The P2Y₁₂ receptor as a target of antithrombotic drugs

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Introduction

The P2Y₁₂ receptor for adenosine diphosphate (ADP) plays a central role in the physiological process of hemostasis and thrombosis. It has proven to be a key target in the prevention of complications associated with atherosclerotic vascular disease especially in the context of percutaneous coronary intervention. Thienopyridines specifically target this receptor and have become the cornerstone of treatment in this setting, showing significant reduction in adverse events in trials that initially compared ticlopidine and aspirin with either aspirin alone or with warfarin and aspirin. The CAPRIE (clopidogrel versus aspirin in patients at risk of ischaemic events) trial was the first large randomized trial that have established the benefit of clopidogrel over aspirin in atherothrombotic patients, by reducing the rate of recurrent myocardial infarction [1]. Clopidogrel showed a better tolerance profile than ticlopidine, and the benefit of a loading dose and long term treatment was then confirmed [2]. A survival effect of clopidogrel against placebo was then shown in a large randomized non-PCI study performed in ST-elevation myocardial infarction (STEMI) patients exposed to aspirin [3] but no single study has shown a reduction in mortality with clopidogrel when used in the

setting of Percutaneous Coronary Intervention (PCI) or in STEMI patients treated by PCI.

Due to the sub-optimal pharmacodynamic and pharmacokinetic profile of clopidogrel, new P2Y₁₂ inhibitors have been developed that are more potent and have a faster onset of action than clopidogrel, characteristics that make them particularly attractive for PCI. Four new P2Y₁₂ inhibitors have now been tested in several clinical studies that recruited STEMI, Non-ST-Elevation-Acute Coronary Syndrome (NSTEMI-ACS) and Stable Coronary Artery Disease patients, predominantly treated with PCI. Each of these antagonists have different properties, targeting the P2Y₁₂ receptor in different ways: prasugrel is an oral prodrug leading to irreversible blockade of the P2Y₁₂ receptor; ticagrelor is a direct acting and reversible inhibitor of the P2Y₁₂ receptor with potentially more pleiotropic effects; cangrelor is an intravenous direct and reversible inhibitor of the P2Y₁₂ receptor providing the highest level of inhibition; and elinogrel is an intravenous and oral P2Y₁₂ antagonist with a direct and reversible action. With the advent of these new therapies comes potential for individualized therapy based on platelet function analysis and genetic testing, of which the feasibility and effectiveness has yet to be confirmed [4, 5] (Table 1).

Platelet activation and the P2Y receptors

Platelet activation and aggregation is an intrinsic component in hemostasis and arterial thrombus formation. ADP plays a crucial role as a key mediator in this process [6]. Atherosclerotic plaque rupture and endothelial activation causes localized adhesion of platelets leading to platelet activation in response to various platelet agonists including thrombin, thromboxane A₂ (TXA₂ and collagen) [7]. ADP is secreted in high concentrations from platelet dense granules and acts to amplify the platelet activation induced

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Table 1 Different agents with mode of action

P2Y ₁₂ receptor inhibitors	Type	Administration	Action	Loading and maintenance dose
Clopidogrel	Thienopyridine (second generation)	Oral	Hepatic transformation in active metabolite/irreversible blockade	300–600 mg 75 mg
Prasugrel	Thienopyridine (third generation)	Oral	Hepatic transformation in active metabolite/irreversible blockade	60 mg 10 mg
Ticagrelor (AZD-6140)	Cyclopentyl-triazolo-pyrimidine	Oral	Direct and reversible inhibition Competitive binding	180 mg 90 mg × 2
Cangrelor (ARC-669931MX)	Analogue de l'ATP	IV	Direct and reversible inhibition Competitive binding	30 µg/kg bolus 4 µg kg ⁻¹ mn ⁻¹ , 2–4 h
Elinogrel	(PRT060128)	IV oral	Direct and reversible inhibition Competitive binding	Bolus from 10 up to 60 mg

by these agents. Its proaggregatory effects are via its interaction with the two G protein-coupled receptors, P2Y₁ and P2Y₁₂. These belong to a family of P2Y receptors whose ligands are purine and pyrimidine nucleotides. They are divided into two distinct subgroups based on structural difference: G_q-coupled subtypes and G_i-coupled subtypes [8, 9].

The P2Y₁ receptor couples to G_{αq} which, in response to ADP, mediates phospholipase activation and calcium mobilization from internal stores leading to platelet shape change and weak and transient aggregation. Concomitant ADP activation of the P2Y₁₂ receptor through its G protein, G_{i2} is essential for complete aggregation [10].

The P2Y₁₂ receptor, previously named P2T_{AC}, P2C_{YC} and P2Y_{ADB} was the last receptor to be identified and cloned but a great deal of knowledge was accumulated previous to this from the observed pharmacocological effects of ticlopidine and clopidogrel [11]. Its presence is limited to platelets, endothelial cells, glial cells and smooth muscle cells thus making it an attractive target for antiplatelet drugs. Structurally, it contains 342 aminoacid residues, including four extracellular Cys residues and exists mainly as homo-oligomers within lipid rafts [12]. The P2Y₁₂ receptor is coupled to G_{αi2} protein and appears to influence platelet activation and aggregation through several intracellular pathways downstream of the receptor. Activation of G_{αi2} leads to inhibition of cyclic adenosine monophosphate (cAMP), which has a facilitating effect on platelet activation by inhibition of the cAMP-dependant protein kinase mediated phosphorylation of the vasodilator-stimulated phosphoprotein (VASP) [13, 14]. VASP is an actin regulatory protein and a negative modulator of the αIIbβ3 integrin activation. Thus, levels of VASP phosphorylation/dephosphorylation reflect P2Y₁₂ inhibition/activation state, which constitutes a sensitive marker to identify the effects of P2Y₁₂ antagonists [15, 16]. The other pathways by which P2Y₁₂ amplifies platelet response

include stimulation of phosphatidyl inositol-3 kinase (PI-3 K) activity [17, 18] leading to sustained aggregation; and activation of small GTPase Rap1b through a PI-3 K dependant mechanism [19, 20]. Finally, P2Y₁₂ also has an effect on the activation of the glycoprotein (GP) GIIb/IIIa receptor which in turn binds fibrinogen and links platelets [21].

The important role of P2Y₁₂ in thrombosis has been further demonstrated in studies of patients with congenital selective defects of the receptor and of ADP-induced platelet aggregation having a history of mild to moderate excessive bleeding [22]. This is also shown in studies of P2Y₁₂ knockout mice. This underlines its relevance as a key target of efficient antithrombotic therapy.

Thienopyridines

Ticlopidine and clopidogrel

The first- and second-generation thienopyridines ticlopidine and clopidogrel are prodrugs that require metabolism to their active forms by the hepatic cytochrome P450 (CYP 450) isoenzymes. Ticlopidine is metabolized by at least five main pathways resulting in a minimum of 13, mostly inactive, metabolites [23, 24]. One of them has been identified to have antiplatelet activity [25]. However, ticlopidine has almost completely been replaced by clopidogrel in clinical practice due its increased pharmacological activity and better safety and tolerability profile. Clopidogrel is largely hydrolysed by esterases in the blood, such that only 15% is metabolized by the cytochrome P450 (CYP) system to generate the active metabolite. Different CYP isoenzymes, including CYP2C19, CYP3A4/5, CYP1A2, CYP2B6, and CYP2C9 are involved in this process [26]. The reactive thiol group of the active metabolite covalently binds the P2Y₁₂ receptor by forming a disulfide bridge with

one or more of the two extracellular cysteine residues (Cys 17 and Cys270) [27, 28]. This irreversible modification of the receptor results in the inhibition of binding of the P2Y₁₂ agonist 2-methylthio-ADP and the ADP-induced down regulation of adenylyl cyclase. This results in inhibition of platelet aggregation for the 7- to 10-day life cycle of the platelet and hence it is recommended to stop the drug at least 5 days before surgery.

In the CAPRIE pivotal trial, clopidogrel was shown to be superior to aspirin in preventing cardiovascular events in patients with symptomatic atherosclerosis [1]. The combination of aspirin and clopidogrel or dual antiplatelet therapy has since become the standard of care for patients with acute coronary syndromes and for patients undergoing percutaneous coronary intervention with stenting [29, 30].

Limitations of clopidogrel

Despite its proven benefit, recurrent clinical adverse cardiovascular events still occur with dual antiplatelet therapy which is largely related to the various limitations of clopidogrel as an antithrombotic agent. Firstly, it has a delayed onset of action which results in sub-optimal platelet inhibition in the acute setting. Secondly, the platelet inhibition is irreversible and there is interindividual variability in the recovery of platelet function leading to higher bleeding risk for patients undergoing surgery including CABG. Thirdly, there is considerable interindividual variability in the pharmacodynamic response to the drug with some patients being termed clopidogrel resistant or as having high on treatment platelet reactivity (HPR). This is largely due to interindividual differences in the metabolism of the prodrug and has been correlated with increased risk of atherothrombotic events [31–34].

Genetic variants particularly in the genes encoding the hepatic cytochrome (CYP) 450 enzymes have been proposed mechanism and data regarding in vitro metabolism and clinical outcomes suggest that reduced-function polymorphisms have an effect on the conversion to active metabolite and hence the degree of platelet inhibition. The liver enzyme, CYP2C19 has been identified to contribute substantially to both of the oxidative steps required for clopidogrel activation. A loss-of-function polymorphism of the CYP2C19 gene (chromosome 10) known as (CYP2C19)*2 allelic variant (or G681A polymorphism) has been identified, accounting for more than 90% of cases of poor metabolism [35–37]. Approximately 30% of the Caucasian population are carriers of this variant and studies have shown that they display reduced pharmacodynamic response to clopidogrel and a higher rate of cardiovascular events compared with noncarriers [38].

CYP2C19 is also inhibited by some proton pump inhibitors with ex vivo studies showing reduced response

to clopidogrel when lansoprazole or omeprazole were co administered [39]. Whether this translates into an increased rate of adverse clinical events is a subject of recent controversy with some population studies [38] suggesting increased risk and no impact on clinical outcomes in the single prospective randomized Clopidogrel and the Optimization of Gastrointestinal Events Trial (COGENT) [40].

There are numerous other genetic variants which have been associated with clopidogrel pharmacodynamics including CYP2C9, CYP2B6, CYP3A5, POR, ABCB1, PON1 currently being investigated. Moreover, the varied response to clopidogrel has also been demonstrated in other subpopulations such as the elderly, diabetics and the obese.

Various strategies have been proposed to reduce the potential ischemic risk posed by the above mechanisms [41]. Giving a loading dose of clopidogrel (300–600 mg) accelerates the onset of action, thus resolving the issue of delayed action to a certain extent. Individualized tailored antiplatelet treatment based on pharmacodynamic and genetic testing rather than a fixed, “one size fits all” strategy is an exciting prospect but requires validation in randomized studies currently being undertaken [4]. Therefore, the use of one of the novel P2Y₁₂ inhibitors that have been designed to effect a more predictable and effective inhibition of platelet function is desirable.

Prasugrel

Prasugrel is a third-generation thienopyridine (2-acetoxy-5-(alpha-cyclopropylcarbonyl-2-fluorobenzyl)-4,5,6,7-tetrahydrothieno[3,2-*c*]pyridine) that has a similar mechanism of action to clopidogrel in that its active form binds covalently to the P2Y₁₂ receptor via a disulfide bond causing irreversible blockade for ADP binding. However, it has much more rapid and consistent inhibitory effects on platelet aggregation than clopidogrel, which has been shown to reflect more efficient in vivo generation of its active metabolite. The prodrug is rapidly hydrolysed by carboxylesterases to a thiolactone, which is then efficiently converted to the active derivative via CYP isoenzymes in a one-step process, thus evading significant genetic influences. The superior pharmacodynamic and pharmacokinetic profile of prasugrel has translated into clinical benefit when compared to clopidogrel. The TRITON TIMI-38 trial evaluated 13,608 high-risk patients with acute coronary syndromes who required PCI. Patients were randomized to receive prasugrel 60-mg loading dose followed by 10 mg/day or clopidogrel 300 mg followed by 75 mg/day for 6–15 months. Prasugrel was associated with few ischemic events and a higher rate of bleeding [42]. The observed clinical efficacy and safety of prasugrel is mostly explained by the reduced rate of “resistance” to the drug. Although it solves many of the problems related to clopidogrel, it does

not issue with irreversibility and thus slow offset of action. Some direct P2Y₁₂ inhibitors may resolve this issue.

Direct P2Y₁₂ inhibitors

Ticagrelor

Ticagrelor (AZD6140) is the first of a new class of antiplatelet family called cyclopentyl-triazolo-pyrimidines (CPTP) and is also the first oral, reversible selective P2Y₁₂ receptor antagonist. Like the thienopyridines, ticagrelor binds the platelet P2Y₁₂ receptor to inhibit ADP's pro-thrombotic effects. However, unlike the thienopyridines, this effect is non-competitive and reversible. Ticagrelor appears to act through an allosteric modulation site and exhibits a conformational change in the receptor by binding independently of ADP. It therefore does not prevent ADP binding but seems to have an effect on ADP receptor-induced signaling and platelet aggregation [43]. It is a direct acting compound and does not require metabolic activation thus obviating any influence of the CYP450 pathway on the antiplatelet response. When administered orally, the agent displays a linear pharmacokinetics profile and has a rapid onset of action with offset of effect more rapid than clopidogrel and so it may be advantageous in clinical scenarios requiring rapid reversal of the antiplatelet effect, for example, in patients requiring CABG. Similar to prasugrel, ticagrelor has shown clinical benefit in head to head phase II and III studies with clopidogrel in ACS showing decreased incidence of adverse cardiac events with a higher rate of non-CABG-related bleeding [44].

Cangrelor

Cangrelor (AR-C69931MX) belongs to a family of ATP analogs that are relatively resistant to the breakdown of ectonucleotidases. It does not require metabolic activation and acts as a reversible, competitive antagonist on the P2Y₁₂ receptor. Administered parentally rather than orally, it has a short half life of <5 min with a rapid onset of effect, inhibiting platelets to a high degree, and a quick offset of effect with resolution of normal platelet function within an hour of cessation of treatment [45–47]. With this pharmacokinetic profile, the major use for cangrelor is in the acute setting where rapid antiplatelet effect with minimal increase in bleeding is needed. While the pivotal trials to date have shown a satisfactory rate of major bleeding side effects, the highly potent cangrelor has not impacted significantly the occurrence of adverse cardiac events. The phase III CHAMPION-PCI and CHAMPION-PLATFORM trials compared cangrelor to clopidogrel (600 mg) in ACS patients scheduled for PCI, with the timing of clopidogrel

dose the major difference between the trials [48]. Both were discontinued prematurely due to insufficient evidence of the clinical effectiveness of cangrelor. There was, however, a reduction in stent thrombosis and death from any cause. Further studies are being carried out currently to assess its effectiveness in the PCI setting (www.clinicaltrials.gov/ct2/show/NCT01156571) and in the setting where it is used as a bridge for patients in whom interruption of thienopyridine therapy is needed due to impending bypass grafting (www.clinicaltrials.gov/ct2/show/NCT00767507).

Elinogrel

Elinogrel (PRT060128) is a potent, direct acting, competitive and reversible P2Y₁₂ antagonist. It can be administered both intravenously and orally. Again, as a direct acting drug, it may avoid the interpatient variability seen with clopidogrel. It is hypothesized that elinogrel has potentially less risk of spontaneous bleeding because it competes directly with ADP for binding at the P2Y₁₂ receptor and it has been shown that ADP concentrations are higher in low-flow environment seen at bleeding sites than high-flow sites. Like cangrelor, its availability in the intravenous form makes it useful in the acute setting of myocardial infarction but with the added advantage of being also available as an oral preparation thus avoiding inconsistent platelet inhibition in transition from IV to oral. Pre-clinical and early-phase clinical testing have shown promising results. Elinogrel has been shown to have consistent inhibitory effects in a first in human study where healthy individuals were administered oral doses, with full inhibition of P2Y₁₂ platelet aggregation at an IC₅₀ of 980 ng/ml (maximum aggregation) and 450 ng/l (at the 6-min endpoint) (Gretler D et al., JACC2007, reversible, abstract). Complete inhibition of P2Y₁₂-dependent ADP-induced platelet aggregation was observed at the 20-mg dose in a study testing intravenous safety and dosing (Lieu HD, 2007, JTH, experience). Elinogrel is currently under evaluation in phase II trials with one trial assessing the safety and feasibility of elinogrel in patients undergoing primary PCI for STEMI prematurely terminated for administrative reasons [49]. Another phase II trial yet to be published is a randomized, double blind trial comparing intravenous and oral administration of elinogrel with clopidogrel in non-urgent PCI [50].

Clinical implications of novel agents

With respect to prasugrel and ticagrelor, the previously mentioned pivotal trials have clearly changed experts' recommendations [51]. Both were given a class 1A recommendation for primary PCI (ST elevation MI presenters) indicating clear evidence of effectiveness (Fig. 1).

Fig. 1 Platelet activation and inhibition mechanisms and the sites of action of antiplatelet drugs

With prasugrel, prespecified landmark analyses demonstrated a 21% reduction of the primary endpoint without bleeding excess in the prospectively defined cohort of STEMI [52], formed by stratification at the time of randomization. These results translate into a number needed to treat (NNT) to prevent one death/MI or stroke of 21.

Similar trends were observed with ticagrelor in STEMI presenters although it did not reach a statistical difference with regard to the primary study endpoint, a composite of death, myocardial infarction or stroke [53]. Although, none

Fig. 2 Overall effect of new P2Y₁₂ inhibitors versus clopidogrel in the setting of percutaneous coronary intervention [54]

ESC Guidelines

Drug	COR	LOE
NSTE-ACS		
Aspirin	I	C
Clopidogrel (600mg ASAP)	I	C
Clopidogrel (for 9-12mo after PCI)	I	B
Prasugrel	Ila	B
Ticagrelor	I	B
STEMI		
Aspirin	I	B
Clopidogrel (600mg ASAP)	I	C
Prasugrel	I	B
Ticagrelor	I	B

Fig. 3 P2Y₁₂ inhibitors in the setting of PCI according to the myocardial Revascularisation guidelines of the European Society of Cardiology [51]

of the individual studies was powered to detect a difference in mortality against clopidogrel, a significant reduction in all cause mortality and cardiovascular death is observed in STEMI patients treated by PCI or by primary PCI, when pooling all the Phase III trials (Fig. 2) [54]. This further emphasizes that STEMI patients are undoubtedly at higher risk and therefore theoretically obtaining greater benefit from rapid platelet inhibition for PCI [54].

Additional analyses of the pivotal trials may help identify preferential targets for these drugs. A similar optimal risk/benefit ratio for STEMI presenters has been observed in the pre-defined group of diabetics, a preferred indication for prasugrel in stented ACS with a striking NNT of 21. In addition, chronic kidney disease patients draw an impressive benefit from ticagrelor with a 23% relative risk reduction of the primary ischemic endpoint compared with a non-significant 10% reduction in patients without CKD, and an even more striking 4.0% absolute and 28% relative risk reduction of all cause mortality [55]. These results translate into an NNT to prevent one death of 25 in CKD patients versus 200 in patients without CKD. Although subjected to bias, these additional analyses have already led experts to issue recommendations on a selective use of the new drugs [51].

These attractive new drugs will not however replace clopidogrel in all ACS patients as the excess of bleeding makes the risk/benefit ratio questionable in some situations; particularly when translating the results from highly selected patients to real life. This is further highlighted by the same meta-analysis pointing out a mortality reduction in all PCI for ACS with a trade-off toward a significant increase in major bleed [54] (Fig. 2). The large randomized CURRENT-OASIS7 trial, double blind for the evaluation of clopidogrel dosing and open label for the evaluation of aspirin dosing, failed to demonstrate a significant benefit of doubling the doses of one or the other oral antiplatelet agents [56]. However, the data also suggest that higher doses of clopidogrel might benefit the patient undergoing rapid PCI, a strategy already recommended and used by many interventionalists.

With generic clopidogrel now becoming widely available, the comparative cost may somewhat temper the enthusiasm for these newer agents in real-life practice. In the only published cost-effective analysis with this group of drugs, prasugrel versus clopidogrel yielded a net in-trial cost of treatment of \$977 per patient and an ICER of \$9,727 per life-year gained when considering the price of clopidogrel as low as \$1 per day [57]. Prasugrel remained an economically dominant strategy when treatment was restricted to the first 30 days follow-up and treatment after this was no longer cost saving but still considered cost-effective with an ICER of around \$20,000. Further analysis in this respect will be needed with ticagrelor, cangrelor and elinogrel.

Conclusions

The P2Y₁₂ receptor is an important target of antithrombotic therapy. In PCI patients, new P2Y₁₂ inhibitors reduce all-cause mortality and major ischemic events, in particular in PCI for STEMI patients (Fig. 3). This further supports that higher level of platelet inhibition than clopidogrel (600 mg) is mandatory for the vast majority of patients. Whether guided treatment with rapidly obtained gene/or platelet function profiles may help improve the clinical risk/benefit ratio for the individual patient and the cost/benefit ratio of therapies for the health care reimbursement systems is an ongoing challenge.

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