P2Y12 Receptor in Cardiovascular Diseases

Elisabetta Liverani *, PhD, Laurie E. Kilpatrick, PhD and Satya P. Kunapuli, PhD

Abstract

Blood platelets are anucleate cells that circulate in blood vessels and play critical roles in hemostasis by providing rapid essential protection against bleeding. Hence upon vessel injury, endothelial disruption leads to the exposure of extracellular matrix, which activates circulating platelets causing them to aggregate and release the contents of their cytoplasmic granules into the surrounding milieu. The P2Y12 receptor is a G-protein coupled receptor that regulates ADP-induced aggregation but can also dramatically potentiate secretion, when platelets are activated by other stimuli. The central role that P2Y12 receptor plays in platelet functional responses underlines its relevance as a key target of efficient anti-thrombotic therapy. This mini-review will focus on the role of P2Y12 receptor during cardiovascular diseases and the advantages and limitations of the current treatments antagonizing this receptor.

Keywords — cardiovascular diseases, P2Y receptor, platelets cardiovascular diseases and thrombosis

Cite this article as: Liverani E., Kilpatrick L.E., Kunapuli S.P. P2Y12 receptor in cardiovascular disease JCVD 2014

I. INTRODUCTION

Platelets are anucleate cells derived from megakaryocytes [1], which under physiological conditions circulate in blood vessels [2]. However upon vessel injury, when the endothelial layer is disrupted, exposure to collagen, Von Willebrand factor (vWF) and vitronectin [3] causes platelet adhesion and aggregation. Platelets contain different cytoplasmic granules, such as α-granules, dense granules rich in thromboxane A2 (TXA2), ADP, serotonin, and lysosomes [4]. Following platelet activation, granule content is released into the surrounding environment, in order to recruit more platelets [2] and/or part of granule content is also incorporated into their plasma membrane [5]. As a result of aggregation, adhesion and platelet recruitment, blood vessel integrity will be restored and rapid cessation of bleeding will occur. Similar events occur at the site of atherosclerotic plaque rupture, when platelet activation leads to thrombus formation and possible vessel occlusion [5]. P2Y12 is a G-protein coupled receptor expressed on platelet membranes, which regulates ADP-induced aggregation [6]. Activation of this receptor can also dramatically potentiate granule release hence amplify platelet aggregation in response to activation by other agonists [7, 8]. Considering the importance of P2Y12 receptor in platelet activation, anti-platelet drugs have been designed which antagonize this receptor hence reduce the risk of anti-thrombotic events [6]. In this mini-review, we will summarize recent findings about the role of P2Y12 receptor in cardiovascular diseases and we will discuss the advantages and limitations of the current treatments antagonizing this receptor in patients with acute coronary syndrome (ACS).

Abbreviations and Acronyms

Acute coronary syndrome (ACS); percutaneous coronary interventions (PCI); Phosphatidylinositol 3 (PI3); Thromboxane A2 (TXA2); vasodilator stimulated phosphoprotein (VASP); Von Willebrand factor (vWF);
leukocytes [15]. Considering the role of platelets in the pathogenesis of acute coronary thrombotic events [2], they are one of the most important targets of the current treatments in patients with ACS [16].

III. P2Y<sub>12</sub> RECEPTOR

ADP-induced aggregation is mediated by both P2Y<sub>12</sub> and P2Y<sub>1</sub> receptors [12] that are respectively G<sub>i</sub> and G<sub>q</sub>-protein-coupled proteins and they are expressed on platelet membranes [17]. P2Y<sub>1</sub> activation leads to intracellular calcium mobilization and protein kinase C (PKC) activation, causing shape change and a weak and transient aggregation [18]. On the other hand, the P2Y<sub>12</sub> receptor couples primarily to G<sub>q</sub> and less prominently to other members of the G<sub>i</sub> family [19]. Its activation causes inhibition of adenylyl cyclase activity hence decreasing cAMP intracellular levels [6]. Furthermore, G<sub>i</sub> signaling leads to activation of the Phosphatidylinositol 3 (PI3) kinase pathway and potassium channels [20, 21]. Studies from our laboratory have identified PI3 kinase as the isoform involved in this signaling [22]. PI3 kinase activation is required for Rap1b [23] and Akt activation [24] that causes a dramatic potentiation of granule release, indicating a critical role for the P2Y<sub>12</sub> receptor in platelet secretion [25]. Indeed, signaling events downstream of the P2Y<sub>12</sub> receptor also potentiate agonist-induced dense granule release, pro-coagulant activity and thrombus formation [6]. In addition, α-granule release and subsequent expression of P-selectin on activated platelets are dependent on P2Y<sub>12</sub> activation [26].

ADP binding to platelets produces selective short-term (5-10 min) desensitization and subsequent receptor internalization [27], hence the responsiveness of P2Y<sub>1</sub> and P2Y<sub>12</sub> receptor in human platelets decreases with ADP exposure. More specifically, recent studies have shown that P2Y<sub>12</sub>-mediated desensitization is mediated by G-coupled receptor kinases (GRK) 2 and 6, leading to their rapid recruitment to clathrin-coated pits in an arrestin-dependent manner [28]. In addition, different PKC isoforms may have distinct roles in regulating platelet both P2Y<sub>1</sub> and P2Y<sub>12</sub> receptor function and trafficking [29]. Following internalization, the receptors are recycled back to the cell surface following receptor dephosphorylation. The C-terminus of the receptor has been implicated as a critical regulator of the trafficking process [30].

P2Y<sub>12</sub> receptor functions have also been investigated in different pathological conditions. Hypercholesterolemia, for example, increases platelet sensitivity to agonist in vitro [31]. Indeed altered cholesterol composition of platelet membrane, in combination with increased TXA<sub>2</sub> generation, was responsible for enhanced platelet reactivity [32]. Previous studies have reported that G<sub>i</sub> signaling mediated by the P2Y<sub>12</sub> receptor is dependent on the cholesterol rich lipid rafts [33]. Interestingly, data from our group have shown that the P2Y<sub>12</sub> receptor pathway was substantially involved in platelet hyper-reactivity associated with mild and severe hypercholesterolemia [34]. In addition, also diabetes and hypertension may increase P2Y<sub>12</sub> receptor functions and hence the risk of thrombosis [35]. Also smoking has been associated with increased platelet reactivity and thrombus formation [36, 37]. Interestingly, exposure to nicotine was able to increase P2Y<sub>12</sub> receptor expression in cell line of endothelial cells and megakaryoblast in vitro [38], which could explain the higher reactivity to platelets in smokers compared with non-smokers. Together these data confirm the central role of P2Y<sub>12</sub> receptor in platelet functions and its relevance in different diseases/conditions.

P2Y<sub>12</sub> antagonism was able to significantly decrease also shear-induced platelet aggregation [39], causing diminished p-selectin expression and microparticle formation initiated by vWF activation [40]. Similar results were observed in a mouse model of atherothrombosis, where pre-treatment with P2Y<sub>12</sub> antagonists, ticagrelor or cangrelor, inhibited thrombus formation and decreased stability [41]. Similar results were observed in ex vivo thrombus formation with human platelets from coronary heart disease patients treated with clopidogrel [42], further confirming the important role of P2Y<sub>12</sub> during thrombus formation and its stability.

Defects in the gene encoding the P2Y<sub>12</sub> receptor are responsible for a congenital bleeding disorder [43]. Patients with defective P2Y<sub>12</sub> receptor functions have normal platelet shape change, but impaired abilities to inhibit adenylyl cyclase activity [44]. Dense granules are normal in both numbers and content, but granule release is decreased.

P2Y<sub>12</sub> receptor expression was thought to be exclusively in the microglia [45] and in platelets [46]. However recent studies have shown its expression in other cells of the immune system, such as lymphocytes [47], monocytes [47] and dendritic cells [48]. These data suggest that P2Y<sub>12</sub> antagonists may have a wider effect than on the vascular system only. P2Y<sub>12</sub> antagonist administration has been studied during inflammatory conditions and these drugs have shown to influence the immune state [49-53] along with their anti-platelet effects. The expression in other cells of the immune system implies that this effect may have direct anti-inflammatory properties rather than solely platelet mediated effects. Furthermore, P2Y<sub>12</sub> receptor expression has also been reported in endothelial cells [54] and smooth muscle cells [55, 56], suggesting that the anti-thrombotic effects may involve other component of the cardiovascular system.

IV. P2Y<sub>12</sub> RECEPTOR ANTAGONISTS

Considering the relevant and well-established role of P2Y<sub>12</sub> receptor in platelet activation, an important category of anti-platelet drugs have been designed to antagonize this receptor [6]. Drug-receptor binding prevents ADP-induced aggregation and consequently thrombus formation [6, 22, 57]. P2Y<sub>12</sub> antagonist therapy can be monitored in patients by a number of methods [58]. The most specific method so far is the measurement of vasodilator stimulated phosphoprotein (VASP), knowing that the phosphorylation of VASP is directly proportionate to the degree of antagonism of P2Y<sub>12</sub> receptor [59]. Alternatively, P2Y<sub>12</sub> antagonist therapy can also be monitored by ex vivo stimulation of platelets with ADP [58].

Ticlopidine was the first designed FDA-approved P2Y<sub>12</sub> receptor antagonists [60] that belongs to the family of Thienopyridine. Ticlopidine is administrated orally and metabolized by at least five main pathways resulting in a variety of different metabolites [61], among which one has been identified to have anti-platelet activity [60].
Clopidogrel also belongs to the family of Thienopyridine and it is a well-established anti-platelet therapy. It binds irreversibly to the P2Y12 receptor and reduces ischemic events in patients with ACS and patients undergoing percutaneous coronary interventions (PCI) [62]. Clopidogrel has largely replaced ticlopidine in clinical practice since it has shown increased pharmacological activity, improved tolerability and reduced side-effect profile, such as less neutropenia and a lower incidence of thrombotic thrombocytopenic purpura [43]. Clopidogrel has been used alone as well as in co-administration with aspirin as a standard treatment in patients with ACS [63]. This drug is administered as a pro-drug, then metabolized in the liver to generate the active form that irreversibly antagonize P2Y12 receptor [64]. About 85% of clopidogrel is hydrolyzed by esterases in the blood, so that only the remaining is metabolized by the cytochrome P450 system to generate the active form [65]. This multi-step process and limited production of the active metabolite partially explains why approximately 30% of patients are non- or poorly responsive to clopidogrel. Furthermore, numerous studies have shown that cytochrome polymorphisms contribute significantly to differential clinical responses to clopidogrel [66]. Furthermore, many other drugs are metabolized through cytochrome P450 in the liver, and therefore may interfere with the effectiveness of clopidogrel. Moreover, clopidogrel resistance may have other causes, such as the increase in the release of ADP, alternate pathways of platelet activation, or P2Y12 polymorphism [67]. Considering clopidogrel multi-step metabolism in the liver, drug-drug interaction should be considered, when clopidogrel is chosen as treatment. For example, co-administration of this P2Y12 antagonist with strong cytochrome P450 inhibitors or inducers should be avoided [68]. Furthermore, also smoking has shown to be relevant for clopidogrel effects, since previous studies have indicated that clopidogrel anti-platelet characteristics were enhanced in smokers compared with non-smokers (PARADOX study) [69]. Such a resistance to clopidogrel in different subsets of patients has highlighted the need for more sustained and reliable anti-platelet agents. That is the reason why another Thienopyridine, prasugrel, was designed and evaluated [70]. This drug has shown highly efficient metabolism to the active form [70]. Indeed, prasugrel is rapidly hydrolyzed and efficiently converted to the active derivative through 1 CYP-dependent step, leading to a much higher concentration of its active metabolite [71]. This rapid conversion is likely to be the reason why this drug has a lower potential for drug-drug interaction [68]. Interestingly, also smoking did not seem to alter the anti-platelet effects of this drug [69], although further analysis need to be carried out [36]. The active form binds covalently to the P2Y12 receptor via a disulfide bond similar to that formed by the clopidogrel active metabolite, irreversibly antagonizing the ADP P2Y12 receptor. Preclinical studies have shown that the potency of prasugrel is 10-fold higher on platelet aggregation inhibition than that of clopidogrel [72]. However, in patients studies while prasugrel was able to decrease the risk of myocardial infarction and stroke more efficiently in CAD [73], it was also responsible for significantly increase life-threatening as well as fatal bleeding [73, 74], suggesting that it should be administered cautiously. Interestingly, previous studies carried out with human plasma have shown that the metabolism of the pro-drug, for both clopidogrel and prasugrel, results in vivo generation of other metabolites [70, 75] that have been considered inactive [75]. Our group has shown that these metabolites and/or the active form could have pleiotropic effects on other receptors, hence influence other cells than platelets [49, 76]. These platelet-independent effects need to be further elucidated in order to understand whether Thienopyridines, the active form and/or other metabolites, target other receptors rather than P2Y12.

Ticagrelor (AZD6140) is the first of a new class of an anti-platelet family called cyclopentyl-triazolo-pyrimidine [77]. These are high affinity ADP analogues that antagonize the P2Y12 receptor. However, unlike Thienopyridines, the effect is non-competitive and reversible [78]. Interestingly, 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 rather alters ADP receptor induced signaling and platelet aggregation [79]. This drug is administered orally and it does not required metabolic activation.

Cangrelor (AR-C69931MX) belongs to a family of ATP analogs and it does not require any metabolic activation [80]. It acts as a reversible, competitive antagonist of P2Y12 receptor. It is administered intravenously rather than orally, which may be potentially advantageous in the PCI settings [81] as the drug has a more rapid onset of action and greater degree of platelet inhibition than clopidogrel [73] and does not significantly increase bleeding time [73, 82].

V. CONCLUSIONS

P2Y12 receptor antagonists have a well-established role as anti-thrombotic agents in the treatment of PCI and acute coronary syndromes (Table 1). As previously discussed, the choice about which P2Y12 antagonists to use should be done on a case-by-case basis [83]. Clopidogrel is most commonly used alone or in co-administration with aspirin. This is probably due to lack of extensive knowledge of newer agents, such as prasugrel and ticagrelor. Hence it is important to investigate the differences between these P2Y12 antagonists, in order to evaluate the best one in use [83]. However, the relatively slow onset of action of clopidogrel and the phenomenon of its response variability and resistance raise the need for other more effective drugs. Prasugrel appears to overcome these problems related with clopidogrel, but the increased bleeding time suggests that it may not be the best substitute. Ticagrelor and Cangrelor are promising alternatives. Finally, recent studies have highlighted interesting new features for the P2Y12 receptor and its antagonists. First, drugs such as clopidogrel and prasugrel produce metabolites that might target other receptors. This observation expands the possibility of therapeutic applications for these anti-platelet drugs that need to be fully characterized. Second, recent studies evaluated P2Y12 receptor expression in other cells rather than platelets and this could explain why P2Y12 receptor antagonists altered inflammation as well as hemostasis. However, there is no pharmacological evidence that this receptor is expressed and functional in any of the cells investigated, hence further studies are required to understand the role of P2Y12 receptor during inflammation.
Table 1: P2Y_{12} receptor antagonists.

<table>
<thead>
<tr>
<th>P2Y_{12} receptor antagonists</th>
<th>Binding to P2Y_{12} receptor</th>
<th>Administration</th>
<th>Co-administration with other drugs</th>
<th>Metabolic activation</th>
<th>Metabolite formation</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thienopyridine - Ticlopidine</td>
<td>Irreversible and non-competitive</td>
<td>Orally</td>
<td>N/A</td>
<td>Multi-step metabolism to the active form</td>
<td>Results in production of other metabolites</td>
<td>[57-58]</td>
</tr>
<tr>
<td>Thienopyridine - clopidogrel</td>
<td>Irreversible and non-competitive</td>
<td>Orally</td>
<td>Often co-administered with aspirin. Concomitant use with CYP inducers and inhibitors should be avoided.</td>
<td>Multi-step metabolism to the active form</td>
<td>Results in production of other metabolites</td>
<td>[40], [59], [61], [63]</td>
</tr>
<tr>
<td>Thienopyridine - prasugrel</td>
<td>Irreversible and non-competitive</td>
<td>Orally</td>
<td>Can be co-administered with CYP inducers and inhibitors.</td>
<td>Highly efficient metabolism to the active form</td>
<td>Results in production of other metabolites</td>
<td>[65], [67-69]</td>
</tr>
<tr>
<td>Ticagrelor (AZD6140)</td>
<td>Reversible and non-competitive</td>
<td>Orally</td>
<td>Concomitant use with CYP inducers and inhibitors should be avoided.</td>
<td>Metabolic activation not required</td>
<td>No other metabolite production</td>
<td>[72-74]</td>
</tr>
<tr>
<td>Cangrelor (AR-C69931MX)</td>
<td>Reversible and competitive</td>
<td>Intravenously</td>
<td>N/A</td>
<td>Metabolic activation not required</td>
<td>No other metabolite production</td>
<td>[68], [75-77]</td>
</tr>
</tbody>
</table>

VI. REFERENCES


vascular rupture or remodeling

P2Y₁₂ on vascular


