the change in TxA_2 production which in turn reduces the effect of sphingosine-1 phosphate (S1P) (19). S1P, a pro-inflammatory lipid molecule, is produced and secreted by endothelial cells and platelets upon their activation (20). Research identified that aspirin by reducing TxA_2 synthesis helps to protect the endothelial integrity by inhibiting S1P-mediated inflammation (21). In addition, aspirin-inhibited COX redirects the arachidonic acid substrate to the lipoxygenase pathway. This relays the production of 'aspirin-triggered lipoxins' (ATL) and 'resolvins' (ATRv) that help to counter the inflammatory profile via reducing the production of IL1β, IL6, IL8, IL17, TNF-α and reactive oxygen species (ROS) (22, 23). The reduction in ROS production by aspirin also reduces the NLRP3 inflammasome-induced damage to endothelial gap junctions and thereby restores normal endothelial permeability (24).

In a healthy endothelium, there is a cytoskeletal lattice present beneath the endothelial cell membrane. This actin cytoskeleton helps to stabilize the vascular lining and is continuously influenced by PGI_2 and NO (25, 26). With an increase in the shear of the flowing blood that can occur within CVD patients, pressure is exerted on the endothelium, thereby activating COX enzymes and eNOS (endothelial nitric oxide synthase) to produce PGI_2 and NO, respectively (27). These mediators synergize to reinforce the actin cortical lattice in endothelial cells to withstand the shearing force of blood flow (Figs. 1 and 2) (28). Therefore, aspirin, by inhibiting PGI_2 production, would also challenge the endothelial protection by limiting the cytoskeletal thickening, thereby rendering the endothelium less able to withstand high shear. With the passage of increased flow of blood, aspirin-induced lack of adaptive actin reinforcement would result in damaging the endothelium (29). Apart from possible denudation, the endothelial damage could result in the release of tissue factor that converts prothrombin into thrombin – a strong platelet activator, or expulsion of vWF from the endothelial stores of Weibel Palade bodies that enable circulating platelets to roll and spread on the collagen matrix to initiate clot formation (30, 31). Furthermore, a lack of endothelial integrity decreases the inhibitory influence of the thrombin–thrombomodulin complex and the associated protein C and protein S along with reduced production of tPA; thereby predisposing for further platelet activation and thrombus formation.

Therefore, understanding how aspirin affects the production of TxA_2 vs PGI_2 is critical to understanding if

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it could have an anti-thrombotic or prothrombotic effect. Depending on the extent to which these mediators traverse the thrombus and the impact they produce on the activity of platelets would then feed in to control the height, graded structure and the extent of thrombus (11).

Complexities of using aspirin

A key part of understanding the potential therapeutic benefit of aspirin is the dose at which it is used. It can be used both in a low- and high-dose form, although preventive cardiology recommends the usage of 75 mg/day of aspirin, which is akin to low-dose aspirin therapy (32). High-dose aspirin has been used to relieve pain, temperature and swelling along with enhanced inhibition of TxA_2 production (33). However, it also led to a significant reduction in PGI_2 production from the vascular endothelial cells (34). This led the regulatory authorities to shift to using low-dose aspirin as it generated a comparable anti-platelet effect to that produced at higher doses of aspirin (32). The low-dose aspirin was also believed not to impact the level of the anti-thrombotic compound – PGI_2 (35). However, later published research showed that even low doses of aspirin also impact both TxA_2 and PGI_2, with a greater inhibiting influence on TxA_2, which then underpins why low-dose aspirin is thought to have an anti-platelet effect (36). Importantly, TxA_2 inhibition should at least be 95% for aspirin to be effective as the remaining COX could compensate to produce TxA_2 and reclaim the patient prothrombotic profile. This highlights the need for individual corrected dosing for patients while considering the factors of aspirin resistance along with patient compliance.

However, recently, the use of aspirin as an anti-thrombotic has been shown to have mixed effects. The anti-thrombotic trial (ATT) identified that the use of aspirin was not beneficial for all patients and in fact may induce a prothrombotic phenotype (37). Similarly, a meta-analysis by Guirguis-Blake *et al.* stressed a negligible and ineffective response to aspirin use in reducing the risk of cardiovascular mortality (38). These claims were strengthened by major clinical trials – Aspirin to Reduce Risk of Initial Vascular Events (ARRIVE), A Study of Cardiovascular Events in Diabetes (ASCEND) and Aspirin in Reducing Events in the Elderly (ASPREE) that probed the effectiveness of aspirin use in CVD (39, 40, 41).

The ARRIVE trial enrolled moderate-risk CVD patients and identified aspirin to increase bleeding while there was no change in major adverse cardiovascular events (MACE) (40). Similarly, ASPREE, a study conducted on patients aged 70 years old or more, also showed no change in MACE, but there was an increase in bleeding tendency and mortality (39). The ASCEND trial was conducted on diabetic patients and showed a reduction in MACE which led to higher bleeding rates (41). Their data identified that prescribing aspirin to different patient groups could induce varying thrombotic responses and challenged its reliance during emergency, primary and secondary prevention for CVD conditions.

Importantly, the age of a patient and the combined risk of developing CVD have critical significance. The current recommendation for primary prevention has a limited age range for aspirin prescription; targeted patients should be aged 40–59 years and have a high 10- year risk of CVD development (42). As for secondary prevention and emergency measures of CVD, aspirin still is considered effective, but the increased bleeding risk lingers (43).

All these factors, along with aspirin resistance, highlight the need to develop a nuanced and personalized approach to anti-platelet therapy that should have greater effectiveness and capability of dealing with different patient groups (44). With revised regulations by the United States Preventive Service Task Force, the previously allowed age group of 60–69 years have now been excluded from starting aspirin (45). The stricter guidelines necessitate the caution that needs to be advised by weighing the ratio of benefits to harm, in accordance with the dose of aspirin to be used.

Alternative anti-platelet medication avenues

Finding an effective medication to manage thrombotic conditions is the key to lessen the reliance on aspirin for an anti-platelet regimen. Due to use of long-term aspirin monotherapy under question, additional therapies were sought and researched for their potential to work either as monotherapy or as combinations in dual anti-platelet therapy (DAPT).

The 2016 ACC/AHA guidelines for DAPT use comprised low-dose aspirin with $P2Y_{12}$ antagonists. The combination lasted for 12 months and was followed indefinitely by aspirin monotherapy (46). Although, DAPT showed better clinical outcomes and continued use of aspirin even at low doses was questionable due to the bleeding and endothelial cell dysfunction tendency. This potentially inclined the patient to an unknown level of thrombotic risk (47). This prompted ADP blockers and anti-GPVI to be included in the DAPT and reduce the thrombotic risk.

The ADP binds to the receptors; $P2Y_1$ and $P2Y_{12}$, with a significant contribution from $P2Y_{12}$. They potentiate platelet activation via engaging downstream Ca^{2+}

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mobilization and granule secretion along with inhibiting the PGI_2–cAMP axis (Fig. 3) (48). Interestingly, recent DAPT guidelines have been proposed where a month of DAPT (aspirin and clopidogrel) is followed by a 12-month clopidogrel monotherapy for ACS patients (49). This shift in guidelines highlights the potential need and strengthens the case for finding improved therapies.

The search for improved ADP receptor blockers includes prasugrel that, similar to clopidogrel, requires prior activation by liver, while ticagrelor has a quicker mode of action, as it does not need activation. The half-life of clopidogrel and prasugrel lasts the lifespan of the platelet (10–14 days); therefore, they act as irreversible inhibitors, while ticagrelor binds for 3–5 days and is therefore classified as a reversible inhibitor (50). Ticagrelor reaches a maximum plasma concentration in 120 min due to its lower bioavailability, while clopidogrel requires 60 min and prasugrel 30 min only (50). Considering both prior activation and attainment of maximum plasma concentration, ticagrelor and prasugrel are comparable to achieve maximum platelet inhibition at 2 and 3 h, respectively, while clopidogrel necessitates an 8 h interval to achieve maximal impact on platelets (50).

Johnston *et al* published a clopidogrel and aspirin comparison with 75 mg of clopidogrel daily to significantly lower annual rate of vascular death, myocardial infarction or ischaemic stroke (51). The delayed onset of clopidogrel along with the concept of clopidogrel resistance however caused an increase in the risk of post-PCI thrombus development (52). This identified the need to compare ticagrelor, a quicker-action ADP receptor blocker, with aspirin in high-risk patients with ACS in the TWILIGHT trial. It identified that ticagrelor alone or in combination with aspirin reduced bleeding tendency along with no higher risk of myocardial infarction or stroke (53). Contrastingly, the PRINCE trial reported similar rates of overall major bleeding along with significant reduction of cardiovascular and all-cause mortality comparing a combination of aspirin and ticagrelor vs aspirin and clopidogrel (54). These varied evidence for major bleeds (as classified by TIMI criteria) highlighted a potential variation in response to ticagrelor which was dependent on the patient cohort.

Ticagrelor and prasugrel have shown better outcomes by comparing them with clopidogrel or aspirin. They have comparable efficacies and safety profiles, with prasugrel having a slight increase in bleeding tendency (55). Ticagrelor, on the other hand, had mild dyspnoea and ventricular pauses as identified in the DISPERSE, DISPERSE-2 and the ONSET/OFFSET trials comparing ticagrelor with clopidogrel in patients with coronary artery disease (56).

Supplementary to the recognized effects of ticagrelor to cause direct platelet inhibition, it also resulted in adenosine uptake inhibition by cells that led to an increased plasma adenosine concentration that prompted further inhibition of platelets (57). In addition, ticagrelor caused an increased production of NO and PGI_2 that promote a healthy endothelium (58). Although with potential clinical variation, the ability of ticagrelor as an anti-platelet does place it a notch above other ADP receptor blockers in the market.

Continuing research on ADP $P2Y_{12}$ receptor blockers have developed their anti-thrombotic impact. Ticagrelor and newer medications in the same drug class have been developed, such as cangrelor, with improved potency and effectiveness for new patients after PCI (59). Similarly, Vicagrel – an analogue of clopidogrel, with far greater efficacy, is under clinical development (60). Selatogrel,

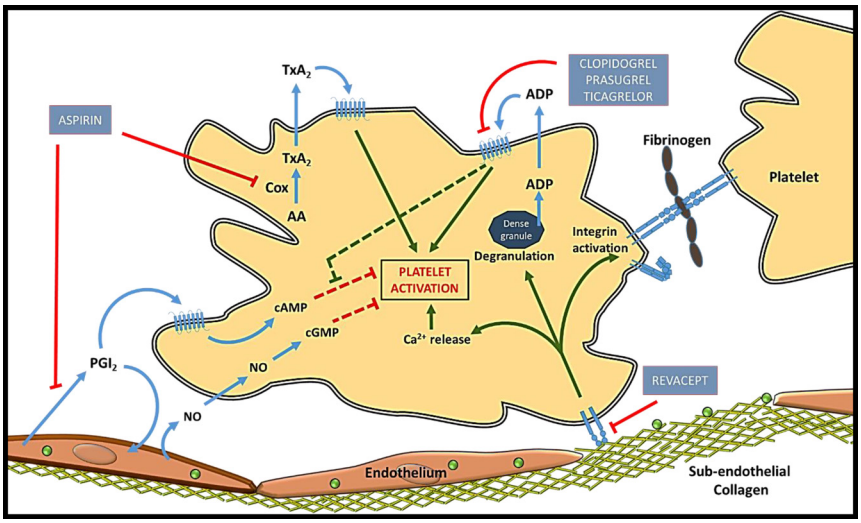

Figure 3
Platelet therapeutic modulations. The figure illustrates the targets of aspirin, ADP receptor blockers and GPVI receptor blockers along with the downstream impact on platelet activation. In the platelet, aspirin inhibits Cox enzyme to prevent the production of TxA_2, a platelet activator. Simultaneously, aspirin also hinders endothelial PGI_2 production that relieves the inhibitory impact on platelets and prevents the endothelial cytoskeletal thickening thereby exposing the endothelium to high pressure of flowing blood that leads to denudation and dysfunction. The ADP receptor blockers (e.g. clopidogrel, prasugrel and ticagrelor) and GPVI receptor blocker (e.g. revacept) help to modulate the platelet activity while sparing the endothelium.

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another $P2Y_{12}$ receptor blocker, is under development, with s.c. administration and less off-target effects (61). Selatogrel is identified to be effective for managing acute cardiovascular events due to its rapid action along with reduced bleeding risk (61).

In addition to the ADP receptors as potential targets for anti-platelet therapy, the collagen receptor – GPVI, is also emerging as a potential anti-platelet target to prevent thrombosis and stroke (Fig. 3). GPVI has been shown to bind to various extracellular matrix proteins, such as fibrinogen, fibrin, laminin, fibronectin and collagen (62, 63). GPVI has an important role in the high-shear environment of arterial thrombosis, although there is increasing evidence that GPVI may also play a role in venous thrombus formation (64). Furthermore, GPVI has also been reported recently to be overexpressed in stroke patients (65). However, GPVI deficiency had a minimal impact on bleeding as it did not affect haemostasis (64), potentially as its role can be compensated by vWF or thrombin. This therefore means that by targeting GPVI, you can effectively reduce arterial thrombosis while potentially reducing the bleeding diathesis associated with other anti-platelet therapies. However, a combination of GPVI Fc antibodies, which block GPVI signalling, alongside aspirin or $P2Y_{12}$ antagonists could be beneficial, as this prevents atherosclerotic plaque-mediated thrombus formation without elevating unwanted bleeding (66).

Multiple anti-GPVI approaches have been considered that either inhibit the GPVI receptor or downregulate GPVI surface expression such as that observed in mice with antibodies – JAQ1 and/or activate GPVI cleavage enzymes (67, 68, 69). Table 1 expands on the anti-GPVI compounds of which revacept, glenzocimab and DZ-697b have proceeded to clinical trials (Phase I or II).

Glenzocimab (ACT017) is a humanized antibody fragment of the O912 antibody used to target mouse GPVI. It is a selective and reversible inhibitor of the GPVI receptor. ACT017 completed a phase 1 placebo-controlled study in 2019 and showed a favourable safety profile (69). It has since proceeded into phase II and III trials – ACTIMIS and ACTISAVE, respectively, which are being carried out on patients with acute ischaemic stroke (69, 70).

Revacept is a GPVI-Fc fusion protein that lacks downstream signalling. It competes with GPVI present on platelets, for the exposed collagen-binding sites in damaged vascular endothelium. By blocking the ability of the GPVI expressed on the surface of the platelet to bind collagen, it can then reduce platelet adhesion and aggregation (71, 72). Unfortunately, the addition of revacept did not reduce myocardial injury in low-risk PCI patient already on DAPT in the phase II ISAR_PLASTER trial for improving the anti-thrombotic risk (66, 72, 73, 74). However, there is potential that as only low-risk patients were used in this trial, revacept may have clinical relevance in higher-risk groups. However, it is also being evaluated within patients who have had a transient ischaemic attack or a stroke due to carotid artery stenosis. It will be interesting to see how revacept affects this patient cohort given the lack of effect within the PCI patient group.

A distinct downside with both revacept and glenzocimab is that as antibody therapies, the drug delivery mechanism requires i.v. injection. This will possibly limit the compliance as many patients take anti-platelet therapy outside a clinical environment. Therefore,

Table 1 List of anti-GPVI approaches. The table enlists currently researched antibodies and small molecule inhibitors that target the interaction of GPVI and collagen that climaxes in platelet activation.

Compound	Strategy	References
Antibodies		
Glenzocimab (ACT-017)	Binds to GPVI active site on platelets and reversibly compete with collagen interaction	(69, 70)
Revacept	Inhibits GPVI receptor downstream signalling	(66, 71, 72, 73, 74)
JAQ1	Downregulates GPVI expression in mice	(67)
Small molecule inhibitors		
DZ-697b	A novel compound that inhibits interactions amongst GPVI + collagen and GPIbα + fibrinogen	(75)
Losartan	Selectively inhibit collagen-mediated platelet activation by binding to GPVI and blocking downstream signalling	(76)
Artesunate	Selectively inhibit collagen-mediated platelet activation by binding to GPVI and blocking downstream signalling	(77)
Mutalysin-II	An enzyme that cleaves GPVI and GPIbα.	(78)

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the development of a GPVI oral inhibitor would be of great benefit.

DZ-697b is an oral antagonist of GPVI that inhibits Fc g chain phosphorylation by collagen. Usefully, it is not a prodrug and therefore does not require metabolism to its active ingredient. Phase I trials of the compound identified its potential as an anti-platelet therapy as it showed a reduced risk of bleeding in comparison to clopidogrel and aspirin (75, 79). However, the compound has not progressed into phase II trials at present.

Mutalysin II is a snake venom that cleaves both GPVI and the vWF receptor GP1b. As it cleaves GPVI, it therefore blocks associated signalling and reduces GPVI-mediated platelet aggregation (78). It is proposed that it has good potential as an anti-platelet but it needs further investigation to see if it can progress into clinical trials.

Although current anti-platelet therapies have shown their ability to manage CVD effectively, an increased bleeding tendency persists as a common side effect. International guidelines have thereby recommended careful evaluation of bleeding risks along with assessing benefit to harm ratio for continuing with the medicament. Moreover, consideration needs to be drawn to other patient factors such as anaemia, low body weight and chronic kidney disease that impart an increased risk of major bleeding, as had been appreciated in TICO randomized trial (80).

Personalised anti-platelet therapy

The idea of personalized anti-platelet therapy is potentially highly useful in CVD patients. It is becoming clearer that there are patients resistant to different anti-platelet therapies, for several different reasons (age, genetic polymorphisms, BMI). Therefore, there is a need to target therapy more effectively to prevent unwanted bleeding, ineffective therapeutic responses and maximize cost-effectiveness of anti-platelet therapy.

There are several different genes (*CYP2C8*, *CYP2C9*, *CYP2C18*, *CYP2C19*2*, *CYP2C19*3*, *CYP2C19*17*, *SLCO1B1*, *UGT2B7* and *CYP3A4*) that have been associated with ineffective patient responses to aspirin, clopidogrel, prasugrel and ticagrelor (81, 82, 83). These therefore have the potential to be used to help screen patients to aid personalized therapy. This notion is under consideration, as the need to assess the extent of anti-platelet impact of these medications is of prime concern. Their sensitivity and specificity are still being screened for worldwide usability, and secondly, cost implications too would pose a major hurdle as lower-middle-income countries are homing the major brunt of the disease.

Of the identified genes, CYP polymorphisms have been the most investigated. Loss-of-function CYP450 polymorphisms, such as CYP2C19, are important especially as clopidogrel is a prodrug and therefore requires metabolism in the liver to produce the active metabolite. However, although there is little dispute over the ability of clopidogrel to induce high platelet reactivity in a subset of patients, the reasons for this are still in dispute. The FDA has indicated that patients that metabolize clopidogrel poorly should preferably be treated with other anti-platelet therapies (84). However, trials that have investigated the use of a personalized therapy based on CYP450 polymorphism have had variable outcomes. The most recent trial (TAILOR-PCI) was unclear on how personalized therapy would benefit CVD patients (85). There were indications the study was underpowered, but overall, it indicated that there was little benefit to a personalized therapy approach based upon a pharmacogenetics approach to CYP450 loss-of-function mutations. However, interestingly, further analysis of this trial dataset has indicated that using the ABCD-GENE score could prove to be beneficial in separating different patient groups (86).

The conceptual need for a personalized medication schedule stands to reason. Further research is starting to identify subsets of patients that could carry further genetic polymorphisms or are part of specific patient subsets that would potentially benefit from this personalized approach (87). Supplementary investigations are required to fully understand the benefit of a personalized therapeutic approach to anti-platelet therapy.

Conclusion

Aspirin is a drug which all can effectively access. However, although aspirin continues to be used in the clinic as a key therapy for managing cardiovascular and thrombotic pathologies, its use is now being challenged. There are questions about aspirin suitability for all age groups; the most beneficial dose to be used; potential issues around how aspirin works within different patient cohorts and the benefits of long-term aspirin. This review identifies the potential benefits of newer therapies, such as ticagrelor, prasugrel and anti-GPVI drugs. Furthermore, there is a need for a more nuanced, personalized approach to anti-platelet therapy that can effectively balance the bleeding and anti-thrombotic risk of these therapies.

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Aspirin along with other anti-platelet medications needs careful consideration and individual assessment for identifying the therapeutic advantage in dealing with thrombotic risk. This highlights that it would be beneficial to weigh up the merits of the available medications and to ensure a targeted personalized treatment in the most effective manner, whether to be used as monotherapy or as DAPT or to be replaced by other anti-platelet medication combinations while addressing emergency thrombotic situations or managing preventive CVD outcomes.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement

HK and MZY conceived the review and made figures. HK, TG, NA, SC and MZY have made substantial contribution to draft the write-up. SC, HK and MRB extended the write-up and helped revise it. All authors have read and approved the final manuscript.

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