The platelet P2Y12 receptor for adenosine diphosphate: congenital and drug-induced defects

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P2Y12, the Gi-coupled platelet receptor for adenosine diphosphate (ADP), plays a central role in platelet function. Patients with congenital P2Y12 defects display a mild to moderate bleeding diathesis, characterized by mucocutaneous bleedings and excessive post-surgical and post-traumatic blood loss. Defects of P2Y12 should be suspected when ADP, even at high concentrations (≥ 10 μM), is unable to induce full, irreversible platelet aggregation. Tests that evaluate the degree of inhibition of adenylyl cyclase by ADP should be used to confirm the diagnosis. Drugs that inhibit P2Y12 are potent anti-thrombotic drugs, attesting the central role played by P2Y12 in platelet thrombus formation. Clopidogrel, the most widely used drug that inhibits P2Y12, is effective both in monotherapy and in combination with acetylsalicylic acid. The most important drawback of clopidogrel is its inability to inhibit adequately P2Y12-dependent platelet function in approximately one-third of patients who are therefore not protected from major cardiovascular events. New drugs, such as prasugrel and ticagrelor, which effectively inhibit P2Y12 in the majority of patients, proved to be more efficacious than clopidogrel in preventing major cardiovascular events. Although they increase the incidence of major bleedings, the net clinical benefit is in favor of the new P2Y12 inhibitors. (Blood. 2011;117(7):2102-2112)

Introduction

Adenosine diphosphate (ADP) plays a key role in platelet function. When it is secreted from the platelet-dense granules where it is stored, it amplifies the platelet responses induced by other platelet agonists1,2 and stabilizes platelet aggregates.3-5 The ADP-induced signal is mediated by P2Y receptors, which are G-coupled 7-membrane-spanning proteins that are present in almost any kind of cell, whose ligands are purine and pyrimidine nucleotides. From a phylogenetic and structural point of view, 2 distinct P2Y receptor subgroups with a relatively high level of structural divergence have been identified: the first subgroup includes the Gq-coupled subtypes (P2Y1, P2Y2, P2Y4, P2Y6, and P2Y11), and the second subgroup includes the Gi-coupled subtypes (P2Y12, P2Y13, and P2Y14).6 Human platelets express 2 distinct receptors for ADP: P2Y1 and P2Y12. The Gq-coupled P2Y1 receptor mediates a transient rise in cytoplasmic Ca2+, platelet shape change, and rapidly reversible aggregation, whereas the Gi-coupled P2Y12 receptor mediates inhibition of adenylyl cyclase and amplifies the platelet aggregation response.2 Concomitant activation of both the Gq and Gi pathways by ADP is necessary to elicit normal aggregation.2,7 P2Y12 receptors exist predominantly as homo-oligomers situated in lipid rafts. On treatment with the active metabolite of clopidogrel (which inhibits P2Y12 function), the homo-oligomers are disrupted into nonfunctional dimers and monomers that are sequestered outside the lipid rafts.9

P2Y12 signaling

ADP and some of its analogs, such as 2-methylthio-ADP and (N)-methanocarba-2-methylthio-ADP, stimulate P2Y12, whereas adenosine triphosphate and its triphosphate analogs act as antagonists.13 The P2Y12 receptor is coupled to inhibition of adenylyl cyclase activity mostly through activation of Gαi2 and has a critical requirement for lipid rafts.14 It must be noted, however that, although inhibition of adenylyl cyclase via Gαi2 is a key feature of platelet activation by ADP, it bears no causal relationship to platelet aggregation.15,16 Therefore, other signaling events downstream of Gαi2 are required for activation of integrin αIIbβ3 and subsequent platelet aggregation. Several studies demonstrated a crucial role for different isoforms of phosphoinositide 3-kinase (PI3K) in ADP-dependent P2Y12 receptor-mediated amplification of platelet activation.5,17-21 In addition, studies of platelets from P2Y1 knockout mice and of normal platelets in the presence of specific P2Y1 antagonists showed that ADP, at higher concentrations than those commonly used to activate platelets, induces slow and sustained PI3K-dependent platelet aggregation, which is not preceded by platelet shape change.22,23

Role of P2Y12 in platelet function

Although ADP by itself is unable to cause the secretion of platelet-dense granules, its interaction with P2Y12 greatly amplifies
platelet secretion induced by agonists, such as thromboxane A₂ and thrombin receptor-activating peptide (Figure 2). This effect, which is probably mediated by PI3K, was observed both at physiologic and micromolar concentrations of extracellular Ca²⁺, in the presence of acetylsalicylic acid (ASA), and independently of the formation of large platelet aggregates, demonstrating that it is a direct effect of P2Y₁₂, rather than secondary to P2Y₁₂-mediated amplification of aggregation.

P2Y₁₂ plays an essential role in the stabilization of platelet aggregates induced by thrombin or thromboxane A₂ (Figure 2), which is mediated by PI3K. Studies of human platelets congenitally lacking P2Y₁₂ and of P2Y₁₂ knockout mice demonstrated that platelet aggregation and secretion induced by a range of platelet agonists were impaired. P2Y₁₂ receptor stimulation by released ADP contributes to inhibition of adenylyl cyclase, activation of serine-threonine kinase Akt in platelets, tyrosine phosphorylation, extracellular signal-regulated kinase 2 activation, Rap1B activation, and Ca²⁺ mobilization induced by other agonists.

P2Y₁₂ shares with P2Y₁ the ability to contribute to collagen-induced platelet microparticle formation in whole blood, and to contribute to the formation of platelet-leukocyte aggregates mediated by platelet surface P-selectin exposure, which results in tissue factor exposure at the surface of leukocytes. However, only the P2Y₁₂ receptor was found to be involved in the exposure of phosphatidylserine by thrombin or other platelet agonists and in tissue factor-induced thrombin formation in platelet-rich plasma.

**Congenital defects of P2Y₁₂**

**Congenital deficiency of P2Y₁₂**

Congenital P2Y₁₂ deficiency is an autosomal recessive disorder. The first patient with severe P2Y₁₂ deficiency (patient 1) was...
described in 1992.30 He had a lifelong history of excessive bleeding, prolonged bleeding time (15-20 minutes), reversible aggregation in response to weak agonists, and impaired aggregation in response to low concentrations of collagen or thrombin. However, the most typical feature was that ADP, even at very high concentrations (>10 μM), did not induce full and irreversible platelet aggregation. Other abnormalities of platelet function were: (1) no inhibition by ADP of prostaglandin E1 (PGE1)-stimulated platelet adenylyl cyclase, but normal inhibition by epinephrine; (2) normal shape change and borderline-normal mobilization of cytoplasmic Ca2+ induced by ADP; and (3) the presence of approximately 30% of the normal number of binding sites for [33P]2MeSADP on fresh platelets39 or [3H]ADP on formalin-fixed platelets (which are associated with the ADP receptor P2Y1).30 Five additional patients with severe P2Y12 deficiency, belonging to 4 kindreds, were subsequently described: one French man (patient 2),34 2 Italian sisters (patients 3 and 4),26 a Japanese woman (patient 5),40 and a British woman of Asian descent (patient 6; Table 1).41

The study of the son of patient 4 allowed the characterization of heterozygous P2Y12 deficiency25: ADP-induced platelet aggregation was reversible for ADP concentrations less than or equal to 10 μM but was full and irreversible for concentrations of ADP more than or equal to 10 μM; the inhibition of PGE1-induced increase in platelet cyclic AMP was impaired, albeit not completely absent; the number of platelet binding sites for [35P]2MeSADP was intermediate between his mother’s and normal subjects’; finally, the platelet secretion was impaired. Because the secretion defect in this patient’s platelets was not associated with impaired production of thromboxane A2 or low concentrations of platelet granule contents, it is very similar to that described in patients with an ill-defined and probably heterogeneous group of congenital defect of platelet secretion, sometimes referred to with the general term “primary secretion defect.”25,42

Molecular defects

The P2Y12 gene of patients 1 and 6 displayed homozygous base pair deletions in the open reading frame, resulting in frameshifts and premature truncation of the protein.41,43 The P2Y12 gene of sisters (patients 3 and 4) displayed an identical single base pair deletion (378delC), resulting in a frameshift and premature truncation of the protein.43 As only alleles encoding the mutated DNA sequence were found by polymerase chain reaction analysis, the patients were considered homozygous for the 378delC mutation. However, a subsequent study revealed that they have the P2Y12 deficiency because of haploinsufficiency and to the 378delC mutation in their remaining allele.44 Patient 5 is homozygous for a single nucleotide substitution in the transduction initiation codon (ATG to AGG).40 The molecular defect that is responsible for P2Y12 deficiency in patient 234 is less well defined45: one mutant allele contains a deletion of 2 base pairs, resulting in a frameshift and early truncation of the protein. Surprisingly, the other allele did not display any mutation: the findings that the patient’s platelets contained P2Y12 transcripts derived from the mutant allele only and that his daughter, who had a heterozygous phenotype, inherited the mutant allele from her father and a normal allele from her mother, suggest that patient 2 has an additional, as yet unknown, mutation that silences his normal allele.

Congenital dysfunction of P2Y12

One patient (patient 9) with congenital bleeding disorder associated with abnormal P2Y12-mediated platelet responses to ADP, whose platelets display normal number of dysfunctional P2Y12 receptors has been described.32 Platelets from this patient displayed reduced and reversible aggregation in response to 4 μM ADP, similar to normal platelets with a blocked P2Y12 receptor. However, the response to 20 μM ADP, albeit still decreased and reversible, was more pronounced and was further inhibited by a P2Y12 antagonist, indicating residual receptor function. ADP failed to lower adenylyl cyclase activity stimulated by PGE1 in the patient’s platelets. Analysis of the patient’s P2Y12 gene revealed, in one allele, a G to A transition changing the codon for Arg256 in TM6 to Gln and, in the other, a C to T transition changing the codon for Arg265 in EL3 to Trp (Table 1). Neither mutation interfered with receptor surface expression, but both altered receptor function because ADP inhibited the forskolin-induced increase of cyclic AMP markedly less in cells transfected with either mutant P2Y12 than in wild-type cells. These observations, in accordance with previous studies of the P2Y1 receptor,36,47 helped to identify regions in TM6 and EL3,
whose structural integrity is necessary for normal receptor function (Figure 1). A heterozygous point mutation in the same region of the molecule, which changed codon 258 coding for proline (CCT) to threonine (ACT; Pro258Thr), was described in a patient with mild bleeding disorder and severely impaired ADP-induced platelet aggregation. Because the proline-to-threonine substitution alters the protein hydrophobicity, size, and rotational mobility, it probably affects the function of P2Y12.

Finally, a heterozygous mutation, predicting a lysine to glutamate (Lys174Glu) substitution in P2Y12, was identified in one patient with mild type 1 von Willebrand disease. Platelets from this patient showed reduced and reversible aggregation in response to ADP, up to 10 μM. The reduced response was associated with an approximate 50% reduction in binding of [3H]2MeS-ADP. Considering that Lys174 is situated in the second extracellular loop of P2Y12, adjacent to Cys175, which may be important for the expression of the ADP binding site receptor, and that a hemagglutinin-tagged Lys174Glu P2Y12 variant showed surface expression in Chinese hamster ovary cells, it is probable that the Lys174Glu mutation is responsible for disruption of the ADP binding site of the receptor.

It is interesting to note that, for reasons that are presently unclear, 2 patients with heterozygous dysfunctional P2Y12 (Pro258Thr and Lys174Glu) display a much more severe impairment of ADP-induced platelet aggregation compared with the 2 patients who are heterozygous for P2Y12 deficiency and to the 2 children of patient 9, who are heterozygous for the Arg265Gln mutation (Table 1).

**Bleeding diathesis**

Patients with defects of P2Y12 experience mucocutaneous bleedings and excessive postisurgical or posttraumatic blood loss. The severity of their bleeding diathesis is variable. The bleeding scores of patient 1 and of the 2 sisters (patients 3 and 4), which was calculated using a standardized questionnaire that was developed to investigate patients with type 1 von Willebrand disease, were 8, 7, and 13, respectively, (normal values ≤ 3; unpublished data). After extensive investigation of hemorrhosis parameters, which included measurement of the activity of clotting and fibrinolytic factors and the search for known polymorphisms of hemorrhosis proteins, we found no explanation for the discrepancy in the severity of bleeding manifestations in the 2 sisters (patients 3 and 4).

The bleeding score of patient 8, the son of patient 4, was normal; however, it must be noted that this young boy had not yet experienced situations that could challenge the hemostatic system at the time of our investigation. His bleeding time, despite the mild defect of P2Y12, was prolonged (13 minutes).

**Diagnosis and treatment**

The diagnosis of P2Y12 defects is rather simple: they should be suspected when ADP, even at relatively high concentrations (≥ 10 μM), is unable to induce full, irreversible platelet aggregation while inducing normal shape change. Tests that evaluate the degree of inhibition of adenyl cyclase by ADP, by measuring either the platelet levels of cyclic AMP or the phosphorylation of vasodilator-stimulated phosphoprotein after the exposure of platelets to PGE1, should be used to confirm the diagnosis (Table 2).

The intravenous infusion of the vasopressin analog desmopresin (0.3 μg/kg) shortened the prolonged bleeding time of patient 1 from 20 minutes to 8.5 minutes. After the infusion of desmopressin, which was repeated twice at 24-hour intervals, the patient underwent a surgical intervention for disc hernia repair, which was not complicated by excessive bleeding. Although the efficacy of desmopressin in reducing bleeding complications of patients with defects of primary hemostasis is anecdotal, its administration is generally without serious side effects.

**Drugs targeting P2Y12**

Drugs that target P2Y12 reduce the incidence of arterial thrombosis, as documented by the results of several randomized clinical trials.
### Table 3. Main results of major double-blind, randomized, controlled clinical trials with ticlopidine or clopidogrel

<table>
<thead>
<tr>
<th>Clinical trial</th>
<th>Reference</th>
<th>Patients</th>
<th>Treatments</th>
<th>Cardiovascular endpoints</th>
<th>Follow-up</th>
<th>Efficacy*</th>
<th>Bleeding events†</th>
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</table>
| CATS           | 55        | Recent thromboembolic stroke | 1. Ticlopidine  
2. Placebo | Stroke, MI, or vascular death | 24 mo | 23.3% (1.0-40.5) | 6.5% |
| TASS           | 56        | Recent transient or mild persistent focal cerebral or retinal ischemia | 1. Ticlopidine  
2. ASA | Nonfatal stroke or death from any cause | 3 y | 12% (–2%–26%) | 10.0% |
| ISAR           | 57        | CAD patients: PCI and stent implantation | 1. Ticlopidine  
2. Heparin/VKA + ASA | Cardiac death or AMI, CABG, or repeated PCI | 4 wk | 0.25 (0.06-0.77) | 0.00 |
| SAR            | 58        | CAD patients: PCI and stent implantation | 1. Ticlopidine  
2. Heparin/VKA + ASA  
3. ASA | AMI, death, repeat PCI, stent thrombosis at angiography | 30 d | 1 vs 2: 0.20 (0.07-0.61) | 1 vs 3: 0.15 (0.05-0.43) |
| CAPRIE         | 59        | Atherosclerotic vascular disease | 1. Clopidogrel  
2. ASA | Ischemic stroke, AMI, or vascular death | 1.9 y | 8.7% (0.3-16.5) | 9.27% vs 9.28% |
| CHARISMA       | 60        | Clinically evident CV disease or multiple risk factors | 1. Clopidogrel + ASA  
2. Placebo + ASA | MI, stroke, or CV death | 28 mo | 0.93 (0.83-1.05) | F: 1.53 (0.83-2.82) | M: 1.25 (0.97-1.61) |
| MATCH          | 61        | Recent ischemic stroke or TIA and ≥ 1 risk factors | 1. Clopidogrel + ASA  
2. Clopidogrel + placebo | Ischemic stroke, MI, vascular death, or rehospitalization for acute ischemia | 18 mo | 6.4% (–4.6–16.3) | LT: 1.26 (0.62-1.88) | M: 1.36 (0.86-1.86) |
| CURE           | 62        | Acute coronary syndromes | 1. Clopidogrel + ASA  
2. Placebo + ASA | CV death, Nonfatal AMI, or stroke | 12 mo | 0.80 (0.72-0.90) | LT: 1.21 (0.95-1.56) | M: 1.38 (1.13-1.67) | T: 1.69 (1.48-1.94) |
| COMMIT         | 63        | Suspected AMI | 1. Clopidogrel + ASA  
2. Placebo + ASA | (1) Death, reinfection, or stroke  
(2) Death from any cause | Up to 28 d | (1) 0.91 (0.86-0.97)  
(2) 0.93 (0.87-0.99) | F: –0.1 (SE: 0.5) | M: 0.4 (0.7) | m: 4.7 (1.7) |
| PCI CURE       | 64        | NSTE ACS undergoing PCI in the CURE study | 1. Clopidogrel + ASA  
2. Placebo + ASA | CV death, AMI, or urgent target vessel revascularization | 30 d | 0.70 (0.50-0.97) | LT: 0.92 (0.38-2.26) | M: 1.13 (0.61-2.10) | m: 1.33 (0.59-3.03) |

Doses of the antiplatelet agents are as follows: ticlopidine, 250 mg twice a day55,56, ASAs, 650 mg twice a day55; 325 mg daily56,59; 100 mg twice a day57; 160 mg daily58; 75 to 325 mg daily60,63; 75 to 160 mg daily57; 75 mg daily57 in all randomized clinical trials, plus 300 mg loading dose in the CURE trial.62,64

MI indicates myocardial infarction; CAD coronary artery disease; AMI, acute myocardial infarction; VKA, vitamin K antagonist; CV, cardiovascular; and TIA, transient ischemic attack.

*Results are reported as relative risk reduction55,56,59,60,63 and relative risk57,58,60,61,62,64 (95% confidence interval).

†When available, data on major (M), life-threatening (LT), fatal (F), moderate (Mod), minor (m), and total (T) bleeding events are reported as total incidence55,56,59 relative risk57,58,60,61,62,64 and excess per 1000 patients63 (95% confidence interval).
Thienopyridines

First- and second-generation thienopyridines: ticlopidine and clopidogrel. Ticlopidine and clopidogrel (Figure 3) are prodrugs that need to be converted in vivo by the hepatic cytochrome P-450 (CYP) enzymatic pathway to active metabolites, which covalently bind to P2Y12 by forming a disulfide bond with cysteine residues, thereby irreversibly inhibiting the receptor.53 Several randomized clinical trials documented the clinical efficacy of these drugs in the prevention of major adverse cardiovascular events (MACEs;55-64 Table 3). Their efficacy was essentially similar to that of ASA in patients with stable disease of cerebral or coronary arteries. For patients with acute coronary syndromes, undergoing medical treatment only or in combination with percutaneous coronary intervention (PCI), the addition of thienopyridines to ASA proved highly efficacious. In contrast, the combination of clopidogrel and ASA was not more effective than monotherapy in low- to moderate-risk patients with stable disease but increased the incidence of bleeding. Because of its toxicity,53 ticlopidine has been almost completely replaced by clopidogrel in clinical practice.

Despite its proven antithrombotic efficacy, clopidogrel has some important drawbacks54: (1) its antiplatelet effects are delayed because of the need for metabolism of the prodrug; (2) there is substantial interindividual variability in platelet inhibition; (3) its ability to irreversibly inhibit P2Y₁₂ may represent a problem for patients who need to undergo coronary bypass (CABG) surgery because the incidence of postoperative bleeding complications is higher than in patients not treated with clopidogrel. Although the onset of action of clopidogrel can be accelerated by giving patients a loading dose of 300 to 600 mg, the solution of the other 2 problems appears more difficult.54

The high interindividual variability of the response to clopidogrel is a clinically relevant issue, as it has been demonstrated that poor responders are not adequately protected from MACE.65 Approximately one-third of treated patients do not display adequate inhibition of P2Y₁₂-dependent platelet function; this condition is associated with loss-of-function mutations of CYP,66-68 with the homozygous 3435C→T mutation of ABCB1, a gene encoding for the efflux pump P-glycoprotein, a key protein involved in thienopyridine absorption,69,70 and may be exacerbated by negative interference with common adjunctive medications, such as proton pump inhibitors.68 Tailored treatment of patients, based on the results of platelet function tests or of CYP genotyping, has been proposed to solve the problem of clopidogrel resistance.54 This approach cannot be recommended in daily clinical practice yet because the best laboratory method to monitor the effects of clopidogrel on platelet function still needs to be identified, standardized (for preanalytical and analytical variables), and validated in the clinical setting. Several recent studies demonstrated that the agreement among different laboratory tests to identify poor responders is rather low and that assessment of platelet response to clopidogrel is highly test-specific.71-76 Recent studies showed that the search for loss of function mutations of CYP is not very accurate in predicting the response to clopidogrel.77,78 In addition, preliminary experiments that evaluated the effects of increasing the dose of clopidogrel in resistant patients gave results that are incompletely satisfactory because many patients remained “resistant” to clopidogrel, even after repeated administrations of high doses of the drug.79,80

Mostly based on the aforementioned consideration, a recent consensus paper concluded that, until the results of large-scale trials of personalized antiplatelet therapy are available, the routine use of platelet function measurements in the care of patients with cardiovascular disease cannot be recommended.51 Therefore, the use of new P2Y₁₂ antagonists that are able to induce predictable
and adequate inhibition of platelet function in all patients is desirable.

**Prasugrel, a new thienopyridine.** Prasugrel is a new thienopyridine, with much more rapid and consistent inhibitory effects on platelet aggregation than clopidogrel. It has a distinct chemical structure, which permits conversion to its active metabolite with less dependence on CYP enzymes than clopidogrel (Figure 3). Consequences of the different metabolism of prasugrel, compared with that of clopidogrel, are \(^{54}\): (1) faster appearance and higher concentration of its active metabolite in circulating blood; (2) faster and greater mean inhibition of P2Y\(_{12}\)-dependent platelet function; (3) no influence of the CYP genotype on its pharmacokinetics, pharmacodynamics, and antithrombotic activity; and (4) much lower interindividual variability in inhibition of P2Y\(_{12}\)-dependent platelet responses and very low prevalence of subjects who display “resistance” to the drug.

The aforementioned more favorable characteristics of prasugrel compared with clopidogrel result in greater clinical benefit, as shown by the results of TRITON TIMI-38, which 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 to 15 months. Prasugrel was associated with fewer ischemic events but more non–CABG-related and CABG-related major and fatal bleedings (Table 4).

Based on the results of the TRITON TIMI-38 trial, prasugrel is generally considered a more potent antiplatelet agent than clopidogrel, to be used only in high-risk patients or for a short period, whereas treatment with clopidogrel should be preferred in the remaining situations. However, it is incorrect to say that prasugrel is more potent than clopidogrel, as both ex vivo and in vitro studies demonstrated that the active metabolites of the 2 compounds have the same potency.\(^{83,84}\) The different clinical efficacy and safety of prasugrel compared with clopidogrel are mostly explained by the fact that very few treated patients are “resistant” to prasugrel. Because protection from thrombotic events and exposure to bleeding risk are a function of the degree of inhibition of P2Y\(_{12}\)-dependent platelet function, the higher efficacy and the lower safety of prasugrel compared with clopidogrel are simply explained by the fact that prasugrel protects from MACE and exposes to the risk of bleeding more patients than clopidogrel. Based on the results of published studies, it can be predicted that, if tailored treatment with clopidogrel were successful in all patients displaying hypersensitivity to the drug, incidences of MACE and bleedings in patients given tailored clopidogrel treatment would be similar to those observed in patients given prasugrel.\(^{54}\)

Therefore, prasugrel appears an attractive solution to some of the problems that are associated with the use of clopidogrel. However, prasugrel does not solve the problem associated with the slow offset of action because, like clopidogrel, it causes irreversible inhibition of P2Y\(_{12}\). Some direct P2Y\(_{12}\) antagonists with such characteristics are currently under development.

### Direct P2Y\(_{12}\) inhibitors

**Ticagrelor.** Ticagrelor belongs to the new chemical class cyclopen-tyl-triazolo-pyrimidines (Figure 4): it does not require conversion to an active metabolite and has a half-life of 7 to 8.5 hours.\(^{85,86}\)
After oral administration, it rapidly and reversibly inhibits P2Y₁₂ via a mechanism that is noncompetitive with ADP, suggesting the existence of an independent receptor binding site.⁸⁷ In phase 2 trials, ticagrelor more rapidly and effectively inhibited platelet aggregation and with less variability than clopidogrel.⁸⁸,⁸⁹ A study that compared the onset and offset of action of clopidogrel and ticagrelor showed that, despite the greater mean antplatelet effect of ticagrelor, inhibition of platelet aggregation at 24 hours after the last dose was equivalent in ticagrelor- and clopidogrel-treated patients, which is indicative of a faster offset of effect.⁹⁰ Considering that, because of its short half life, ticagrelor needs to be administered every 12 hours, which might negatively affect the compliance of patients under chronic treatment, these data have relevant practical implications because they suggest that patients who miss one dose of ticagrelor will have a level of platelet inhibition at 24 hours after the last dose that is not inferior to that of patients undergoing chronic clopidogrel therapy. Dyspnea was reported in 10% to 20% of patients treated with ticagrelor in phase 2 trials, although none of the incidents was considered to be serious.⁹⁰,⁹¹ The pathogenesis of dyspnea during ticagrelor treatment is unclear, although it has been hypothesized that it may be mediated by adenosine.⁹² The results of PLATO trial, in which ticagrelor (180 mg loading dose [LD], 90 mg twice a day maintenance dose [MD]) was compared with clopidogrel (300-600 mg LD, 75 mg daily MD) for prevention of MACE in patients with non-ST or ST elevation acute coronary syndromes (two-thirds of them underwent PCI) showed that ticagrelor decreases the incidence of MACE compared with clopidogrel (Table 4).⁹¹ Very importantly, ticagrelor also decreased the incidence of cardiovascular and total mortality. There was a higher incidence of TIMI major non–CABG-related bleedings in patients who received ticagrelor compared with those treated with clopidogrel. The incidence of major CABG-related bleedings was similar in the 2 groups. Therefore, similarly to the TRITON-TIMI 38 trial, the PLATO trial showed that a more consistent, adequate inhibition of P2Y₁₂-dependent platelet function than that achieved with standard doses of clopidogrel is associated with greater antithrombotic efficacy and higher risk of non–CABG-related major bleedings. The higher incidence of clinically irrelevant dyspnea in ticagrelor-treated patients was confirmed in the PLATO study.⁹¹

**Cangrelor.** Cangrelor (Figure 4) belongs to a family of analogs of adenosine triphosphate that are relatively resistant to breakdown by ectonucleotidases and display high affinity for the P2Y₁₂ receptor, which is reversibly inhibited by the drug.⁹³ Cangrelor does not require conversion to an active metabolite and is immediately active after intravenous infusion, with a half-life of 3 to 6 minutes.

Two trials, which compared cangrelor with clopidogrel in patients requiring PCI, were prematurely terminated because of insufficient evidence of superiority of cangrelor.⁹²,⁹³ Cangrelor is still being studied as a bridge for patients who need to suspend thienopyridines before surgery.

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**Bleeding events associated with treatment with P2Y₁₂ inhibitors**

As already mentioned, patients with congenital P2Y₁₂ defects have a bleeding diathesis of variable severity: it was therefore not unexpected that drugs targeting P2Y₁₂ increase the incidence of bleedings. Although the value of laboratory tests of hemostasis for the prediction of bleeding events in patients with acute coronary syndromes under antithrombotic treatment is extensively being evaluated, a recent study showed that a simple bleeding score, based on 6 readily available clinical and laboratory variables (female sex, advanced age, elevated serum creatinine and white blood cell count, anemia, and type of acute coronary syndrome), plus the anticoagulation regimen used, may provide a rapid tool to predict the rate of major bleeding in these patients.⁹⁴

**Major bleedings and the risk of mortality**

Severe bleeding complications during antithrombotic therapy have negative consequences, not only because they may be fatal, disabling, and expose the patients to the risks that are associated with blood transfusion: they are also associated with poor prognosis of the patients, whose risk of death is increased during a follow-up of up to 1 year.⁹⁴,⁹⁵ The nature of the relationship between major bleeding complications and long-term mortality is unclear. Although this association remained statistically significant after adjustment for confounders, it is still possible that bleeding may simply be a marker of an underlying severe disease state, which exposes the patient to increased risk of mortality. A direct causal effect of major bleeding on the long-term risk of death is doubtful, and it is ruled out by the observation that major bleeding after CABG surgery is not associated with increased mortality.⁹⁴,⁹⁶ Yet, the possibility that non–CABG-related major bleeding may be indirectly causally associated with increased mortality of patients under treatment with antithrombotic drugs is biologically plausible. Antithrombotic drugs are usually withheld in patients who experience major bleeding, and this exposes them to high risk of MACE. In keeping with this hypothesis is the observation that major bleeding was also associated with increased risk of ischemic events, such as myocardial infarction and stroke.⁹⁷

**Old thienopyridines versus ASA**

Randomized clinical trials that compared old thienopyridines (ticlopidine or clopidogrel) with ASA showed that the risk of bleeding was not different between the 2 treatment arms (Table 3). This observation is somewhat surprising, considering that in vitro and in vivo experiments demonstrated that ADP plays a more important role in platelet thrombus formation than thromboxane A₂. Although there are many plausible explanations for these unexpected results, the high prevalence of nonresponders to old thienopyridines, who are not exposed to the risk of bleeding, is the most plausible.

**Combined treatment with clopidogrel and ASA**

Combined treatment with clopidogrel and ASA is associated with increased bleeding compared with monotherapy. If this is a fair price to pay when treating patients with ACS, in consideration of the net clinical benefit associated with combined therapy, it is unacceptable for secondary prophylaxis of patients with stable disease or for primary prophylaxis of patients at risk because the higher bleeding risk is not counterbalanced by antithrombotic efficacy in these settings (Table 3).

**New P2Y₁₂ inhibitors versus clopidogrel**

The higher incidence of bleeding complications that was observed in patients treated with the new P2Y₁₂ antagonists prasugrel and ticagrelor, compared with clopidogrel (Table 4), is mostly explained by the fact that the new drugs effectively inhibit P2Y₁₂-dependent platelet function in the great majority of treated
patients, who will not only be protected from MACE, but will also be exposed to higher risk of bleeding, compared with patients treated with clopidogrel who do not respond adequately to the drug. This hypothesis is biologically plausible and is supported by the observation that patients who respond adequately to clopidogrel not only are better protected from MACE, but also experience an increased incidence of bleedings, compared with poor responders.

P2Y12 inhibitors and CABG-related bleeding

A variable percentage of patients with acute coronary syndromes (< 10%) need to undergo CABG surgery. It has been demonstrated that clopidogrel treatment within approximately 4 days of the procedure is associated with increased blood loss, reoperation for bleeding, increased transfusion requirements, and prolonged intensive care unit and hospital stays. For this reason, when the clinical conditions of the patients allow it, clopidogrel is usually withheld for 5 days before CABG, to restore the hemostatic competency of the patient. This procedure was followed for all patients undergoing CABG in randomized clinical trials that compared the new P2Y12 antagonists to clopidogrel. For this reason, the incidence of CABG-related bleeding complications should not be considered when evaluating the risk of bleeding associated with the new anti-P2Y12 drug, for the simple reason that patients were off-treatment when they underwent CABG. Differences among P2Y12 antagonists in this setting should be evaluated on the basis of the time needed to withhold treatment before surgery to restore hemostatic competency. Considering that withholding antiplatelet treatment exposes patients to high risk of MACE, it is obvious that drugs with reversible mechanism of action and short half-life, such as ticagrelor, may be preferable to drugs that irreversibly inhibit the receptor.

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