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Anti-platelet therapy: ADP receptor antagonists

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The $P2Y_{12}$ receptor on platelets with which ADP interacts has an important role in promoting platelet function and thereby platelet involvement in both haemostasis and thrombosis. Agents that act as antagonists at this receptor are thus likely to provide effective antithrombotic therapy, provided that there are no adverse effects on haemostasis. Here we describe the ADP receptor antagonists that are available and in development. We also consider their mode of action and ask whether there are additional mechanisms through which they exert their inhibitory effects on platelet function.

Introduction

Adenosine diphosphate (ADP) interacts with two different purinergic receptors on platelets, known as $P2Y_1$ and $P2Y_{12}$ (Figure 1). These are two receptors of the seven transmembrane (7TM) class of receptors [1]. Interaction with $P2Y_1$ receptors initiates the platelet response while interaction with $P2Y_{12}$ receptors promotes the response. Blockade of the effects of ADP at either of these receptors results in a marked reduction in the overall effect of ADP on platelet function [2, 3].

The initial response to ADP is a change in the shape of the platelet whereby disc shaped cells convert into a spherical form from which pseudopodia emerge. This change, which is mediated by the $P2Y_1$ receptor, involves Ca^{2+} influx, intracellular Ca^{2+} mobilization and actin polymerization. Interaction of ADP with the $P2Y_{12}$ receptor results in inhibition of adenylate cyclase, which is accompanied by platelet aggregation [4]. The latter is mediated by a change in conformation of glycoprotein complexes on the platelet surface known as GPIIb/IIIa complexes, such that they become receptors for fibrinogen (Figure 1). Fibrinogen is a bivalent molecule present in high concentrations in blood plasma. Once bound to some of the 50 000–100 000 GPIIb/IIIa complexes on a single platelet, fibrinogen links the GPIIb/IIIa on adjacent platelets together. The result is that individual single platelets are transformed into a large mass in which many hundreds of thousands of platelets have aggregated together [5, 6].

ADP appears along with adenosine triphosphate (ATP) in the circulation consequent to cell and tissue damage. ADP can also be produced from ATP by the action of CD39 on endothelium and white cells (Figure 1) [7, 8]. ATP itself can also promote platelet function under certain conditions through interaction with another purinergic receptor, the $P2X_1$ receptor [9]. Other important agents that result in platelet activation and platelet aggregation are collagen and thrombin. In addition to causing platelet shape change and aggregation, collagen and thrombin promote secretion by platelets of the contents of storage granules which include ADP and ATP. The ADP, acting via $P2Y_1$ and $P2Y_{12}$ receptors on the platelet surface amplifies the effects of the primary stimulus provided by the collagen or thrombin [10].

Other platelet functional responses that are amplified by released ADP include synthesis of thromboxane A_2 (TXA_2), which also promotes platelet aggregation acting via TP receptors (Figure 1) [11]. ADP also promotes secretion of other materials from intracellular granules including P-selectin which is the prime mediator of platelet interactions with leucocytes [12]. In addition, platelet

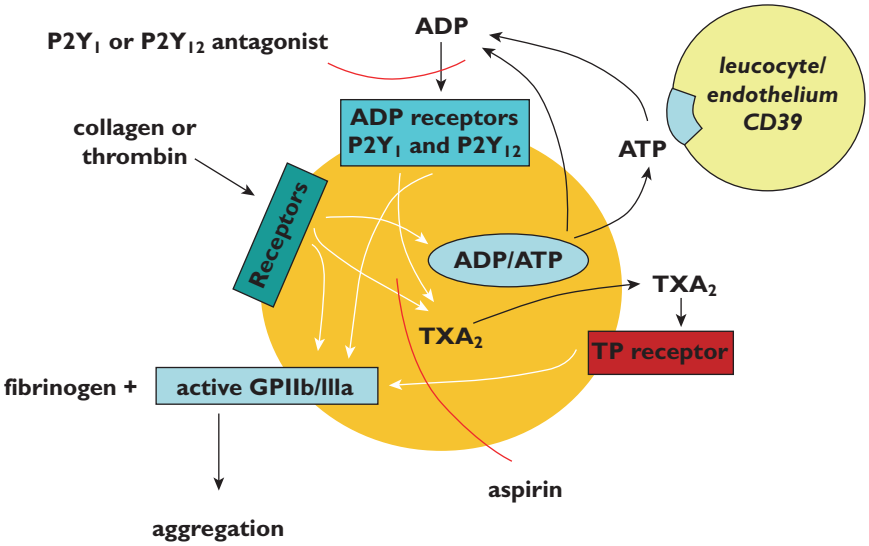

Figure 1

Some of the receptors and pathways involved in platelet aggregation. The points at which $P2Y_1$ or $P2Y_{12}$ antagonists and aspirin inhibit platelet aggregation are shown

microparticles are formed which provide procoagulant surfaces that accelerate the coagulation cascade [13]. Collectively all these mechanisms contribute to haemostasis, thrombosis and inflammation.

ADP is thus an important contributor to platelet function through the $P2Y_1$ and $P2Y_{12}$ receptors. Agents that reduce the effects of ADP at either of these could provide effective antithrombotic therapy.

$P2Y_1$ antagonists

Studies in $P2Y_1$ knockout mice have shown that absence of the $P2Y_1$ receptor leads to impaired platelet aggregation in response to ADP and resistance to thromboembolism [14, 15]. A number of pharmacological agents have been identified that act as platelet $P2Y_1$ antagonists [16–21]. These have been investigated experimentally and clear inhibition of ADP-induced platelet function has been shown. However, given the ubiquitous expression of $P2Y_1$ receptors in other tissues of the body, the clinical potential for the $P2Y_1$ receptor to be used as an antithrombotic target is questionable [22]. As far as we are aware, there have been no clinical studies evaluating the use of $P2Y_1$ antagonists as antithrombotic agents in human subjects. Consequently this review will focus on $P2Y_{12}$ receptor antagonists.

$P2Y_{12}$ antagonists

There are several agents that interact with the $P2Y_{12}$ receptor on platelets, thereby reducing platelet function. Some are already in use as antithrombotic agents and others are in development. The drugs we will discuss here are clopidogrel, prasugrel, ticagrelor, cangrelor and elinogrel. These drugs are routinely used in conjunction with aspirin which inhibits TXA_2 synthesis in platelets and thus provides an additional means of reducing platelet function.

Clopidogrel

The $P2Y_{12}$ antagonist that is currently most widely used as an antithrombotic agent is clopidogrel. It is recommended in current clinical guidelines for prevention of further thrombotic events following acute coronary syndrome and ischaemic stroke [23]. Clopidogrel is a thienopyridine and succeeded another thienopyridine, ticlopidine, which has been withdrawn gradually from clinical use due to a high incidence of haematological side effects including thrombotic thrombocytopenic purpura, neutropenia and aplastic anaemia [24].

Clopidogrel is a prodrug and its effects on platelets following oral administration derive from generation *in vivo* of an active metabolite [25]. This binds to $P2Y_{12}$ receptors irreversibly, rendering the receptor unable to respond to ADP, thus reducing platelet function. The irreversible effect at the $P2Y_{12}$ receptor is consequent to covalent binding to cysteine sulphydryl residues within the receptor. Its effect on platelet function lasts for the lifetime of the affected platelet, which is between 7 and 10 days.

Although it is clear that clopidogrel provides significant protection against thrombotic episodes when administered to patients at risk of thrombosis (e.g. the CAPRIE study [26]) and especially when used in combination with aspirin (e.g. the CURE study [27]) the drug does have some

drawbacks. Its onset of action as an inhibitor of platelet function is rather slow following initiation of standard therapy, taking several days for a full effect to be realized, and also its inhibitory effects on platelet function are variable in different people with some patients continuing to have high platelet reactivity despite treatment.

Multiple factors may contribute to this high on-clopidogrel platelet reactivity. Genetic factors include polymorphisms of the *ABCB1* gene affecting intestinal absorption [28], and polymorphisms of hepatic cytochrome P450 enzymes including CYP2C9 and CYP2C19 that are involved in the generation of the clopidogrel active metabolite [29]. Recently, variation in another enzyme, paraoxonase-1, has been shown to affect clopidogrel metabolism [30]. Other factors that could affect clopidogrel efficacy include body mass index, gender, ethnicity, and comorbidities such as liver disease and insulin resistance. A number of drug–drug interactions have also been implicated [29]. As well as inter-individual variability, it has recently been shown that there is also large intra-individual variability in the response to clopidogrel during long term therapy [31].

High on-clopidogrel platelet reactivity has been shown to be an important predictor of adverse thrombotic outcomes [32]. Hence it is important that this issue is adequately addressed in clinical practice. Unfortunately, despite some genetic associations, it is not easy to predict how an individual will respond to clopidogrel due to the multiple factors involved. Platelet function testing methods are now becoming available that enable the degree of antiplatelet effect to be determined [33–36].

Recent clinical trials have looked at the effects of increasing the dose of clopidogrel in patients with inadequate inhibition of platelet function on standard dose treatment. In the recently published GRAVITAS trial [37], a point of care platelet function test was used to identify patients with high on-clopidogrel reactivity. In these patients, a modest improvement in platelet inhibition was noted when randomized to high dose clopidogrel treatment compared with a comparison group receiving standard doses. Despite this, no difference in clinical outcome between the groups was seen. It must be noted that patients received different doses of aspirin in this trial, but there were no data on whether these were equally randomized between the groups. It is also possible that the low event rate in the GRAVITAS trial may have masked any difference in outcome. Also, as with previously published data [38], some patients continued to have very high platelet reactivity on higher doses of clopidogrel in this study [37], which may have skewed the outcome data.

Further work is needed to evaluate the potential use of platelet function testing clinically. Questions have been raised about defining the cut off for high platelet reactivity, the optimal time of measuring platelet function on treatment, and whether serial platelet function measurements may be useful [39]. Patients with high on-clopidogrel platelet reactivity may particularly benefit from newer, more effective drugs. Encouraging results to this effect have been seen in an earlier study where the use of intensified platelet inhibition with the GPIIb/IIIa inhibitor tirofiban was shown to improve significantly outcomes in patients who had poor responsiveness to clopidogrel as determined using point-of-care platelet function testing [40]. Further clinical trials are underway evaluating the potential role of personalized antiplatelet therapy [39].

There is also an economic decision to be made when deciding on antiplatelet treatment, because clopidogrel is now widely available as various generic salt formulations that are relatively inexpensive [41], whereas other more effective antiplatelet agents and the newer $P2Y_{12}$ inhibitors (see below) would cost the health service much more to administer. However despite introduction to the market, there are very little data comparing the newer, generic clopidogrel salts with the original clopidogrel bisulphate. Some limited evidence from small studies on healthy subjects have shown absence of an overall difference in bioequivalence or antiplatelet effect of clopidogrel besylate compared with clopidogrel bisulphate [42–44]. However in one cross-over study it was shown that there was marked inter- and intra-individual variability between the two different clopidogrel salts [44], and there is a general lack of patient data. We have been unable to find any peer reviewed publications on other commercially available clopidogrel salts, such as clopidogrel hydrochloride. The limited availability of data about formulations of a drug that already shows notable variation in patient response in its original form, is concerning. This also highlights the potential importance of platelet function testing and monitoring of antiplatelet therapy.

In the future it is likely that platelet function testing will become obligatory if only to ascertain which patients are receiving adequate treatment on clopidogrel, which patients in whom higher doses of clopidogrel might be tried, and which patients would be best receiving a newer, more expensive antiplatelet agent. Using platelet function testing to tailor therapy according to each individual patient's response may offer a cost-effective strategy in future antiplatelet therapy.

Prasugrel

Prasugrel is also a thienopyridine and prodrug which, like clopidogrel, needs to be converted to an active metabolite to effect inhibition of platelet function [45]. As with clopidogrel, the prasugrel active metabolite binds to cysteine sulphydryl groups in the $P2Y_{12}$ receptors irreversibly, rendering the receptors unable to respond to ADP and producing inhibition of platelet function for the lifetime of the affected platelet. Prasugrel differs from clopidogrel, however, in that it has a more rapid onset of action after oral administration, it achieves greater and more consistent platelet inhibition in individual patients [46, 47] and

thus its antiplatelet effects are much more predictable. This is achieved mainly because the metabolism of the drug differs from that of clopidogrel and far greater and more predictable amounts of active metabolite are produced. For example, polymorphisms in CYP2C9 and CYP2C19 genes do not affect prasugrel metabolism, unlike the metabolism of clopidogrel described above [48].

All this translates into a more effective anti-thrombotic therapy as compared with clopidogrel as revealed in the TRITON-TIMI study [49]. This was a head-to-head comparison of prasugrel and clopidogrel in patients with acute coronary syndromes with scheduled percutaneous coronary intervention (PCI). The group treated with prasugrel had a significantly better outcome in terms of a composite of adverse events comprising death from cardiovascular causes, nonfatal myocardial infarction and nonfatal stroke (hazard ratio 0.81, 95% confidence interval 0.73, 0.90, $P < 0.001$). There was also a significant reduction in stent thrombosis compared with the clopidogrel group, although this was achieved at the expense of an increased incidence of bleeding. The clinical benefit of prasugrel over clopidogrel was particularly marked in patients with diabetes. It was noted that risks of bleeding outweighed the clinical benefit in patients with a previous history of stroke, the elderly (≥75 years), and those with a body weight of less than 60 kg. A higher incidence of adverse events related to colon cancer was also noted among patients treated with prasugrel in this study. The reasons for this are unclear, but it is suggested that this may be due to possible earlier recognition of otherwise subclinical lesions because of the higher incidence of bleeding secondary to prasugrel.

Despite its predictable and more effective action compared with clopidogrel, a factor limiting the widespread use of prasugrel clinically is its cost. At present, the retail price of prasugrel is about 10 times higher than generic clopidogrel. In the United Kingdom, prasugrel is currently recommended as the preferred antiplatelet agent in patients with low risk of bleeding who are undergoing primary percutaneous coronary intervention for ST segment elevation myocardial infarction [50].

Ticagrelor

Ticagelor (also known as AZD6140) is a cyclopentyltriazolo-pyrimidine [51] and is a very different molecule to clopidogrel and prasugrel. Ticagrelor is also metabolized and forms an active metabolite, ARC124910XX, but this has very similar pharmacokinetics to the parent compound. Like the thienopyridines ticagrelor interacts with $P2Y_{12}$ receptors on platelets rendering them unable to interact with ADP [52], but in this case the drug does not necessarily require metabolic conversion to interact with the receptor [53]. This is likely to at least partly account for the higher efficacy of ticagrelor over clopidogrel in patients irrespective of genetic differences in hepatic enzyme activity [54].

The mode of action of ticagrelor and its metabolite is reversible, in contrast to the irreversible effect of thienopyridines. Consequently, ticagrelor can no longer inhibit platelet function once its concentration in the blood is reduced on clearance of the drug from the circulation. The onset of platelet inhibition is rapid and predictable as ticagrelor is rapidly absorbed following oral administration [55]. Its off-rate is surprisingly quite slow despite its reversible mode of action. Although residual platelet inhibition is less than 50% at 24 h after a dose, approximately 20% inhibition remains at 3 days [56]. This may be because ticagrelor binds to plasma proteins in the circulation [57], which can reduce its rate of clearance from the blood. Like clopidogrel, ticagrelor is also a CYP450 substrate and hence there is the potential for drug–drug interactions [58].

Ticagrelor has been shown to produce a more effective anti-thrombotic effect compared with clopidogrel. The PLATO trial [59] was a head-to-head comparison of ticagrelor and clopidogrel in patients admitted to hospital with an acute coronary syndrome. The end result was a significant reduction in the composite of cardiovascular death, nonfatal MI and stroke with ticagrelor (hazard ratio 0.84, 95% confidence interval 0.77, 0.92, $P < 0.001$), without any difference in the overall incidence of fatal bleeding or major bleeding as defined by PLATO criteria as well as using TIMI (Thrombolysis In Myocardial Infarction) bleeding scores [60]. There was, however, an increase in major bleeding not related to coronary artery bypass grafting. Of particular interest, for the first time with a new agent, a significant reduction in the overall death rate was noted using ticagrelor in place of clopidogrel. The PLATO PlateLET substudy demonstrated that antiplatelet effects were greater and more consistent with ticagrelor over clopidogrel both at initiation of treatment and during maintenance therapy [61]. An unwanted side-effect was an increased incidence of dyspnoea in the ticagrelor-treated group compared with the clopidogrel-treated group (discussed later in this review).

Another potential problem was a geographical variation in the results of the PLATO trial. The benefits of ticagrelor appeared to be attenuated in patients enrolled from North America. The reasons for this difference are unclear. This may, of course, have just been a result of the play of chance, but it is also possible that geographical confounding factors may have been involved. It is of note that higher doses of aspirin were used in the North American patients compared with the rest of the world. High doses of aspirin may lead to inhibition of synthesis of natural modulators of platelet function such as the vascular prostaglandins, PGI_2, PGD_2 and PGE_2, while recent work (see below) has shown that $P2Y_{12}$ antagonists can promote the inhibitory effects of these same natural modulators of platelet function [62]. The possibility that this interaction with high doses of aspirin could explain the reduced antithrombotic benefits seen in the North American subgroup warrants careful consideration.

Ticagrelor has recently been approved for use in Europe for prevention of atherothrombotic events in

patients with acute coronary syndrome [63], and is currently under review by the FDA (Food and Drugs Administration) regarding approval in the United States.

Cangrelor

Cangrelor (also known as AR-C69931MX) is an ATP analogue and is another direct acting inhibitor of platelet function acting via the $P2Y_{12}$ receptor. Cangrelor also acts in a reversible manner [64]. Unlike ticagrelor, cangrelor cannot be administered orally, but is being developed for intravenous use. The half-life of cangrelor is very short and its effects on platelet function disappear within minutes following infusion [65]. This agent may therefore be a useful therapeutic tool for achieving targeted antiplatelet cover for defined periods of time according to clinical need [66].

If cangrelor is to be used in this way, intravenously for short periods of time, caution will need to be applied when switching treatment from cangrelor to another $P2Y_{12}$ antagonist. This stems from the different modes of action of different antagonists at $P2Y_{12}$ receptors. For example, it has been shown that cangrelor, while in contact with the $P2Y_{12}$ receptor, limits the ability of the clopidogrel active metabolite or the prasugrel active metabolite to act as irreversible inhibitors of platelet function [67, 68]. This could have important clinical implications when switching treatment from cangrelor infusion to oral clopidogrel or prasugrel. If oral therapy is initiated early, cangrelor would still be occupying the $P2Y_{12}$ receptor at the time that the active metabolite of clopidogrel or prasugrel appeared in the blood, and irreversible $P2Y_{12}$ blockade would not be achieved. Once the cangrelor was withdrawn the platelets would then become uninhibited. If, alternatively, administration of the oral drug is delayed until after cangrelor is withdrawn this too could lead to a period of absent $P2Y_{12}$ blockade between clearance of the reversible agent and availability of active metabolites from the oral agent. Such a period of incomplete platelet inhibition could potentially increase the risk of thrombosis during this period. Further work is needed to determine the optimal timing and sequence when switching between different $P2Y_{12}$ antagonists.

The definitive trials needed to demonstrate the superiority of cangrelor over other $P2Y_{12}$ antagonists have not yet been completed or published. Two studies in which this was attempted had disappointing outcomes [69, 70] that may have resulted from inappropriate trials designs. The CHAMPION PHOENIX trial is currently underway, evaluating the efficacy and safety profile of cangrelor compared with clopidogrel, in patients who require percutaneous coronary intervention [71].

Elinogrel

A newer $P2Y_{12}$ receptor blocker currently undergoing phase II clinical trials is elinogrel (PRT060128), a sulphonylurea derivative that too is a direct acting, reversible $P2Y_{12}$ antagonist. It is the first $P2Y_{12}$ antagonist being developed that can be used both as an oral and intravenous agent [72, 73].

This drug is at a relatively early stage in its development. In the new phase II trial, INNOVATE PCI, it was reported that both the intravenous and oral forms of elinogrel produced greater and more rapid antiplatelet effects than clopidogrel in stable cardiac patients undergoing percutaneous coronary intervention. This trial, however, did not demonstrate any difference in the clinical efficacy of elinogrel over clopidogrel, which may be due to insufficient statistical power in the study for efficacy outcomes. It is encouraging to note that there was no excess in minor or major bleeding with elinogrel, although there was a dose-dependent increase in minor bleeds when it was used during the peri-procedural phase [74].

Further considerations regarding the mode of action of $P2Y_{12}$ antagonists

We have seen above that all the drugs discussed have a common mechanism of action in that they interact with the $P2Y_{12}$ receptor on platelets, reduce the ability of ADP to interact with that receptor, and thereby reduce platelet function. Thus there is marked inhibition of platelet function induced by ADP itself, and also by agents such as collagen and thrombin, where ADP secreted from platelets in response to stimulation by these agents contributes to the overall functional response. $P2Y_{12}$ antagonists reduce platelet aggregation, platelet secretion, platelet-leucocyte interaction and the contribution of platelets to coagulation. $P2Y_{12}$ antagonists also reduce TXA_2 synthesis by platelets and thereby the amplification of platelet functions mediated by this molecule [75].

But is this the only means through which such drugs exert their antiplatelet and antithrombotic effects? Also, are there any additional mechanisms through which particular drugs might affect platelet function differently compared with other $P2Y_{12}$ antagonists?

Are there additional mechanisms through which $P2Y_{12}$ antagonists affect platelet function?

It is interesting to consider whether adenosine production is relevant to the effects of $P2Y_{12}$ antagonists. ADP can be rapidly broken down to AMP and then to adenosine in blood plasma via ecto-ADPases such as CD39 and CD73 (Figure 2) [76, 77]. Adenosine is an inhibitor of platelet function acting mainly via A_{2A} receptors on platelets through which there is stimulation of adenylate cyclase and an increase in intracellular cAMP. In a recent communication [78] it was clearly demonstrated that adenosine derived from ADP breakdown in blood plasma inhibits platelet function and that adenosine is particularly effec-

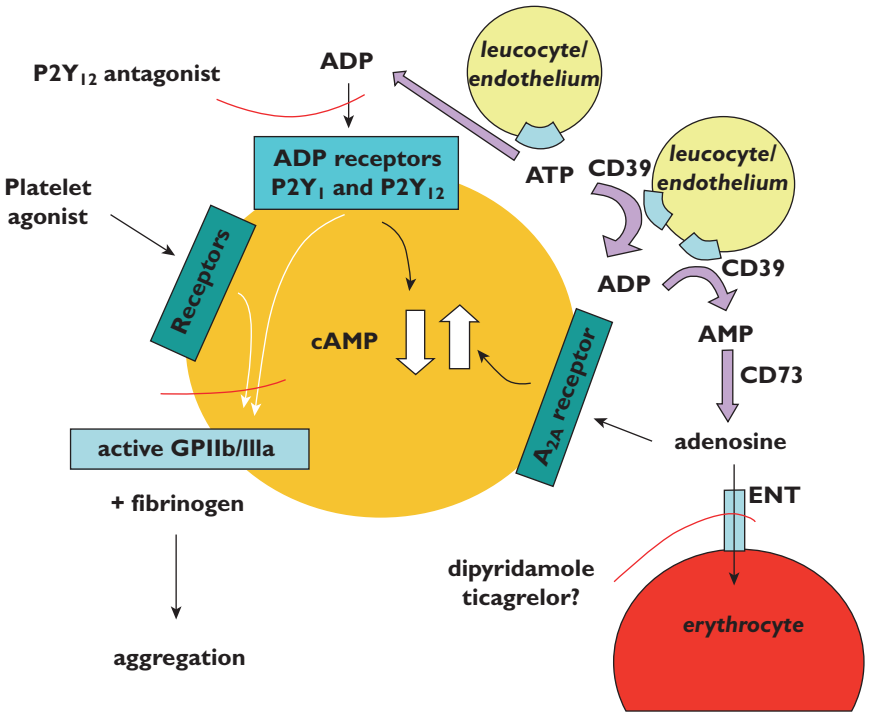

Figure 2

Mechanisms involved in adenosine production and removal, and the way in which adenosine interacts with platelets to increase cAMP and inhibit platelet aggregation. The points at which a $P2Y_{12}$ antagonist and dipyridamole influence the pathways are shown. Recent research does not support an effect of ticagrelor at the equilibrative nucleoside transporter (ENT) [78]

tive as an inhibitor of platelet function in the presence of a $P2Y_{12}$ antagonist [78]. $P2Y_{12}$ antagonists reduce inhibition of adenylate cyclase (Figure 2), so perhaps it is not surprising that an agent that increases cAMP does so more readily in the presence of a $P2Y_{12}$ antagonist and this does prove to be the case. So might part of the action of a $P2Y_{12}$ antagonist, and indeed the clinical benefit to be derived from their use, be mediated via adenosine?

An important consideration is that adenosine is rapidly transported into red cells via the equilibrative nucleoside transporter and this makes the adenosine unavailable to interact with platelets (Figure 2). Thus experiments performed in whole blood (i.e. with red cells present) provided very different results to those obtained in platelet-rich plasma (i.e. with red cells absent). In whole blood any inhibitory effects of adenosine produced from ADP could not be seen. Inhibition of platelet aggregation could only be seen in whole blood when the action of the equilibrative nucleoside transporter was blocked using dipyridamole [78]. There is still the possibility, though, that adenosine could be important *in vivo*, possibly at sites of thrombus formation or haemostasis where, for haemodynamic reasons, red cells may be excluded.

In considering a possible role for adenosine in determining the effects of $P2Y_{12}$ antagonists on platelet function, it is pertinent to consider separately the situation with ticagrelor. This drug has been reported to inhibit the equilibrative nucleoside transporter in addition to its ability to act as a $P2Y_{12}$ antagonist (Figure 2) [79]. Indeed, this is believed to be a possible explanation of the occurrence of dyspnoea observed in clinical trials involving this drug, a condition that is thought could be a consequence of raised adenosine concentrations in the circulation [80]. So, in theory, by both blocking the equilibrative nucleoside transporter and by acting as a $P2Y_{12}$ antagonist ticagrelor should provide more effective inhibition of platelet aggregation in whole blood than other $P2Y_{12}$ antagonists. However, when this was looked for [71], it was found that the effects of ticagrelor on platelet aggregation in both platelet rich plasma and in whole blood were exactly the same as for cangrelor and the prasugrel active metabolite. For all three $P2Y_{12}$ antagonists studied, adenosine produced from ADP inhibited platelet aggregation in platelet-rich plasma but not in whole blood. This finding does not support an additional mode of action of ticagrelor on platelet function via inhibition of adenosine uptake.

$P2Y_{12}$ antagonists may also promote the inhibitory potency of some other natural modulators of platelet function in addition to adenosine (Figure 3). In this regard, it has already been shown that prostaglandin E_1 (PGE_1) is a better inhibitor of platelet aggregation in the presence of cangrelor or clopidogrel than in the absence of one of these $P2Y_{12}$

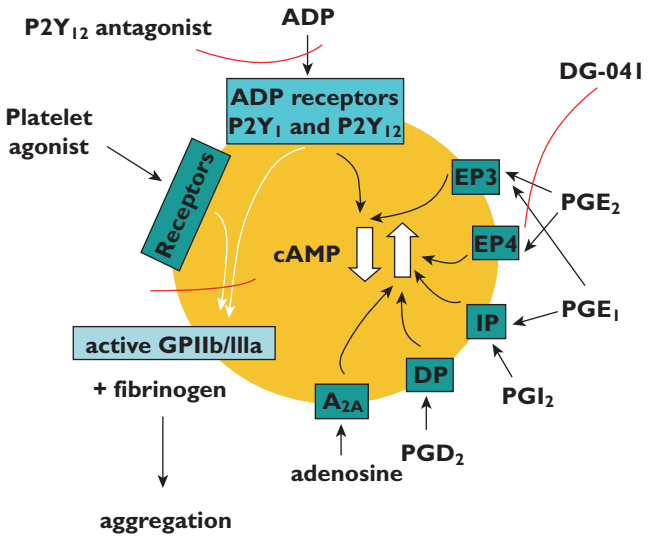

Figure 3

The receptors with which some natural modulators of platelet function interact to either increase or decrease cAMP and thus inhibit or promote platelet aggregation. The points at which a $P2Y_{12}$ antagonist and the EP3 antagonist DG-041 influence the pathways are shown

antagonists [81]. Also prostaglandin I_2 (PGI_2) acting through the IP receptor has been shown to be a better inhibitor of platelet aggregation in the presence of cangrelor [82]. More recently further studies were performed on the effects of cangrelor on the ability of various prostaglandins, PGD_2, PGE_1, PGE_2, PGE_3, PGI_2 and the PGI_2 mimetic iloprost to inhibit platelet aggregation. In all cases cangrelor potentiated the dose-dependent inhibition of platelet aggregation by some 3–10-fold, meaning that much lower concentrations of the prostaglandins were needed to inhibit the aggregation response [62]. This effect was not limited to cangrelor since ticagrelor and the prasugrel active metabolite produced similar potentiating effects. Interestingly, potentiation of the effects of these natural prostaglandins in the presence of a $P2Y_{12}$ antagonist outweighed the ability of a $P2Y_{12}$ antagonist used alone to inhibit platelet aggregation. This may be a major finding regarding the mechanism through which $P2Y_{12}$ antagonists bring about clinical benefit, and requires further investigation. We have already speculated (above) on the possible implications of this finding for the geographically different outcomes of the PLATO study.

Another interesting finding in the study described above [62] was the ability of the EP3 receptor antagonist DG-041 to potentiate the inhibitory effects a $P2Y_{12}$ antagonist used in combination with either PGE_1 or PGE_2. Previous observations on the ability of DG-041 to modulate the effects of PGE_2 on platelet function and thrombus formation have suggested that this EP3 antagonist may produce a useful additional therapy for atherothrombosis [83–85].

There are a number of potential additional effects of particular $P2Y_{12}$ antagonists that have received consideration and investigation, but so far there is no certainty that any such additional effects exist or have any bearing on the effectiveness or otherwise on the outcomes of therapy.

The mode of action of clopidogrel and prasugrel at $P2Y_{12}$ receptors could mean that these drugs also affect other receptors in addition to $P2Y_{12}$ receptors. Other receptors in the 7TM class have cysteine residues within them that could interact with the active metabolites of these two drugs [86]. Effects at such receptors could either promote or inhibit their function. This possibility prompted a study [87] to look for any evidence of effects of the prasugrel active metabolite at a number of G-receptors on platelets and to compare the results with those for ticagrelor and cangrelor. The authors looked for any ability of the $P2Y_{12}$ antagonists to modify the effects on platelet aggregation of a number of agonists and antagonists known to act at a variety of such receptors, all under circumstances where the role of $P2Y_{12}$ receptors were diminished by working under conditions where any ADP or metabolites of ADP were purposely removed. However, no effects of any of the $P2Y_{12}$ antagonists at receptors other than $P2Y_{12}$ receptors could be detected. The receptors that were unaffected by the $P2Y_{12}$ antagonists were the PAR1 receptor, the TP receptor, the IP receptor, the A_{2A} receptor and the EP receptors. These mediate the effects on platelets of thrombin, TXA_2, PGI_2, adenosine and PGE_2, respectively,

The lack of effects of cangrelor at receptors other than $P2Y_{12}$ is also worthy of note because it had been reported previously that cangrelor was able to inhibit platelet aggregation through interaction with an unidentified G_s-coupled receptor leading to increased cAMP concentrations [88]. Indeed these authors argued that this may be the main mode of action of cangrelor rather than through $P2Y_{12}$ blockade. However Iyu *et al*. [87] were unable to replicate the marked inhibition of aggregation induced by agents other than ADP or the increases in cAMP observed previously and thereby found little evidence for a direct effect of cangrelor at receptors other than $P2Y_{12}$ receptors on platelets. They concluded that large quantities of ADP in the platelet preparations may have lead to misleading interpretations of the data obtained previously.

Conclusions

The $P2Y_{12}$ receptor antagonists clopidogrel, prasugrel and ticagrelor are already in clinical use as antithrombotic agents. Variability in antiplatelet effectiveness translating into poor clinical outcomes is a particular problem with clopidogrel, the most widely used and economical ADP receptor antagonist available at present. This makes it crucial to be able to measure the antiplatelet response in individual patients. Platelet function tests are becoming available for clinical use, and these together with the

choice of different ADP receptor antagonists available, may allow tailored antiplatelet therapy to be given to individual patients for optimal effect to reduce thrombotic risk. Newer agents with distinct properties are in development and may offer additional therapeutic options. Research is ongoing to gain more information on interactions between $P2Y_{12}$ receptor antagonists and natural agents that may contribute to inhibition of platelet function, and also to look for any differences in mechanism of action between the different agents that are already available and in development.

Competing Interests

Stan Heptinstall, on behalf of the University of Nottingham, has received a grant for a platelet function workshop from Lilly and a grant for research from AstraZeneca. He is also director of Platelet Solutions Ltd, a new company that is developing simple to use kits for platelet function testing. Yanushi Dullewe Wijeyeratne has no competing interests.

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