THIENOPYRIDINES AND TICAGRELOR

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Thienopyridines and Ticagrelor

ANCC Accredited NCPD Hours: 2.3 hrs

Target Audience: RN/APRN

Need Assessment

Thienopyridines are a class of drug targeting the platelet adenosine diphosphate-2 receptor. Blockage of the receptor results in reduced platelet activation. They significantly reduce platelet activity and are therefore clinically beneficial in settings where platelet activation is a key pathophysiological feature, particularly myocardial infarction. Thienopyridines play a key role in the cardiovascular arena. Their application covers the wide spectrum of patients with stable angina, acute coronary syndrome and /or those undergoing percutaneous coronary interventions. In these settings they reduce the restenosis rate, the risk of thrombosis and adverse cardiac events.

collaborating as an inter-professional team to achieve optimal patient results. An interprofessional approach, with a multifaceted and targetted approach to treatment, is necessary to improve patient outcomes with antiplatelet medications.

Objectives

- Discuss the mechanism of action of thienopyridines
- Describe the pharmacokinetic profile of Ticagrelor
- Discuss the limitations of Ticagrelor
- Identify the two widely used antiplatelet medications

Goal

The goal of this article is to provide a comprehensive summary of the pharmacokinetic, pharmacodynamic and pharmacogenetic profiles of thienopyridines and ticagrelor including their potential to mediate anti-inflammatory effects, to conserve vascular function

Introduction

Coronary artery disease (CAD) and acute coronary syndrome (ACS) are leading causes of mortality in the United States. Interventions that may reduce the incidence of acute coronary syndrome or mitigate its sequelae are therefore of substantial societal benefit. However, the populations evaluated in most randomised cardiovascular outcomes trials do not reflect the broad range of ethnicities to which the therapies studied may be subsequently applied in clinical practice. Engaging under-represented communities in research and identifying patient subgroups whose response to a given therapy may differ from that of the average patient in a trial are priorities for scientific research and investment. Ticagrelor is an oral platelet P2Y12-receptor antagonist that significantly reduces major cardiovascular events after acute coronary syndrome compared with clopidogrel, driven by reductions in myocardial infarction and cardiovascular death, without a significant increase in all-cause major bleeding, although non-coronary artery bypass grafting related bleeding is increased. [1, Rank 5]

Antiplatelet Therapy

Platelets stop bleeding from damaged blood vessels and initiate repair processes. They contain many important components for these functions, of which surface glycoproteins are critical for two processes, adhesion and aggregation (as shown in figure 1. Vascular damage such as rupture or erosion of an atherosclerotic plaque can trigger platelet aggregation. Platelets normally circulate through the vasculature in a non-adhesive state. Upon the detection of an exposed sub endothelial matrix, platelets are induced to come into close contact with the vessel wall and roll, then arrest, at the site of the vessel injury. The process of adhesion is orchestrated by the platelet adhesion receptors GPVI and GPIb-IX-V. The release of soluble agonists, such as adenosine diphosphate and thromboxane A2 amplify platelet activation. Platelet adhesion and activation results in the formation of a platelet plug. The resultant thrombus may lead to vascular occlusion, causing hypoxia and tissue damage. Thrombus occlusion of a coronary artery, which may result in an acute myocardial infarction, is the proximate cause of acute coronary syndrome (ACS). With platelets thus playing a central role in atherothrombosis and consequent pathologies, antiplatelet therapy has

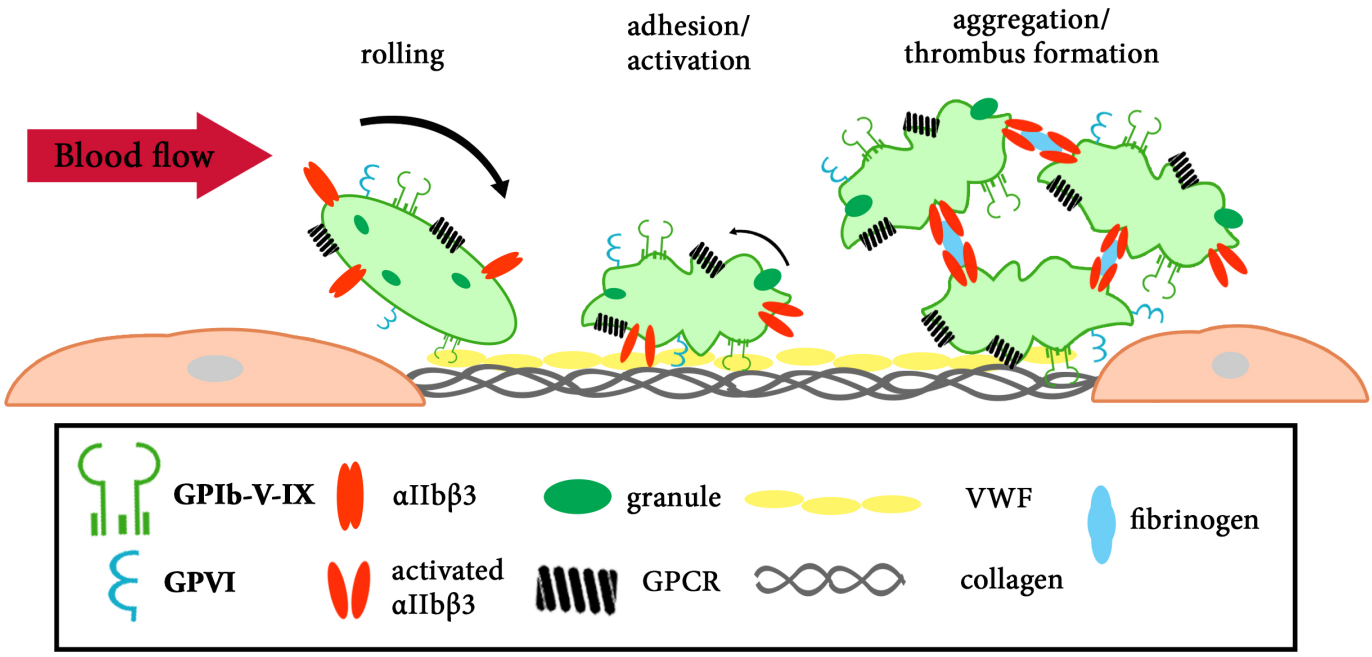


Figure 1: Platelet adhesion and aggregation

emerged as a cornerstone of treatment for acute coronary syndrome patients. [2, Rank 4]

The current standard antiplatelet therapy consists of aspirin, targeting the thromboxane-induced pathway of platelet activation (as shown in figure 2), combined with a P2Y12 receptor antagonist that inhibits the adenosine diphosphate-induced platelet activation. Aspirin irreversibly inhibits platelet cyclooxygenase 1 preventing the formation of prostaglantin H2 and therefore thromboxane A2.

The most frequently used P2Y12 receptor antagonist is clopidogrel, a thienopyridine, administered at a daily dose of 75 mg. However, clopidogrel has drawbacks (as shown in figure 3) such as delayed onset of action, interpatient variability in response or even resistance to clopidogrel,

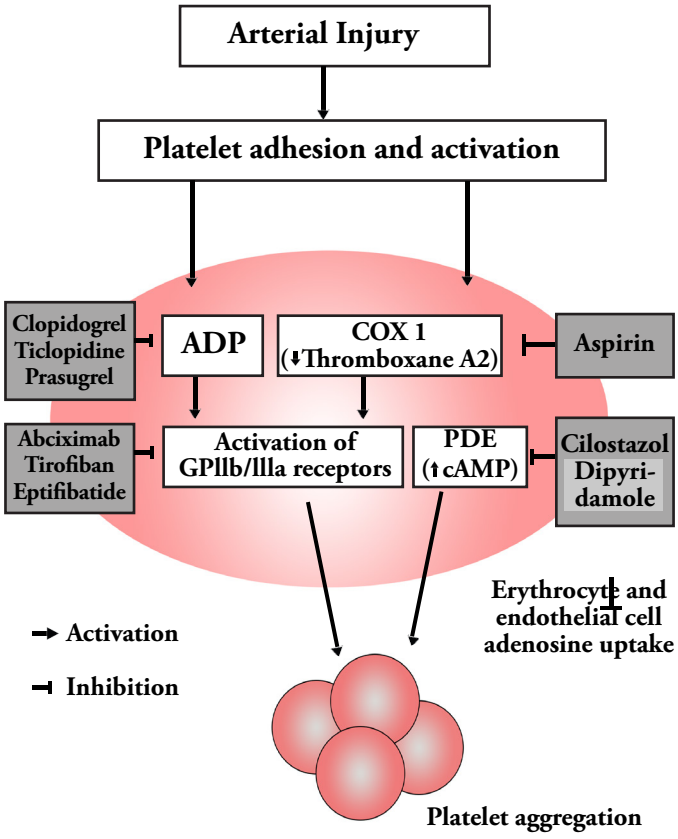


Figure 2: Thromboxane A2 platelet activation pathway

and irreversibility of its inhibitory effect, which increases the risk of bleeding in patients requiring surgery.

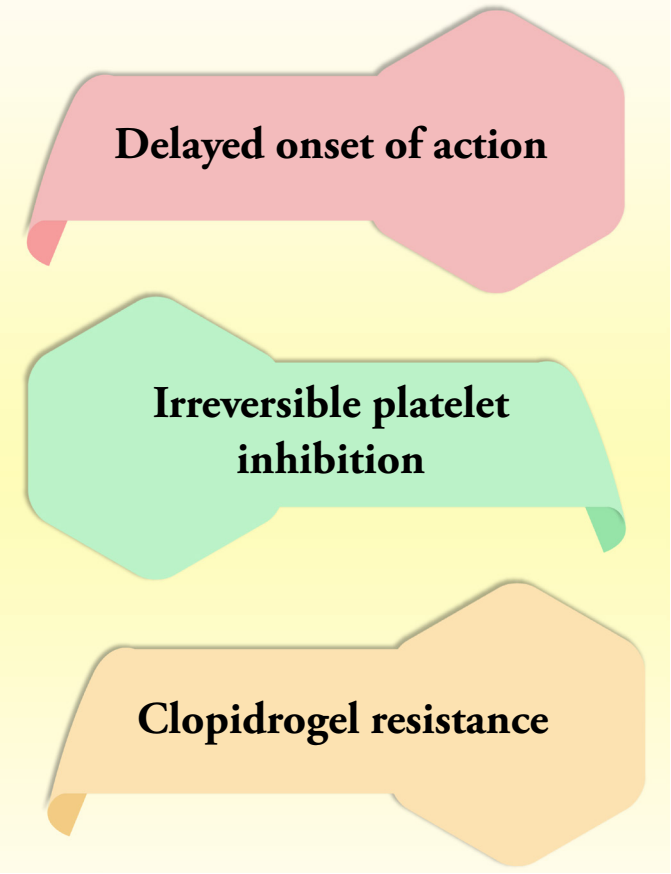


Figure 3: Drawbacks of clopidogrel

Antiplatelets can be classified based on the mechanism of action (as shown in figure 4):

- Platelet aggregation inhibitors such as aspirin and related cyclooxygenase inhibitors, oral thienopyridines such as clopidogrel, ticagrelor, ticlopidine and prasugrel.
- Glycoprotein platelet inhibitors like abciximab, eptifibatide, tirofiban.
- Protease-activated receptor-1 antagonists like vorapaxar.
- Miscellaneous drugs like dipyridamole, cilostazol.

P2Y12 receptor blockers are a group of antiplatelet drugs. This group of drug includes clopidogrel, ticlopidine, prasugrel and cangrelor (as shown in figure 5).Recently, more potent antiplatelet drugs have been developed, such as ticagrelor which is the first of a new chemical class of oral cyclopentyl-triazolo-pyrimidine antiplatelet agents. Ticagrelor is a direct acting and reversibly binding P2Y12 antagonist that is able to overcome non-responsiveness to clopidogrel. Treatment with ticagrelor, as compared with clopidogrel, has been shown to reduce significantly the rate of death from vascular causes, myocardial infarction or stroke in acute coronary syndrome patients. These beneficial effects were achieved without a significant increase in the rate of overall major bleeding, although an increase in the rate of non-procedure-related bleeding was noted. The reversible binding of ticagrelor and its plasma half-life of 7–8.5h mandate twice daily dosing (usually 90mg per dose)

“Thromboxane A2 is a positive feedback lipid mediator produced following platelet activation.

Thromboxane A2 is a powerful vasoconstrictor and promoter of platelet aggregation”

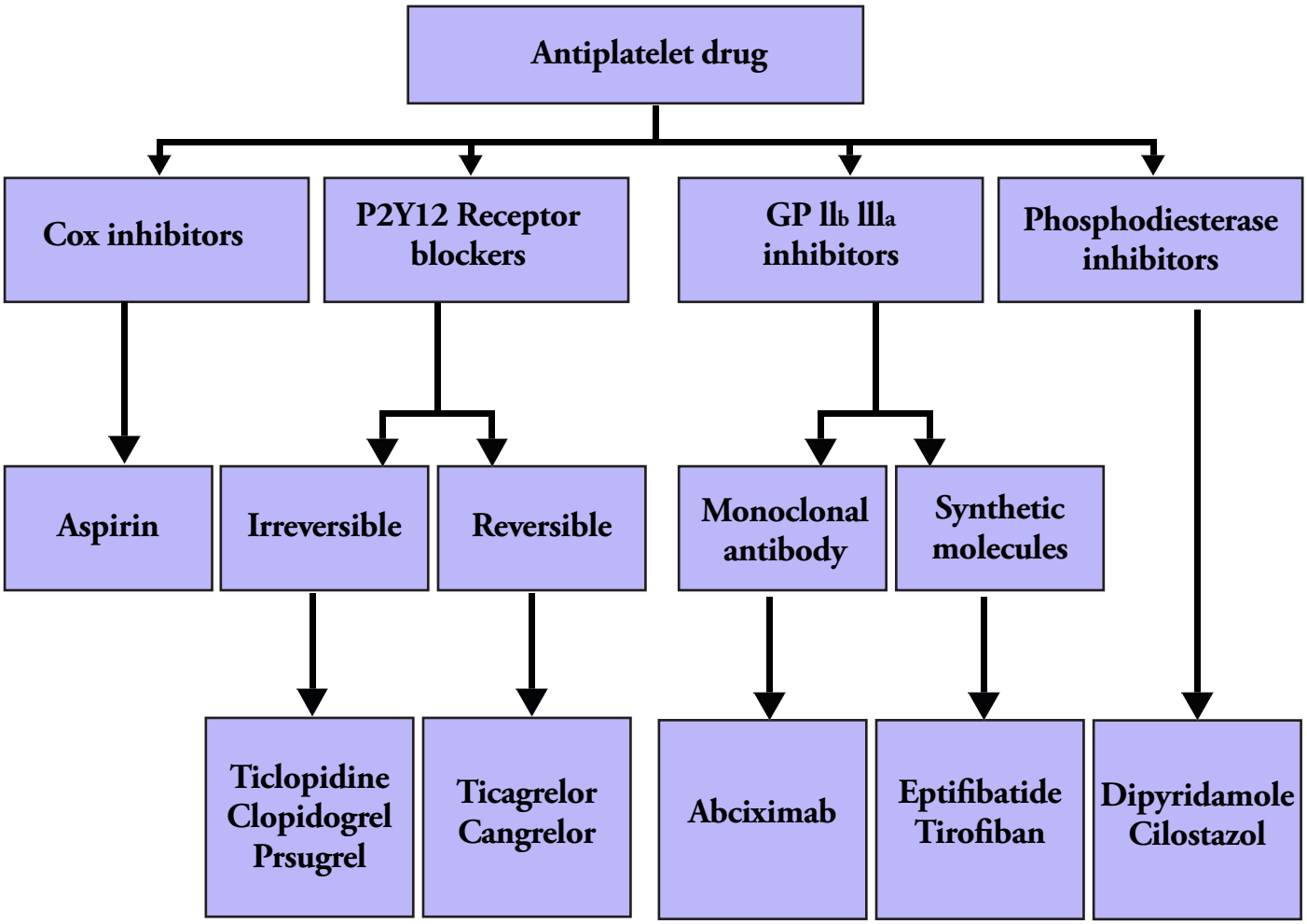


Figure 4: Antiplatelet drugs

Many studies have shown that patients on a once daily dosing regimen take a somewhat higher percentage of prescribed doses than patients on a twice daily dosing regimen. However, the pertinent therapeutic question to be addressed is how these missed doses affect the clinical benefits of the drug. One intermediate endpoint that can be assessed is the extent to which drug action, i.e. platelet inhibition, is maintained in the face of occasionally delayed or missed doses during twice daily or once daily dosing. The ability of drugs to maintain therapeutic activity in patients with non-adherent dosing behaviour is referred to as 'drug forgiveness', whereby drugs with duration of action far exceeding their dosing interval are considered more 'forgiving'. [3, Rank 5]

Assessment of the continuity of platelet inhibition requires an integrated analysis of dosing history data together with the pharmacokinetic (PK) and pharmacodynamic (PD) properties of the drugs being compared. Electronic methods for compiling drug dosing histories are now the recognized standard for quantifying adherence and they provide a high

> "Clopidogrel is most widely used and it causes irreversible alternations in P2Y12 receptor mediated platelet inhibition"

temporal resolution. When combined with established pharmacometric methods, these dosing histories allow the projection of drug concentration levels and of drug action in the body. [4, Rank 3]

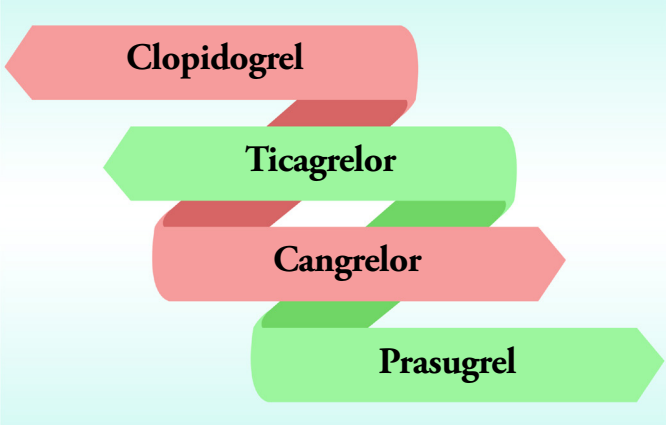


Figure 5: P2Y12-ADP receptor antagonist

Thienopyridines and Ticagrelor

Current standard treatment for acute coronary syndrome (ACS) is clopidogrel in combination with aspirin. Clopidogrel is the only thienopyridine antiplatelet therapy currently recommended by the National Institute for Health and Clinical Excellence (NICE) for the management of non-ST-segment elevation acute coronary syndrome in people at moderate-to-high risk of myocardial infarction or death. However, clopidogrel has been shown to produce an overall low degree of inhibition of platelet aggregation (IPA) and wide response variability (approximately 15–48% of patients have a poor platelet inhibition response to clopidogrel). Furthermore, growing evidence suggests that persistence of enhanced platelet reactivity despite the use of clopidogrel is associated with adverse clinical outcomes, including an increased risk for recurrent ischaemic events. Some of the limitations of clopidogrel have been addressed by prasugrel, a recently approved thienopyridine that has demonstrated more consistent platelet inhibition. However, prasugrel was associated with significantly increased risk of major bleeding, including life-threatening and fatal bleeding. There is therefore still a need for new antiplatelet therapies for acute coronary syndrome that provides high and consistent inhibition of platelet aggregation. [5, Rank 4]

Ticagrelor (AZD6140) is the first reversibly binding oral P2Y12 receptor antagonist, which is in clinical development for the treatment of acute coronary syndrome. In vitro studies indicate that ticagrelor exerts its antiplatelet activity by binding to the P2Y12 receptor at a site distinct from the adenosine diphosphate (ADP) binding site, thereby inhibiting adenosine diphosphate-induced receptor signalling in a non-competitive manner.

> "Ticagrelor is a direct acting oral antagonist of P2Y12-adenosine receptor blocker with reversibility and without catabolic activation, which can have a substantial impact on faster and greater platelet inhibition than clopidogrel"

Ticagrelor undergoes extensive hepatic metabolism, with one known active metabolite (AR-C124910XX) present at a concentration approximately 30% of the parent drug. Unlike the thienopyridines, however, ticagrelor does not require metabolic activation to exert its effect, which may account for its fast onset of action. Based on mass balance data, renal clearance of ticagrelor is limited and elimination occurs mainly through metabolism in the liver and biliary excretion.

Studies suggest that ticagrelor may provide improvements in inhibition of platelet aggregation compared with clopidogrel. Compared with clopidogrel, ticagrelor offered a wider separation between antithrombotic effects and bleeding time in preclinical models and, in phase II studies, provided greater and more consistent inhibition of adenosine diphosphate-induced platelet aggregation without an increase in major plus minor bleeding. The efficacy and safety of ticagrelor has been evaluated in comparison with clopidogrel in a broad population of acute coronary syndrome patients, in the double-blind, randomized phase III PLATO (PLATelet inhibition and patient Outcomes) study. After 12 months, a lower proportion of patients receiving ticagrelor than clopidogrel reached the primary endpoint of death from vascular causes, myocardial infarction or stroke (9.8% vs. 11.7%; $P < 0.001$), with no increase in the rate of overall major bleeding and an increase in the rate of non-procedure-related bleeding. [6, Rank 2]

Single ascending dose studies in healthy volunteers indicate that ticagrelor has linear pharmacokinetics and, at doses ranging from 100 to 400 mg, produces near-complete inhibition of platelet aggregation at 2 h post-dose. Inhibition of platelet aggregation gradually decreased with declining plasma concentrations starting around 12 h post-dose, indicating that the inhibition of platelet aggregation associated with ticagrelor is concentration dependent and reversible. [7, Rank 2]

Pharmacokinetic Profile

Absorption, Distribution, Metabolism and Excretion

The pharmacokinetic profile of ticagrelor has been evaluated in healthy volunteers and in patients with coronaryartery

disease, atherosclerosis and acute coronary syndrome. A regional absorption study in healthy volunteers showed that the proportion of ticagrelor that was absorbed decreased the further down the gastrointestinal tract that the dose was released. In healthy volunteers, single oral doses of ticagrelor 0.1–400 mg were rapidly absorbed, with a median time to reach the maximum plasma concentration (tmax) of approximately 1.3–2 h. Similarly, the median tmax for AR-C124910XX (the active metabolite of ticagrelor) was 1.5–3 h. Alternative methods of administration have been shown to increase the rate of absorption of ticagrelor. In healthy volunteers, administration of ticagrelor as a crushed tablet (dosed orally or via a nasogastric tube) increased plasma concentrations of ticagrelor and AR-C124910XX at early time points (0.5 and 1 h post-dose) relative to oral administration of a whole tablet. The ticagrelor tmax was shorter following crushed versus whole tablet administration (1 vs. 2 h). Further studies are warranted to assess whether administration of crushed tablets provides a pharmacodynamic or clinical benefit in scenarios in which the absorption of ticagrelor occurs less rapidly than predicted, e.g. in patients with acute coronary syndrome, in whom tmax values of 3 and 4 h have been reported for ticagrelor and AR-C124910XX, respectively, following a 180 mg loading dose. [8, Rank 1]

Mean and maximum plasma concentration (Cmax) values of ticagrelor and AR-C124910XX increase linearly and predictably in a dose-dependent manner and are stable at steady state in healthy volunteers and in patients with atherosclerosis, coronary artery disease and acute coronary syndrome. The effect of food on exposure to ticagrelor and AR-C124910XX is small and considered to be of minimal clinical significance. Therefore, ticagrelor can be administered with or without food. [9, Rank 3]

In vitro studies evaluating the metabolism of ticagrelor have been conducted in hepatocyte and microsomal preparations from several animal species. Multiple metabolites were identified and the major metabolites across all species were AR-C124910XX and AR-C133913XX. Cytochrome P450 (CYP) 3A4 and CYP3A5 were primarily responsible for the formation of AR-C124910XX, whereas the formation of AR-C133913XX most likely occurred via CYP3A4, with a lesser contribution from CYP3A5. Consequently, potential interactions between ticagrelor and inducers or inhibitors of CYP3A4 were evaluated in clinical pharmacology studies. [10, Rank 4]

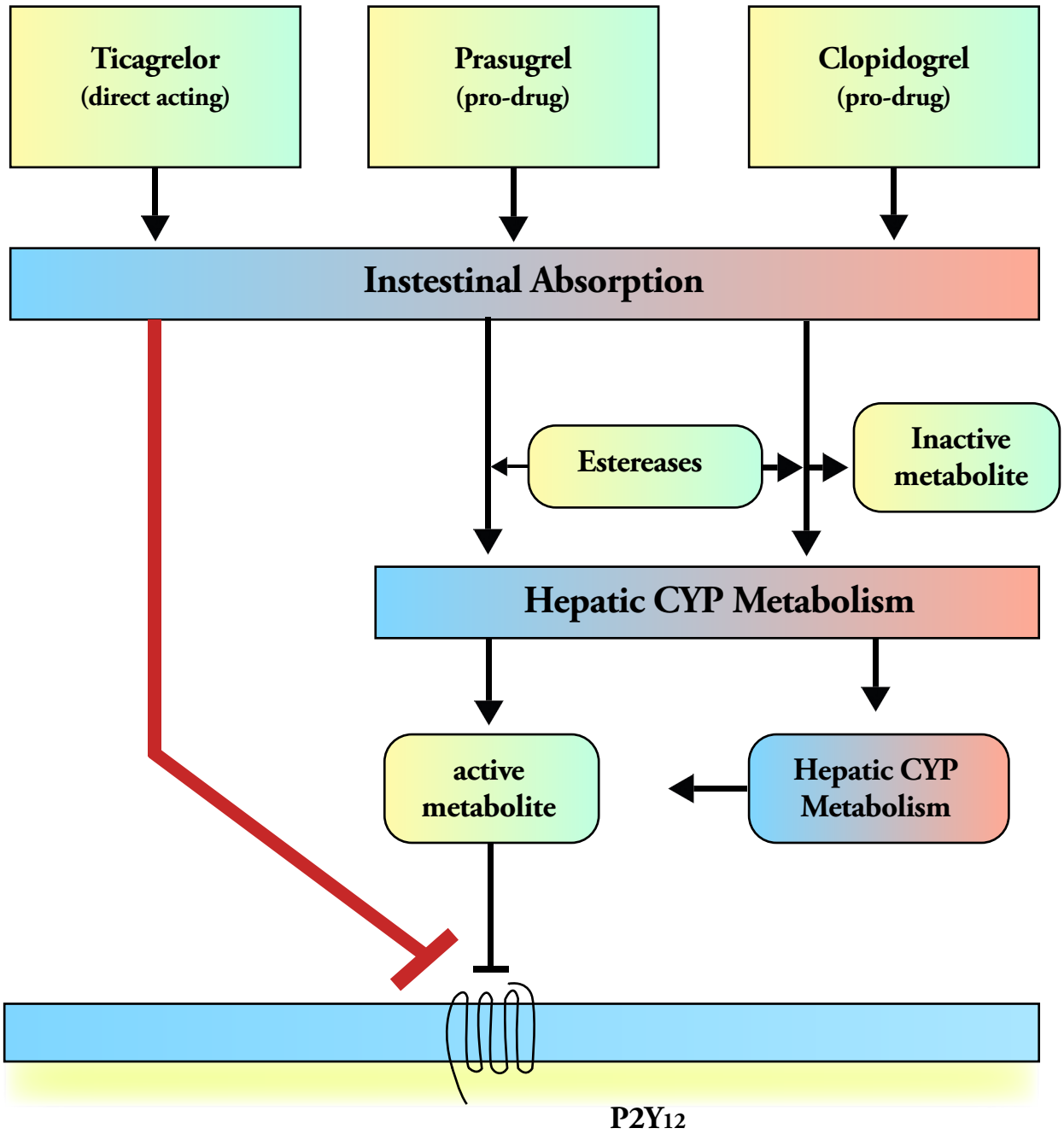


Figure 6: Pharmacokinetic profile

Experiments conducted with radio labelled ticagrelor in six healthy male volunteers identified ten discrete metabolites, with unchanged ticagrelor and AR-C124910XX being the predominant entities observed in the plasma. AR-C124910XX is present at approximately 30–40 % of the concentration of the parent compound. Following administration of [14C]-ticagrelor, 58 % was recovered in the faeces and 27 % in the urine; the levels of unchanged ticagrelor and AR-C124910XX in the urine were <0.05 %. These data indicate that ticagrelor is mainly excreted in the faeces, with renal excretion playing only a minor role; the

primary route of excretion for the active metabolite is most likely biliary secretion.

The mean elimination half-life of ticagrelor is 7.7–13.1 h, whereas the mean elimination half-life of AR-C124910XX is 7.5–12.4 h.[11, Rank 5]

Pharmacokinetics in Special Populations

In 40 healthy volunteers who received ticagrelor 200 mg, systemic exposure to ticagrelor and AR-C124910XX was approximately 40–60 % higher in elderly versus young subjects and in women versus men. However, no dose adjustment is considered necessary on the basis of either age or gender; nor is dose adjustment required in patients with severe renal impairment or mild hepatic impairment. The use of ticagrelor is contraindicated in patients with severe hepatic impairment. As ticagrelor metabolism occurs in the liver, exposure to the parent drug will probably increase in severe hepatic impairment. In addition, the bleeding risk is increased in severe hepatic impairment because of reduced synthesis of coagulation proteins. No pharmacokinetic data are available in patients with moderate hepatic impairment (need to consider the risk–benefit of ticagrelor due to probable increase in exposure) or patients receiving renal dialysis. [12, Rank 3]

In PLATO, the incidence of dyspnoea was higher with ticagrelor compared with clopidogrel. Ticagrelor inhibits platelet activity via P2Y12 receptor inhibition and also via adenosine. A potential mechanism of ticagrelor-induced dyspnoea involves increased endogenous levels of adenosine. Adenosine can induce dyspnoea in normal and asthmatic subjects. Thus, two randomized, double-blind, placebo-controlled, two-way crossover studies were conducted to evaluate the pharmacokinetics, pharmacodynamics and safety of ticagrelor in subjects at risk of respiratory impairment (healthy elderly) and in patients with respiratory impairment (mild asthma or mild to moderate chronic obstructive pulmonary disease [COPD]). Although exposure to ticagrelor (at steady state) and AR-C124910XX (after a single dose of ticagrelor and at steady state) appeared to be lower in patients with asthma or chronic obstructive pulmonary disease compared with healthy elderly volunteers, no consistent relationship was observed between exposure and minute ventilation, respiratory rate, tidal volume, spirometry, pulse oximetry or dyspnoea. Furthermore, ticagrelor did not appear to alter pulmonary function at rest or during exercise; bronchospasm (as determined by spirometry and pulse oximetry) was not observed in any cohort. [13, Rank 3]

The pharmacokinetics of ticagrelor has also been assessed in several different ethnic groups. Ticagrelor and AR-C124910XX exhibited linear and predictable pharmacokinetics in healthy Chinese volunteers at doses of 90 and 180 mg. Exposures to ticagrelor and AR-C124910XX at steady state were slightly higher in Chinese volunteers compared with Caucasian volunteers in other studies. The pharmacokinetics of ticagrelor and AR-C124910XX in Japanese volunteers was assessed in two ethnicity-bridging studies. Pharmacokinetic profiles were broadly comparable between Japanese and Caucasian volunteers following single ascending doses (50–600 mg) and multiple ascending doses (100 or 300 mg) of ticagrelor. After adjustments to account for differences in body weight between the ethnic groups, exposure to ticagrelor and AR-C124910XX was approximately 20 % greater in Japanese versus Caucasian participants. These findings were consistent with observations in a similar study of Japanese patients with coronary artery disease. In DISPERSE (Dose Confirmation Study Assessing Anti-Platelet Effects of AZD6140 vs. Clopidogrel in Non-ST-segment Elevation Myocardial Infarction), after treatment with ticagrelor (45 or 90 mg twice daily) for 28 days, the area under the plasma concentration–time curve (AUCτ) and Cmax of ticagrelor and AR-C124910XX were 1.3- to 1.5-fold greater in Japanese patients than in Caucasian patients. The observed differences in exposure to ticagrelor in Chinese and Japanese versus Caucasian patients are not of sufficient magnitude to warrant dose adjustment on the basis of ethnicity. [14, Rank 1]

Pharmacodynamics

Ticagrelor has more consistent and increased platelet inhibition as compared to clopidogrel (as shown in figure 7). The specifics of the onset of platelet inhibition and duration of inhibition of both ticagrelor and clopidogrel were investigated in the ONSET/OFFSET (A Study of the Onset and Offset of Antiplatelet Effects Comparing Ticagrelor, Clopidogrel, and Placebo With Aspirin) study via light transmittance aggregometry (LTA), VerifyNow® (Accumetrics, San Diego, CA, USA), and vasodilator-stimulated phosphoprotein, phosphorylation assay in patients with stable coronary artery disease. Greater platelet inhibition was seen 0.5, 1, 2, 4, 8, and 24 hours after the loading doses of each agent and at the end of the maintenance dose phase of the study at 6 weeks ($P < 0.0001$ at all time periods). Two hours following the loading dose, 98% of subjects in the ticagrelor group had >50% platelet inhibition

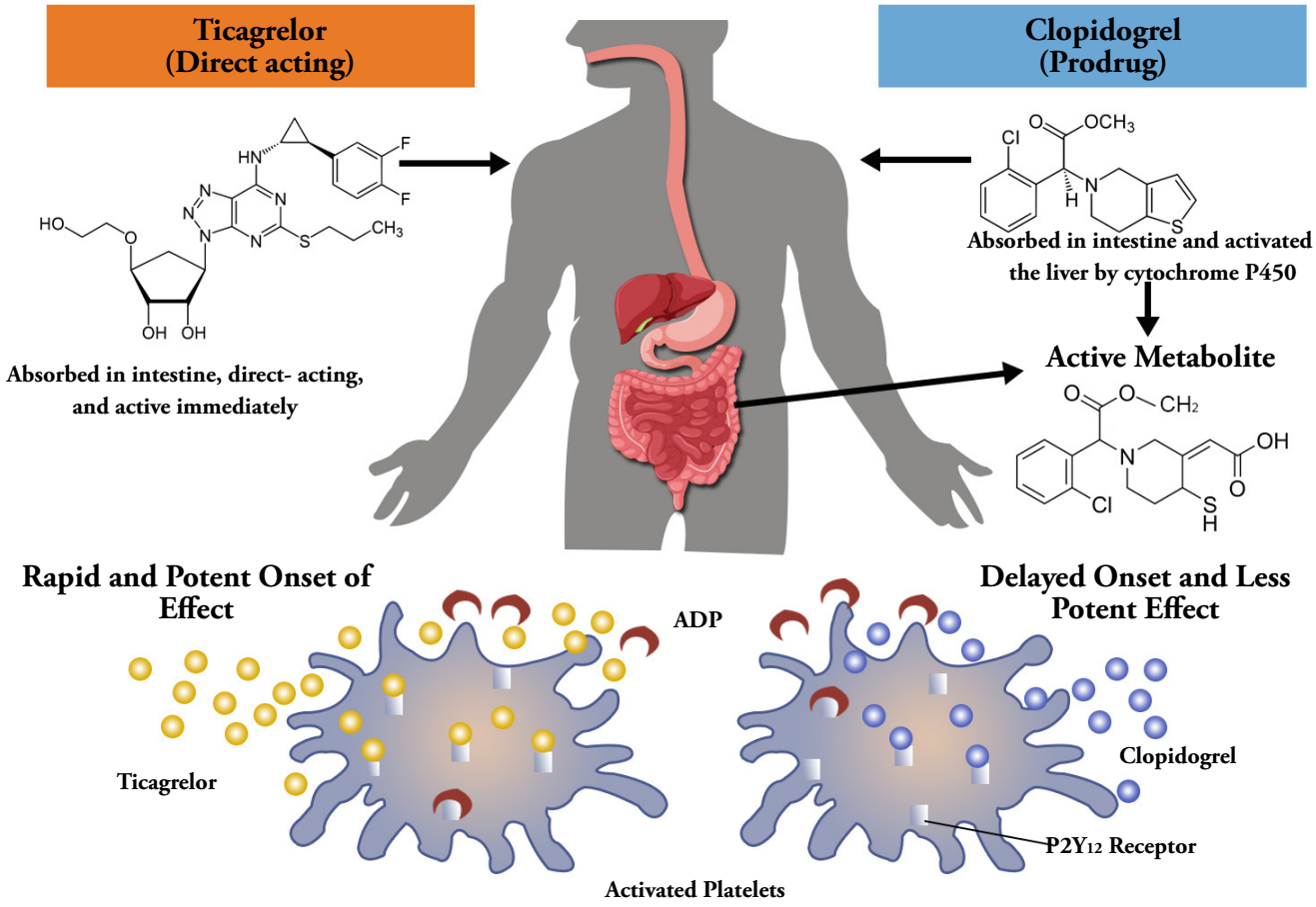


Figure 7: Pharmacodynamics of Ticagrelor and Clopidrogel

compared to 31% in the clopidogrel group and 90% of subjects in the ticagrelor group had >70% platelet inhibition compared to 16% in the clopidogrel group (P< 0.0001 for both comparisons). The study found that the offset of ticagrelor was also more rapid, with similar platelet inhibition on day three of ticagrelor compared to day five of clopidogrel. Similarly, platelet inhibition of ticagrelor at day five and clopidogrel at day seven were similar to placebo. [15, Rank 2]

In DISPERSE (Dose Confirmation Study Assessing Antiplatelet Effects of AZD6140 Versus Clopidogrel in NSTEMI) and DISPERSE-2, the platelet inhibition of ticagrelor and clopidogrel were evaluated via optimal aggregometry in patients with stable atherosclerosis. Ticagrelor exhibited maximal platelet inhibition 2–4 hours post dose (90%–95%), whereas platelet inhibition with clopidogrel was minimal during this timeframe (60%). DISPERSE-2 compared the antiplatelet effect of ticagrelor in patients previously exposed to clopidogrel and those that were clopidogrel naïve. Ticagrelor produced greater platelet inhibition regardless of previous exposure to clopidogrel and to an extent similar to that of the DISPERSE trial. [16, Rank 3]

The RESPOND (A Study of the Antiplatelet Effects Comparing Ticagrelor With Clopidogrel Responders and Non responders) study investigated the response to ticagrelor in patients with stable coronary artery disease who were identified as responders or non-responders to a 300 mg loading dose of clopidogrel. Responsiveness was based on adenosine diphosphate-induced platelet aggregation measured before and 6–8 hours after the dose. Non responders were identified when the absolute change in platelet aggregation was ≤10%. Inhibition of platelet aggregation (via LTA, Verify Now, and vasodilator-stimulated phosphoprotein phosphorylation assay) was significantly greater However, in a pharmacokinetic analysis in 1159 patients participating in the TRITON-TIMI 38 trial, the systemic exposure to prasugrel was not appreciably affected by diabetes mellitus status. Of note, a separate analysis of prasugrel active metabolite kinetics for insulin-treated diabetic was not performed. It seems therefore that our findings of a neutral and a negative impact of non insulin-treated diabetes mellitus and insulin-treated diabetes mellitus on platelet reactivity respectively, could be attributed to platelet dysfunction in the latest high risk subgroup which cannot be entirely overcome by the potent antiplatelet thienopyridine prasugrel. In the largest so far pharmacodynamic study of patients under prasugrel maintenance treatment, an HPR rate of 10-15 % has been described, although not stratified by diabetes mellitus status. In the present study, HPR rate under prasugrel therapy was slightly lower, while a trend for a progressive increase according to diabetes mellitus status and type of treatment was apparent. [27, Rank 2]

In non-responders treated with ticagrelor compared to clopidogrel ($P < 0.05$). Platelet inhibition decreased in patients switched from ticagrelor to clopidogrel and increased in those switched from clopidogrel to ticagrelor. This was demonstrated with ticagrelor's ability to overcome non responsiveness to clopidogrel with a >10%, >30%, and >50% decrease in platelet aggregation from baseline in 100%, 75%, and 13% of patients, respectively. [17, Rank 2]

The pharmacodynamic effect of P2Y12 receptor antagonists, on or off target, is related to the exposure of the active drug. The active metabolites of the thienopyridines can only be detected in the systemic circulation for about 4 h following intake of the prodrug and then only at levels below their in vitro IC50values, determined as the concentration providing 50% inhibition of adenosine diphosphate-induced platelet aggregation. One can therefore argue that the thienopyridines have limited or no systemic exposure and

one may hypothesize that the platelet inhibition by thienopyridines mainly takes place in the hepatic circulation where the active metabolites are formed and therefore likely present at higher concentrations. Because of their irreversible mode of action, however, thienopyridines can overcome the shorter duration of action of their active metabolite so that they still can provide platelet inhibition over 24 h. [19, Rank 2]

In contrast to thienopyridines, ticagrelor does not require hepatic enzyme-dependent conversion into an active metabolite. That said ticagrelor has a major circulating metabolite, AR-C124910XX, with plasma exposure of 30–40% and similar potency versus P2Y12 as the parent ticagrelor. Ticagrelor is given twice a day, which provides a biphasic increase in the plasma concentration of ticagrelor and AR-C124910XX over 24 h. Given that both ticagrelor and its metabolite are active and reversibly binding to the platelet P2Y12 receptor, the main pharmacodynamic effect, platelet inhibition, is directly related to the combined exposure of ticagrelor and AR-C124910XX in the systemic circulation. As predicted from the in-vitro potency data, a high and continuous platelet inhibition is achieved in patients as the plasma exposure of ticagrelor is well above its in vitro IC50throughout 24 h. The presence of an active drug in the systemic circulation throughout 24 h provides what can be referred to as a systemic potential. [20, Rank 3]

Pharmacogenomics

A limitation of the conventional thienopyridine P2Y12 receptor inhibitors is the fact that both clopidogrel and prasugrel are pro-drugs requiring hepatic metabolism via various CYP isoenzymes to convert to their active metabolites. In particular, clopidogrel requires a two-step process for hepatic metabolism, in which the CYP2C19 isoenzyme is responsible for more than half of the first-step formation. There are at least three major genetic polymorphisms of the CYP2C19 isoenzyme. CYP2C19*1 corresponds to normal function metabolism and CYP2C19*2 and *3 are loss-of-function alleles accounting for 85% and 99% of reduced function alleles in Caucasians and Asians, respectively. A patient with two loss-of-function alleles is referred to as a poor metabolizer. Package labeling for clopidogrel was updated to include a boxed warning informing health care providers of its potential for diminished effectiveness and risk for higher cardiovascular event rates in poor metabolizers of CYP2C19 isoenzyme. It states that genotyping is available and alternative treatment or treatment strategies could be considered

in those identified as CYP2C19 poor metabolizers. The American College of Cardiology Foundation and American Heart Association (AHA) also released a clopidogrel clinical alert statement addressing the FDA's boxed warning and emphasized that clinicians must be aware that genetic variability in CYP enzymes can alter clopidogrel metabolism, affecting its inhibition of platelet function, and clinical outcomes. However, similar to the FDA, they did not mandate or require genetic testing to allow for flexibility in clinical decisions. They suggest that genetic testing could be used in patients at moderate or high risk for poor outcomes (i.e., undergoing elective, high-risk percutaneous coronary intervention procedure) to determine if the patient is a poor metabolizer and may not benefit from clopidogrel. [21, Rank 4]

Prasugrel requires a single CYP-dependent step for conversion to its active metabolite predominantly through CPY3A4 and CYP2B6, thus not impacting patients with CYP2C19 polymorphisms. Observational studies have demonstrated no significant decrease in plasma concentrations of prasugrel or platelet inhibition activity in carriers of at least one loss-of-function allele of the CYP2C19 isoenzyme. Ticagrelor does not require transformation to an active metabolite and thus would avoid any loss of platelet inhibition activity that could be present in the presence of a loss-of-function CYP2C19 allele. [22, Rank 3]

There are three genetic studies underway to evaluate ticagrelor in acute coronary syndrome patients. One study seeks to enroll patients identified with both HTPR using standard-dose clopidogrel and with a loss-of-function allele CYP2C19*2 and assign them to standard maintenance doses of ticagrelor or prasugrel or higher maintenance dose of clopidogrel 150 mg daily for 1 month and evaluate platelet reactivity and major cardiac outcomes. Another study will evaluate the utility of genetic testing, in which the control group will have no genetic testing done and receive conventional therapy with clopidogrel compared to the intervention group utilizing genetic testing to identify those with or without two loss-of-function alleles (CYP2C19*2 and *3); the loss-of-function cohort will switch to ticagrelor and evaluate major cardiac outcomes at 1 year. One last study similarly seeks to evaluate the utility of genetic testing where the control group receives no genetic testing and standard therapy of ticagrelor, prasugrel, or clopidogrel at the physician's discretion compared to an algorithm-based modification of P2Y12 receptor inhibitor therapy based on genetic testing identifying loss-of-function alleles CYP2C19*2 or ABCB-1 C.58 The study

protocol states that ABCB-1 C carriers have been found to have reduced clopidogrel absorption and higher risk for ischemic adverse events if treated with clopidogrel. The study does not publish the phenotype–genotype P2Y12 algorithm; however, patients are maintained on their antiplatelet therapy for 1 year when platelet reactivity and major cardiac and bleeding outcomes will be evaluated. These studies will help provide further information on the utility of genetic testing and even the utilization of the combination of genetic testing and HTPR identified through point-of-care assays in the potential for more personalized medical use of P2Y12 inhibitors. [23, Rank 5]

ENT1 inhibition by ticagrelor

Adenosine is formed from adenosine triphosphate and adenosine diphosphate released locally at sites of ischaemia, tissue damage and inflammation. The t1/2 of adenosine in the circulation is a matter of seconds due to its rapid cell uptake and intracellular metabolism. Ticagrelor inhibits the ENT1 transporter (as shown in figure 8) and thereby reduces the cellular uptake of adenosine resulting in its prolonged local t1/2 and extracellular presence. Ticagrelor has been shown to increase the plasma levels of adenosine in acute

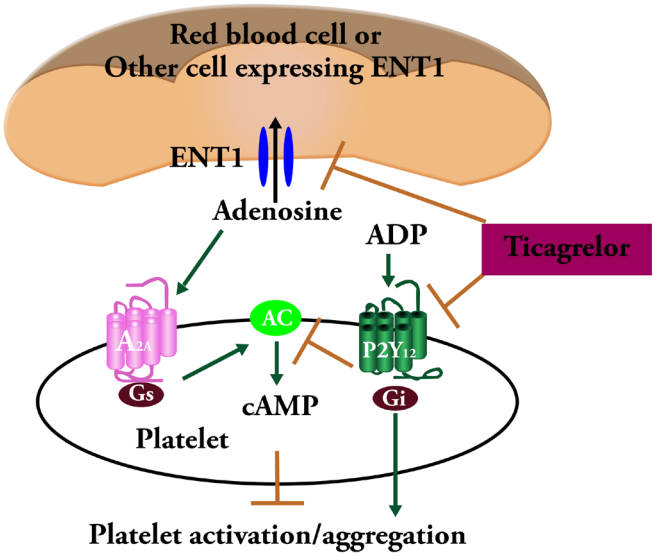


Figure 8: ENT1 inhibition by ticagrelor

coronary syndrome patients and to augment adenosine-induced physiological responses, including increases in coronary blood flow, platelet inhibition, neutrophil migration and the sensation of dyspnoea. Importantly, and unlike P2Y12 receptors, only ticagrelor and not AR-C124910XX significantly inhibits ENT1. In addition to ENT1 inhibition, ticagrelor induced adenosine triphosphate release from human erythrocytes in vitro; it remains unknown whether this effect of ticagrelor on erythrocytes occurs also in vivo. If present, both mechanisms would work together to further enhance the local concentration of adenosine as adenosine triphosphate is rapidly converted into adenosine monophosphate via the ectonucleoside triphosphate diphosphohydrolase 1 (CD39), with subsequent conversion into adenosine via the ecto-5 -nucleotidase (CD73) [24, Rank 2]

Juvenile platelets

Patients with increased platelet turnover have higher levels of immature platelets and an increase in the immature platelet count has been found to be associated with worse cardiovascular outcome supporting the hypothesis that these juvenile platelets are more pro-thrombotic. A continuous systemic exposure of an active compound, as true for ticagrelor, should theoretically inhibit newly formed, juvenile platelets equally well as the old platelets. This should not be the case for the thienopyridines as the platelets formed after elimination of the active metabolites from the circulation will not be inhibited until the next dose of pro-drug on the next day. In support, high immature platelets count has been found associated with lower anti-platelet response to clopidogrel. A rat study has shown that the recovery of platelet function after ticagrelor administration differs from that after clopidogrel treatment. Following ticagrelor administration, all platelets (old and newly formed) were inhibited, and their function gradually recovered over time in parallel with drug elimination. Following clopidogrel treatment, there was a gradually emerging subpopulation of uninhibited platelets, probably juvenile platelets. This difference in the platelet inhibition properties is masked by conventional platelet aggregation tests, which evaluate the net response of the total platelet population, but was revealed by thrombus formation measurement under flow where the juvenile un-inhibited platelets, formed at later time points after clopidogrel treatment, appeared to support thrombus formation.

In another study a mixture of uninhibited and prasugrel-inhibited platelets was studied to model the role of juvenile platelets formed each day. Twenty percent prasugrel-inhibited plus 80% uninhibited platelets were used to mimic diabetics that have a high platelet turnover with an approximate platelet lifespan of 5 days (thus about 20% new platelets should be formed each day). By imaging of adenosine diphosphate-induced aggregates of these mixtures, the authors showed that uninhibited platelets supported a core of aggregated platelets, which was surrounded by prasugrel-inhibited platelets. Another recent study explored platelet function after discontinuation of prasugrel in acute coronary syndrome patients. They found that prasugrel discontinuation resulted in the formation of an emerging subpopulation of adenosine diphosphate-responsive juvenile platelets, which contributed to platelet aggregation and thrombus formation under flow. Finally, a clinical study explored platelet

function in acute coronary syndrome patients on ticagrelor or prasugrel treatment. They found that the number of juvenile platelets (immature platelet count) correlated with platelet aggregation in patients treated with prasugrel, but not in those receiving ticagrelor, indicating that juvenile platelets contribute to increased platelet aggregation in prasugrel-treated, but not with ticagrelor-treated patients. [25, Rank 3]

Platelet Reactivity under Thienopyridine Treatment in Diabetes

Antiplatelet therapy with P2Y12 receptor inhibitors (as shown in figure 9) has become the cornerstone of medical treatment in patients with acute coronary syndrome, after percutaneous coronary intervention and in secondary prevention of atherothrombotic events. Previous studies of factors affecting platelet reactivity while on prasugrel therapy provided conflicting or unclear results concerning the impact of diabetes mellitus on it, without analyzing the type of diabetes mellitus treatment effect. Among 444 prasugrel-treated patients with acute coronary syndrome undergoing percutaneous coronary intervention and assessed 2 to 4 weeks after hospital discharge, patients with diabetes mellitus had higher vasodilator-stimulated phosphoprotein (VASP) index than non-diabetes mellitus patients, but this effect was not present in multivariate analysis. In a previous analysis, by our group, of 234 patients under prasugrel maintenance dose, constituting part of the present cohort, and assessed by the Verify Now, diabetes mellitus had a significant effect on platelet reactivity with 36.3 % increase compared to non-diabetes mellitus patients. In the present larger cohort, diabetes mellitus effect on prasugrel pharmacodynamics is further elucidated and seems to be mostly confined in insulin-treated diabetic patients. [26, Rank 4]

Several explanations could be discussed for the above findings. Platelets of diabetic patients present with a decreased sensitivity to insulin, up regulation of the P2Y12 pathway and increased reactivity. Mechanisms like increased exposure to adenosine diphosphate, increased cytosolic levels of calcium, and increased platelet turnover may also be implicated in the response to P2Y12 receptor blockers in diabetes mellitus patients. These abnormalities are likely more pronounced in the insulin-treated diabetic patient and may partially explain the observed impact on platelet reactivity under prasugrel. Moreover, and concerning the other thienopyridine, clopidogrel, active metabolite kinetic profile-and

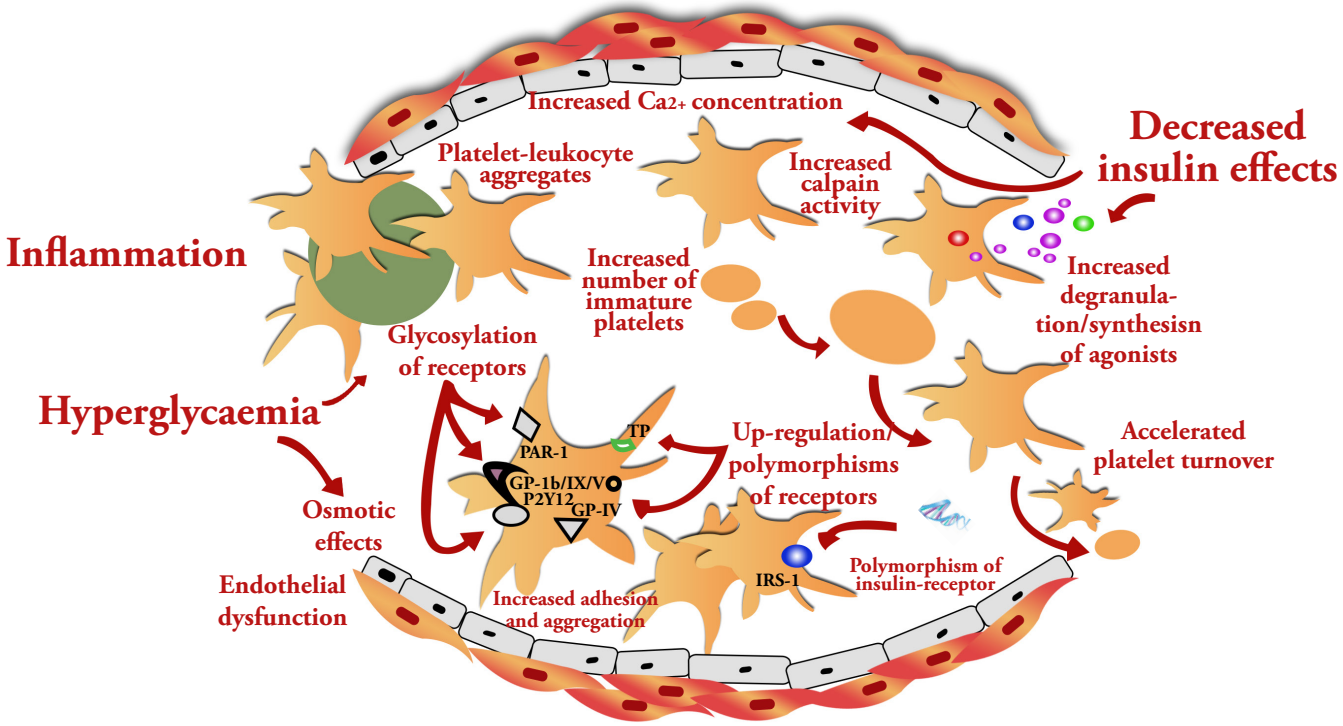


Figure 9: Antiplatelet therapy with P2Y12 receptor inhibitor

to a lesser degree platelet dysfunction-seem to be mostly responsible for the overall impaired platelet P2Y12 receptor blockade mediated in diabetes mellitus patients.

However, in a pharmacokinetic analysis in 1159 patients participating in the TRITON-TIMI 38 trial, the systemic exposure to prasugrel was not appreciably affected by diabetes mellitus status. Of note, a separate analysis of prasugrel active metabolite kinetics for insulin-treated diabetic was not performed. It seems therefore that our findings of a neutral and a negative impact of non insulin-treated diabetes mellitus and insulin-treated diabetes mellitus on platelet reactivity respectively, could be attributed to platelet dysfunction in the latest high risk subgroup which cannot be entirely overcome by the potent antiplatelet thienopyridine prasugrel. In the largest so far pharmacodynamic study of patients under prasugrel maintenance treatment, an HPR rate of 10-15 % has been described, although not stratified by diabetes mellitus status. In the present study, HPR rate under prasugrel therapy was slightly lower, while a trend for a progressive increase according to diabetes mellitus status and type of treatment was apparent. [27, Rank 2]

> **"Prasugrel and ticagrelor is more potent and predictable P2Y12 inhibitor compared to clopidogrel"**

Platelet Reactivity under Ticagrelor Treatment

In a patient-level data meta-analysis of 8 studies involving 445 ticagrelor-treated patients, diabetes mellitus did not emerge as a factor predicting platelet reactivity, although independently associated with lower probability for platelet reactivity <10 PRU. In line, in the present analysis, among ticagrelor-treated patients, no sign of any influence on platelet reactivity by diabetes mellitus was seen, even in the high risk group of insulin-treated diabetic patients. Hence, insulin-treated diabetes mellitus status may impact the thienopyridines clopidogrel and prasugrel action, but not ticagrelor's one, which is a cyclopentyl-triazolo-pyrimidine. Moreover, ticagrelor is a reversible P2Y12 adenosine diphosphate receptor blocker, administered twice daily, which may be more optimal for providing consistent inhibition for patients with high platelet turnover rates such as those with diabetes mellitus. Although it is unknown whether diabetes mellitus status modulates plasma levels of ticagrelor and its metabolite (AR- C124910XX), the described absence of any impact on its pharmacodynamics makes it extremely unlikely. In no case we advocate absence of platelet abnormalities following ticagrelor treatment. Only 1 signaling pathway, the P2Y12 one, is blocked by ticagrelor, leaving multiple other signaling pathways, many known to be unregulated in diabetes mellitus patients, uninhibited. [28, Rank 4]

Clinical Relevance

As the great majority of prasugrel- and all of ticagrelor-treated patients respectively had platelet reactivity levels below the threshold known to be accompanied by ischemic events, our results do not provide a potential explanation why diabetic patients and particularly insulin-treated ones have worse outcomes, despite treatment with novel antiplatelet agents. The observed detrimental impact of insulin-treated diabetes mellitus on platelet reactivity under prasugrel may simply reflect platelet dysfunction of unclear clinical significance, considering the excellent performance of this agent in insulin-treated diabetes mellitus in TRITON-TIMI 38- with a 37 % reduction in the primary endpoint compared to clopidogel. Furthermore, the described impact of insulin-treated diabetes mellitus on platelet reactivity under prasugrel is not in discordance with the relative greater benefit provided by prasugrel in the insulin-treated diabetic cohort versus non-insulin treated diabetic. This,

most likely reflects a considerable 'weakness' of clopidogrel in insulin-treated diabetic patients. In the diabetes mellitus subgroup of the TRITON-TIMI-38 trial, there was no difference in major bleeding between prasugrel and clopidogrel treated patients, regardless diabetes mellitus treatment type. Even with the insulin detrimental effect, platelet reactivity values in our prasugrel-treated diabetes mellitus patients were much lower than provided by clopidogrel and are, therefore, unlikely to provide an explanation for bleeding rates observed in diabetes mellitus cohort of TRITON-TIMI-38 trial. [29, Rank 3]

HPR under prasugrel, even in insulin-treated diabetic patients was very low. Nevertheless, its identification might enable a better understanding of their individual risk profile and allow the future development of targeted treatment strategies for these patients. Overall, our study offers a better understanding of diabetes mellitus status and treatment influence on novel antiplatelets' pharmacodynamic behaviour, while it demonstrates a differential effect of insulin-treated diabetes mellitus on PR according to the administered antiplatelet agent. [30, Rank 5]

The PLATO Trial

The PLATelet inhibition and patient Outcomes (PLATO) trial compared ticagrelor with contemporary regimens of clopidogrel in patients with acute coronary syndromes (ACS) and demonstrated superiority of ticagrelor in reduction of recurrent ischemic events. There was also an observed reduction in total mortality in the ticagrelor group compared to the clopidogrel group (4.5% vs. 5.9%; hazard ratio (HR) 0.78, 95% confidence intervals (CI) 0.69–0.89), including both a significant reduction in cardiovascular death (4.0% vs. 5.1%; HR 0.79, 95% CI 0.69–0.91), which included deaths of unknown cause, and a trend towards reduction in non-cardiovascular mortality (0.4% vs. 0.8%; HR 0.71, 95% CI 0.49–1.04) [31, Rank 1].

Ticagrelor is an oral, reversibly-binding platelet P2Y12 receptor inhibitor belonging to a novel chemical class that also inhibits adenosine reuptake. This is in distinction to clopidogrel and other thienopyridines that are prodrugs acting through hepatic metabolites that bind irreversibly to the P2Y12receptor and are not known to have any effect on adenosine metabolism. Ticagrelor yielded greater inhibition of platelet aggregation than clopidogrel in the PLATO study, which might at least partly

> "Ticagrelor is a more effective alternative than clopidogrel for the continuous prevention of ischemic events, stent thrombosis and death in the acute and long term treatment of patients with ACS"

explain its superiority in reducing the incidence of myocardial infarction and associated cardiovascular death. Platelets play a key role in the inflammatory response to vascular injury and also contribute to innate immune responses. Since the platelet P2Y12 receptor markedly amplifies the release of pro-inflammatory chemokines from platelet granules, it has a dominant role in supporting platelet-mediated inflammation. Adenosine not only has an inhibitory effect on platelet responses but also has wide-ranging effects on other pathways involved in innate immunity and may have a protective effect against pulmonary injury. In order to explore whether the mortality benefit of ticagrelor compared to clopidogrel in the PLATO study may be related to mechanisms in addition to more effective prevention of arterial thrombotic events, we therefore performed a post hoc analysis of adverse events (AEs) attributable to pulmonary infection and sepsis.

Higher levels of inflammatory markers such as neutrophil count, C-reactive protein (CRP) and interleukin-6 (IL-6) have been associated with adverse outcomes and mortality in previous studies of acute coronary syndrome patients. We performed an analysis of serial blood leukocyte counts and inflammatory markers in patients participating in the PLATO study to assess their impact on clinical outcomes and were able, in the current study, to assess any differences of ticagrelor compared to clopidogrel on leukocyte counts and acute and chronic inflammatory responses following acute coronary syndrome. [32, Rank 2]

Clinical Outcome Studies of Ticagrelor

Ticagrelor was evaluated in an early prospective, randomized, double-blind; Phase II DISPERSE-2 study in 990 patients with non ST elevated myocardial infarction. 60 Participants were randomized to ticagrelor 90 mg twice daily, 180 mg twice daily, or clopidogrel 300 mg followed by 75 mg daily for up to 3 months. Patients in the ticagrelor group were also randomized to receive a 270 mg loading dose of ticagrelor or no loading dose. The primary objective was to assess the safety and tolerability of ticagrelor versus clopidogrel in addition to aspirin. The primary endpoint was major and

minor bleeding through 4 weeks. Major and minor bleeding was 8.1% for clopidogrel, 9.8% for ticagrelor 90 mg, and 8.0% for ticagrelor 180 mg (P = 0.43 and P = 0.96, respectively, versus clopidogrel). Major bleeding rates were 6.9% for clopidogrel, 7.1% for ticagrelor 90 mg, and 5.1% for ticagrelor 180 mg (P = 0.91 and P = 0.35, respectively, versus clopidogrel). There were also non significant favourable trends for decreased rates of myocardial infarction in the ticagrelor group (5.6% for clopidogrel, 3.8% for ticagrelor 90 mg, and 2.5% for ticagrelor 180 mg; P = 0.41 and P = 0.06, respectively, versus clopidogrel). Non hemorrhagic adverse effects were comparable between clopidogrel, ticagrelor 90 mg, and ticagrelor 180 mg except for differences in rates of dyspnoea, diarrhoea, hypotension, and asymptomatic ventricular pauses ≥ 2.5 seconds. Specifically, the rate of dyspnoea was significantly higher for ticagrelor 180 mg compared to clopidogrel (15.8% versus 6.4%; P < 0.002) as was the incidence of ventricular pauses with ticagrelor 180 mg compared to clopidogrel (7.9% versus 4.3%; P = 0.14). Ticagrelor 90 mg twice daily had similar efficacy and safety compared to clopidogrel while ticagrelor 180 mg twice daily had worse safety compared to clopidogrel. Thus, the dose of ticagrelor 90 mg twice daily was pursued further in clinical development. [33, Rank 1]

Ongoing Clinical Outcome Studies

A Phase IV, prospective, randomized, double-blind study will be conducted in 1770 patients to determine the efficacy and safety of pre hospital versus in-hospital initiation of ticagrelor in ST elevated myocardial infarction patients planned for percutaneous coronary intervention.77 Patients will receive ticagrelor 180 mg loading dose either in the field or at the hospital followed by 90 mg twice daily for 30 days. The primary outcome measures are TIMI flow Grade III of myocardial infarction culprit vessel at initial angiography and ST-segment resolution up to pre-percutaneous coronary interventions ≥ 70%. This study is expected to be complete by May 2013.

A prospective, randomized, double-blind, double-dummy, Phase III study is being conducted in 800 Asian/Japanese patients to assess the safety and efficacy of ticagrelor for acute coronary syndrome.30 Patients will be randomized to ticagrelor 90 mg twice daily versus clopidogrel 75 mg daily in addition to aspirin. The primary outcomes are time to first total major bleeding event up to 12 months and time to first occurrence of event from the composite of death from vascular causes, myocardial

infarction, and stroke up to 12 months. This study was expected to be completed in July 2012; however, no results have been published for this study yet. [34, Rank 2]

Platelet aggregation exerts an important role in ischemic complications in patients submitted to percutaneous coronary intervention (PCI). Both the atherosclerotic plaque instability and factors related to the procedure itself (endothelial trauma) and contact of thrombogenic structures and the blood are responsible for this platelet aggregation in patients submitted to percutaneous coronary intervention. Despite thromboxane A2 and adenosine diphosphate (ADP) act synergistically in platelet activation, adenosine diphosphate interaction with its receptors, especially P2 receptors, enhances and sustains this activation. For this reason, these receptors have been the main target of current antiplatelet drugs.

Platelets are non-nucleated fragments of megakaryocytes that circulate in the blood stream and have a flattened disk conformation when not activated. They participate in a number of biological processes, from fighting infectious agents to initiating tissue repair by activating angiogenesis. Thus, their functions go well beyond the participation in the coagulation cascade, and they play a key role in modulating the whole tissue repair process. [35, Rank 4]

In the presence of vascular injury or trauma, sub endothelial proteins, such as Von Willebrand factor and collagen, are exposed and platelet adhesion occurs with the objective of promoting tissue healing. The interaction between these proteins and platelets is mediated by a number of receptors at the platelet surface (GPIbα, GPVI α 2β1), and platelet activation occurs concomitantly to adhesion. Multiple metabolic pathways are stimulated, leading to an increase in calcium intracellular concentration. This increase activates phospholipase A2 and actin-myosin adenosine triphosphatase, leading to thromboxane A2 formation and platelet conformational change, respectively. When activated, platelets release their granules (containing adenosine diphosphate, adenosine triphosphate, serotonin, calcium, fibrinogen, Von Willebrand factors, cytokines and pro-thrombotic factors), increasing their volume and reactivity. In order to form this aggregate, platelets interact through the binding of fibrinogen and glycoprotein IIb/IIIa receptor. [36, Rank 4]

Platelet Aggregation and Antiplatelet Selection

Platelet antiaggregation is essential in the management of patients submitted to percutaneous coronary interventions. The risks of bleeding and thrombotic events must guide antiaggreagation therapy intensity. The more intense the antiaggregation, higher the peri and post-surgery bleeding risks. These risks must always be evaluated, since bleeding complications per se lead to a worse prognosis. In the group of patients with chronic renal dysfunction, for example, dual platelet antiaggregation therapy greatly increases hemorrhagic events, reducing or even overriding percutaneous coronary intervention benefit on a medium term basis. The bleeding risk can be evaluated by clinical prediction scores such as CRUSADE, but these scores do not have good prediction values, limiting proper evaluation of the hemorrhagic risk.

Antiplatelet selection must thus be carefully evaluated taking into account all adverse events, since its discontinuation imposes and increased risk of ischemic events to patients submitted to percutaneous coronary intervention. Accessing antiplatelet reactivity may allow anti-aggregation therapy individualization. However, tests for evaluating the response to platelet anti-aggregation drugs are still expensive, lack sensitivity and still require robust evidence showing clinical benefit. [24, Rank 2]

ADP and P2 platelet receptors

Secreted by red blood cells, endothelial cells and released in platelet granules, adenosine diphosphate is an important mediator of the activation and platelet aggregation amplification (as shown in figure 10). Adenosine diphosphate interacts with the platelet surface through receptors of the P2 family, whose two subtypes can be differentiated by the intracellular activation pathway: P2X (binded to ion channels) and P2Y (coupled to G protein). There is currently a new classification based on the agonist type: P2X1, activated by adenosine triphosphate; P2Y1 and P2Y12, activated by adenosine diphosphate (as shown in figure 11).

The P2X1 receptors are responsible for a transient conformational change in platelets, which is associated to the rapid calcium influx. Thus, though not capable of sustaining platelet aggregation, they contribute to collagen-induced activation. [37, Rank 4]

P2Y1 receptors can be found in multiple tissues, including the heart, blood vessels, smooth muscular cells, nervous tissues,

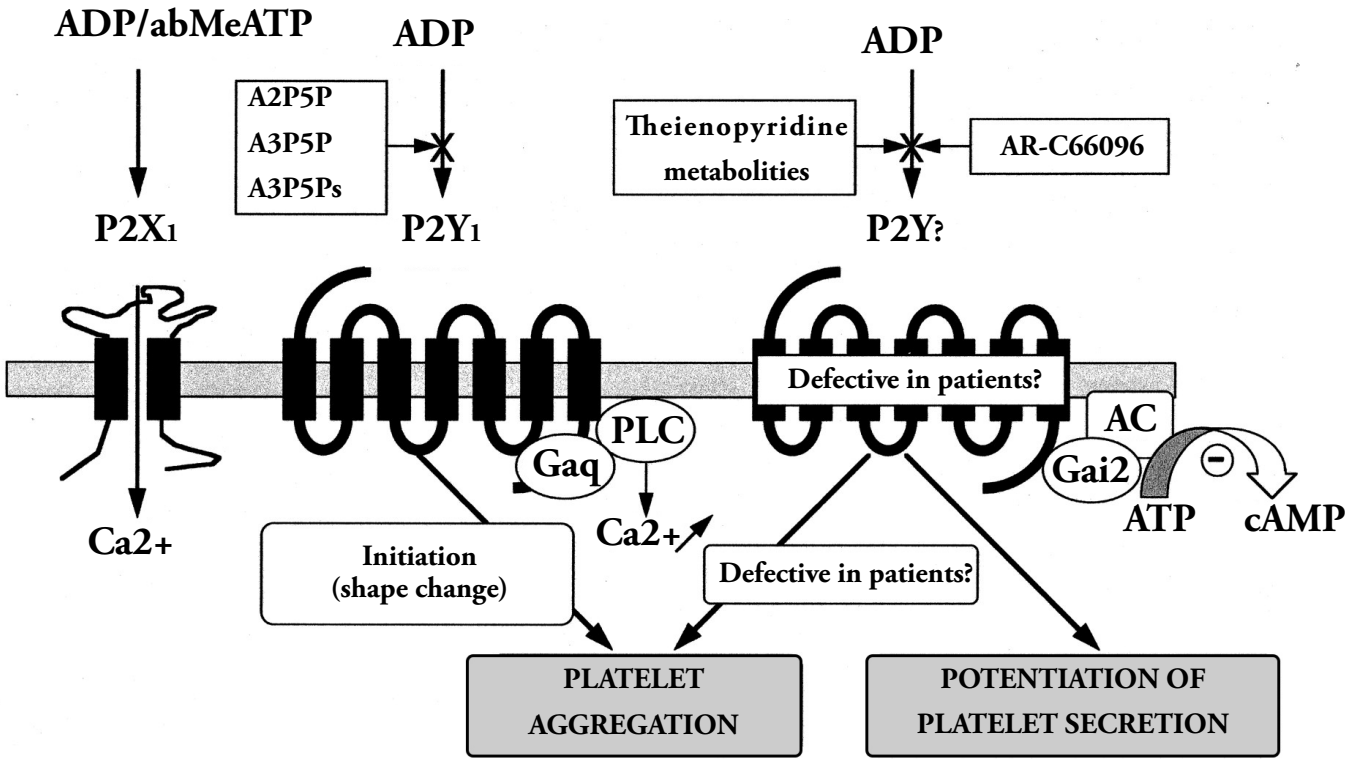


Figure 10: ADP-induced platelet activation.

testicles, prostate and ovaries. In response to adenosine diphosphate-mediated activation, calcium is mobilized from platelet storage, leading to conformational change and transient aggregation. This receptor has a key role in the beginning of adenosine diphosphate-induced activation, but, for the effective stabilization of platelet thrombus, the activation of other receptors is required. [38, Rank 3]

P2Y12 receptors, besides being found in platelets, are also present in the microglia, endothelial cells and smooth muscle cells. These receptors have a central role in the amplification of the aggregation induced by all platelet agonists, such as collagen, thrombin, thromboxane A2, adrenaline and serotonin. Despite that, the agonist with the highest affinity, as observed with P2Y1 receptors, is adenosine diphosphate. The intracellular response to its activation is the inhibition of cAMP (cyclic adenosine monophosphate) production, vasodilator-stimulated phosphoprotein (VASP) dephosphorylation and GTPase Rap1B and phosphoinositide 3-kinase (PI3-K) activation. The activation of both P2 receptors is important to adenosine diphosphate-induced aggregation, since the selective inhibition of one receptor leads to an important reduction in platelet aggregation. [39, Rank 4]

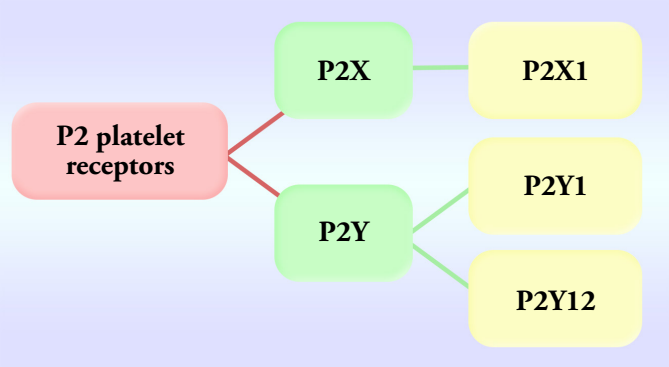


Figure 11: P2platelet receptors

P2Y12 receptor inhibitors

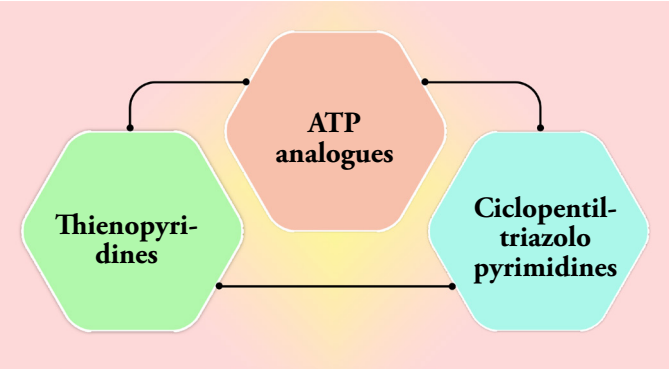


Figure 12: Classes of P2Y12 receptors

Antiplatelet drugs are essential in the management of patients submitted to percutaneous coronary intervention. There are three groups of antiaggregation drugs with proven clinical efficacy: cyclooxygenase inhibitors (AAS), P2Y12 receptor inhibitors and glycoprotein IIb/IIIa antagonists. The P2Y12 receptor is the main target of oral inhibitory agents, since it is directly involved in the amplification of the platelet reactivity required for thrombus formation. There are three classes of P2Y12receptors: thienopyridines, adenosine triphosphate analogues and ciclopentil-triazolo pyrimidines (as shown in figure 12). [40, Rank 3]

Thienopyridines

Clopidogrel

The first and the second generation of thienopyridines are represented by ticlopidine and clopidogrel, respectively. Ticlopidine's utilization is limited by a greater incidence of hematologic adverse effects, such as neutropenia and agranulocytosis. Clopidogrel is a pro-drug that must be metabolized in a two-step process by cytochrome P450 (CP450) in the liver to an active metabolite, which will irreversibly bind to the P2Y12 receptors. The majority of the absorbed clopidogrel (85-90%) is hydrolyzed in inactive carboxylic acid and the remaining is rapidly metabolized by CP450. The 75-mg clopidogrel dose starts acting after two hours, but three to seven days are needed to achieve maximum platelet inhibition. The time for achieving its peak action, however, can be reduced with the utilization of loading doses. With a 300 mg- or 600 mg loading dose, maximum inhibition is achieved in 12 and 3 hours, respectively

It is worth highlighting that these are mean population values which do not reflect the necessary individual aggregation degree, since a number of pharmacokinetics studies were performed in normal

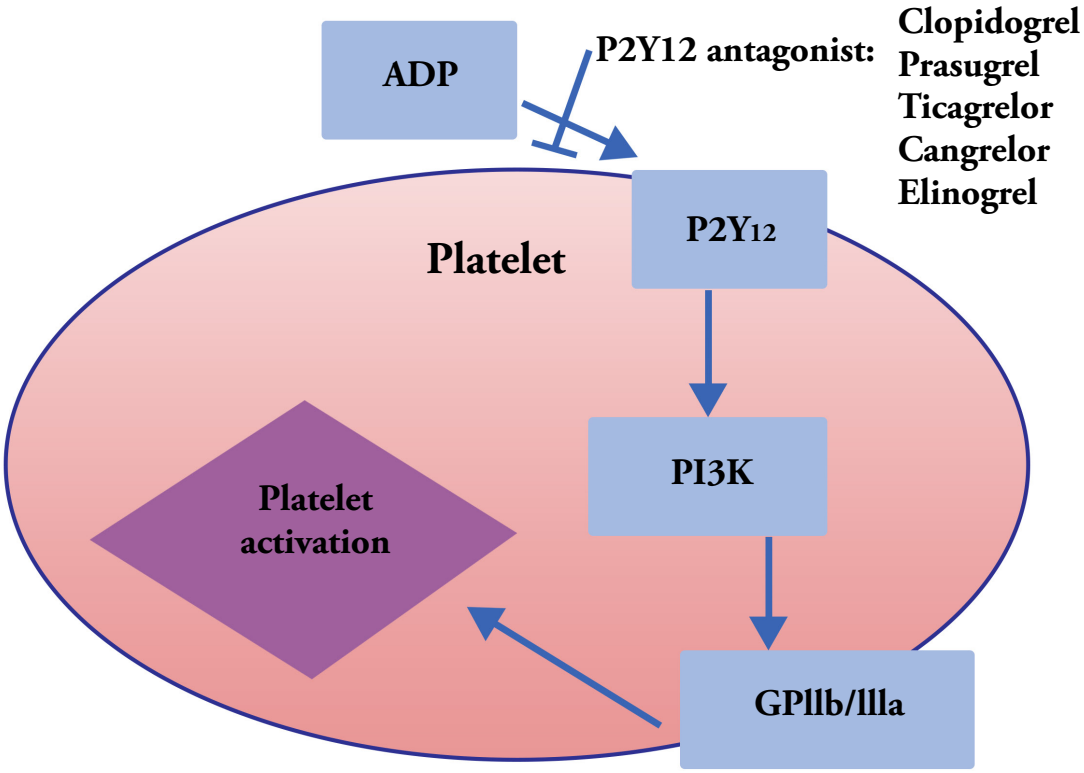


Figure 13: Platelet inhibition agents

individuals without coronary artery disease (CAD) and no damage to organs responsible for drug excretion or metabolization. Doses higher than 600 mg did not lead to more antiaggregation, since these doses did not lead to an increase in the concentration of the active metabolite.

Clopidogrel, despite showing efficacy, cannot be considered the ideal antiaggregant. Its main inconveniences are its great individual variability, due to metabolic differences(as shown in figure 13), the irreversible inhibition of the receptors, increasing bleeding risk specially in patients submitted to surgical procedures, and its latency to achieving the peak of action (reducing its benefit to acute coronary syndrome (ASC) patients needing fast platelet activity inhibition. [37, Rank 3]

Approximately 30% of the patients taking conventional doses of clopidogrel develop resistance or low response to the drug. This percentage represents a clinically vulnerable population with a high risk of major cardiovascular events, including acute myocardial infarction, stent thrombosis and death. Various factors influence this individual variability, including obesity, diabetes mellitus, acute coronary syndrome, age and mutations in the genes coding P450 cytochrome enzymes.

The patients that are homozygous for mutant alleles of CYP2C19 present a high risk of cardiovascular events; mainly stent

thrombosis. Due to this evidence, the FDA issued an alert recommending that the utilization of other antiaggregation agent or unusual doses of clopidogrel be considered for these patients, individualizing platelet antiaggreagation.

Individualized therapy is common in clinical cardiology. Various drug classes are dosed according to the clinical or laboratory response of the patient, such as anti-hypertensive and anticoagulant drugs, respectively.

The utilization of laboratory exams that allow a more precise evaluation of the individual variability in antiaggregant response is thus necessary. Currently, two test groups are available for this purpose: genetic and platelet reactivity tests. Since the genotype is constant, its evaluation is not capable of adequately measuring the cumulative influence and the dynamics of the various factors that interfere in platelet reactivity; thus, despite still limited by technical factors, it is more appropriate to evaluate the final phenotype than the genotype. [36, Rank 5]

Ticagrelor

The most recent P2Y12 receptor inhibitor class is the ciclopentil-triazolo pyrimidines, represented by ticagrelor. Unlike the thienopyridines, ticagrelor does not need to be metabolized by the liver, interacts with platelet receptors reversibly and has faster onset and peak of action. Action onset and peak are similar to clopidogrel in non-responding patients.

> **"Ticagrelor is the first of a new class of antiplatelet agents, the ciclopentyl-triazolo-pyrimidines.**
>
> **Ticagrelor is an oral P2Y12receptor antagonist that exerts antiplatelet effects by blocking ADP"**

Both efficacy and safety of ticagrelor were evaluated in the PLATO study, in which 18,624 acute coronary syndrome patients were randomized for receiving clopidogrel (75 mg/day, with a loading dose of 300 to 600 mg) or ticagrelor (90 mg twice daily, with a 180 mg loading dose). The primary combined endpoint (mortality due to vascular cause, acute myocardial infarction or stroke) in 12 months was significantly smaller in the ticagrelor group (9.8% vs 11.7%). No significant difference was verified on major bleeding rates when the study's major bleeding rate was used. However, when TIMI's major bleeding criteria were used, the bleeding rate was higher in patients not submitted to myocardial bypass in the ticagrel or group (2.8%

vs. 2.2%; p = 0.03).The isolated analysis of acute myocardial infarction, vascular mortality and all-cause mortality rates showed a statistically significant rate in patients taking ticagrelor. In this study, the main adverse effects were dyspnoea and bradycardia. [33, Rank 2]

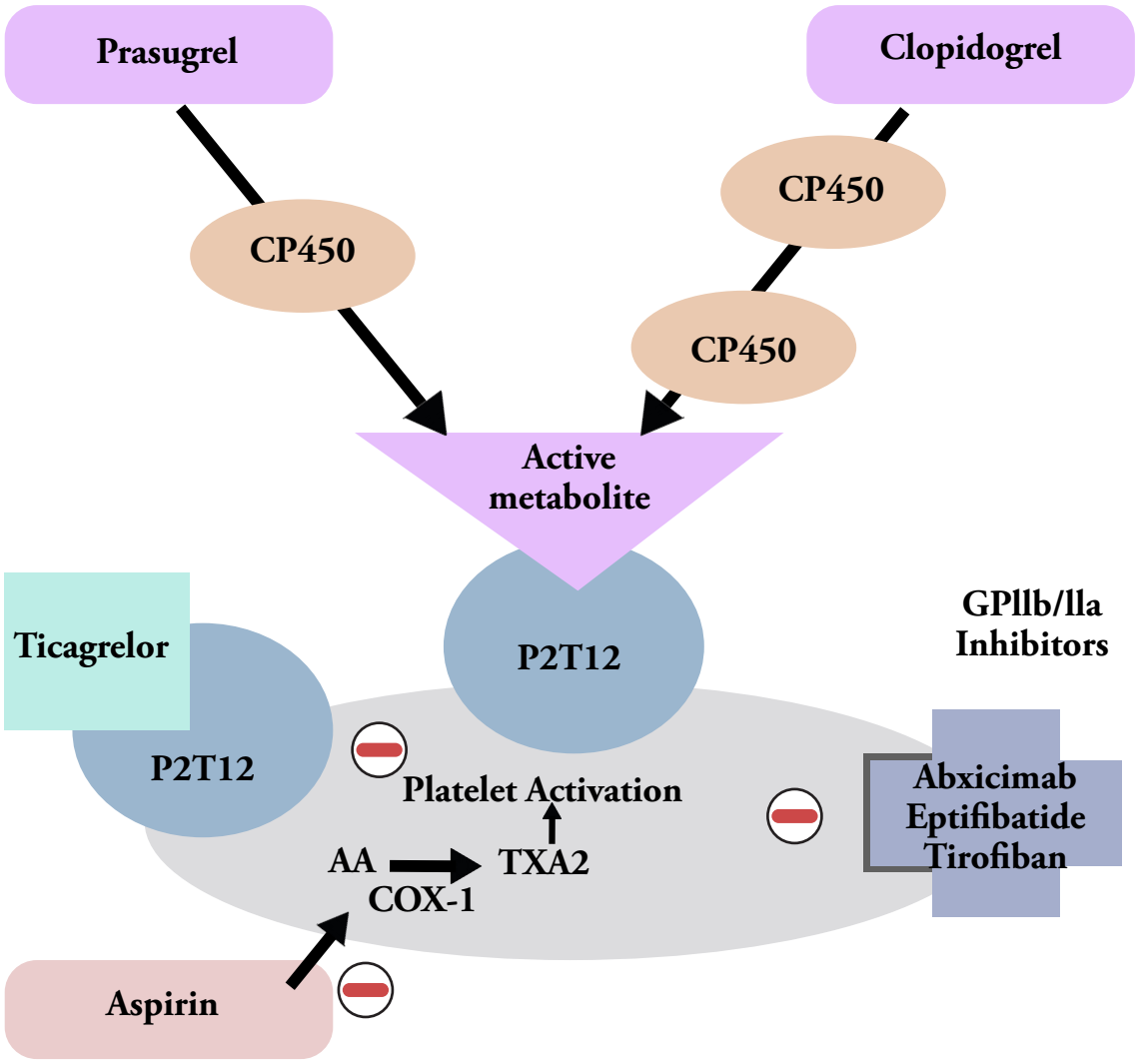
One possible hypothesis for explaining the mortality reduction obtained with ticagrelor is that this drug has other effects besides antiplatelet action. Ticagrelor inhibits adenosine reuptake by red blood cells and is structurally related to adenosine, suggesting that the latter may be one of its metabolites. Adenosine has cytoprotective, anti-inflammatory, antifibrotic and cardio protective properties, which could explain ticagrelor benefits. Adenosine can also justify the main adverse effects of this drug, such as dyspnoea and ventricular pauses. More evidence is required, though, for confirming the direct association of ticagrelor and adenosine.

Due to its short half-life, platelet reactivity returns to basal levels after 3 to 4 missing doses. This is important for patients requiring surgical intervention. However, bad drug adherence quickly exposes the patient to the risk of ischemic events. [31, Rank 3]

Pharmacology/Mechanism of Action

Clopidogrel is a thienopyridine- a prodrug requiring two cytochrome P450 dependent steps to generate an active metabolite-which binds irreversibly to the P2Y12 adenosine diphosphate receptor on platelets. Prasugrel is a third generation thienopyridine sharing the same active metabolite as clopidogrel and despite partial reliance on CYP2C19, achieves faster and faster and more potent platelet inhibition (as shown in figure 14).

Ticagrelor is a member of a class of agents known as the cyclopentyl-triazolo-pyrimidines. These agents are relatively resistant to enzymatic degradation by ectonucleotidases, which rapidly degrade adenosine triphosphate (ATP) in vivo. Resistance to this enzymatic degradation is critical because although adenosine triphosphate serves as a natural competitive antagonist to adenosine diphosphate (ADP) at the P2Y12 receptor, it is not a useful pharmacologic approach to P2Y12 antagonism secondary in part to its poor stability. Efforts to develop stable adenosine triphosphate analogs led to the discovery of cangrelor. Further modifications of this molecule included the elimination of phosphates and a change in the core purine and sugar



AA=arachidonic acid; COX-1=cyclooxygenase-1;CYP450=cytochrome P450;
P2Y12=purinergic receptor P2Y; Gpllb/llla= glycoprotein llb/llla; TXA2=thromboxane A2

Figure 14: Molecular targets of drug therapy on the activated platelets

moieties, leading to the development of ticagrelor. It is not considered an adenosine triphosphate analog because of the changes in the purine and sugar moieties.

Ticagrelor is the most clinically advanced P2Y12 inhibitor in its class, and its chemical structure distinguishes it from the thienopyridines. Similar to the currently available irreversible P2Y12 inhibitors, ticagrelor is orally active and is selective for the P2Y12 receptor. However, in contrast to thienopyridine agents, it is a reversible inhibitor of the P2Y12, which may afford specific advantages and perhaps disadvantages. [35, Rank 5]

Ticagrelor exerts its action via binding to the P2Y12 receptor in a manner distinct from adenosine diphosphate, resulting in a reversible conformational change of the receptor. The ligand inhibition of the adenosine diphosphate receptor and subsequent signaling affect

> "Ticagrelor, cyclopentyl triazolopyridine drug and clopidogrel , second generation thienopyridine drug are antiplatelet drugs indicated for the prevention of thrombotic events in patients with acute or chronic coronary syndromes"

downstream processes. These include the conversion of cyclic monophosphate from adenosine triphosphate, dephosphorylation of phosphorylated vasodilator-stimulated phosphoprotein (VASP), and activation of phosphoinositide 3-kinase. Inhibition of these processes results in reduced exposure of fibrinogen-binding sites to the GP IIb/IIIa receptor and thereby impairment of platelet aggregation. Interestingly, ticagrelor inhibits platelet aggregation despite increasing concentrations of adenosine diphosphate, demonstrating that this receptor inhibition is non competitive. Therefore, even in the setting of increased adenosine diphosphate concentrations, there would be no reduction in the level of platelet inhibition. Likewise, the effects of ticagrelor on platelet function correlate with plasma drug concentrations. In addition; ticagrelor's effects on non-platelet-bound adenosine diphosphate receptors may also produce off-target effects on vascular smooth muscle via inhibition of vasoconstriction. Coronary blood flow through inhibition of adenosine uptake by erythrocytes may also be affected. These pleiotropic effects together with ticagrelor's unique reversible inhibition of the P2Y12 receptor may result in both unique advantages and disadvantages. [29, Rank 3]

Prasugrel vs. Ticagrelor

There are no studies comparing these drugs clinically; thus, care must be taken when extrapolating data from different papers. The study TRITON randomized the great majority of its patients (99%) with knowledge of their coronary anatomy, while PLATO, on the other side, randomized patients at the emergency room. The clopidogrel dose allowed in the two studies was also different. Approximately 50% of the patients in the PLATO study and 33% of the TRITON study patients were taking proton pump inhibitors. A final diagnosis of acute coronary syndrome with ST elevation was made in 37% of the patients in the PLATO study and 26% of the patients in the TRITON study. In PLATO, with the exception of patients submitted to thrombolysis, all therapeutic regimens were evaluated (interventionist,

surgical and clinical), while in TRITON patients were randomized after intervention was indicated. Thus, the two studies have different populations and designs, and it is not possible to compare the drugs. [25, Rank 1]

Role of Platelets in acute coronary syndrome and Importance of Antiplatelet Therapy

Platelets protect vascular integrity and play an important role in haemostasis. However, rupture of an atherosclerotic plaque causes a platelet-dependent thrombus formation leading to occlusion of a coronary artery resulting in acute myocardial infarction. Thus platelets play a central role in pathogenesis of acute myocardial infarction. Strong evidence which suggest that acute myocardial infarction is a platelet related disease is the capability of antiplatelet therapy to reduce morbidity and mortality in this clinical setting.

Many landmark trials of aspirin and thienopyridines have established the role of oral antiplatelet agents in the management of acute coronary syndrome. Aspirin is the oldest of the antiplatelet drugs and has stood the test of time as an integral part of management of acute coronary syndrome. The use of thienopyridines, which act by blocking the P2Y12receptor on the platelet surface, has shown benefit when added to aspirin in this setting. Thus, dual antiplatelet therapy is the current standard of care for patients of acute coronary syndrome which is currently recommended for the period of at least 1 year. However, in spite of currently available antiplatelet therapy there remains a significant risk of arterial thrombosis and post acute coronary syndrome mortality grows over a period of time. Thus there is a need for novel antiplatelet agents which can overcome limitations of current antiplatelet therapies like slow onset of action, low level of platelet inhibition, high inter patient variability at the cost of clinically acceptable bleeding events. [24, Rank 3]

Ticagrelor

Molecular Discovery

Adenosine triphosphate (ATP) competitively antagonizes adenosine diphosphate-induced platelet aggregation. However unfavourable properties of adenosine triphosphate, such as low potency and poor stability do not allow its use as P2Y12 receptor antagonist. Efforts were directed towards formulating adenosine

triphosphate analogues with high potency and more stability. However because of retention of triphosphate group these agents had very short plasma half life and they need to be given intravenously. Subsequent modification of these compounds lead to discovery of selective and stable non-phosphate P2Y12 receptor antagonist AZD6140 (ticagrelor) belonging to a new chemical class Cyclo Pentyl Triazolo Pyrimidine (CPTP). Although adenosine triphosphate structure was used as basis for designing of ticagrelor, it does not contain an adenosine group and therefore is distinct from true adenosine triphosphate analogues such as Cangrelor. [23, Rank 3]

Mechanism of Action

It is an oral, reversible and directly acting inhibitor of P2Y12 receptor. Like thienopyridines, ticagrelor inhibit prothrombotic effects of adenosine diphosphate by blocking the platelet P2Y12 receptor. However, unlike thienopyridines, the binding and effect is reversible and it does not require metabolic activation before its action. It has a rapid onset of action, produces high and consistent inhibition of platelet aggregation with minimal inter patient variability. It binds at a site distinct from adenosine diphosphate binding site, causing locking of the receptor in an inactive state thereby inhibiting adenosine diphosphate signalling and receptor conformational changes. Unlike other thienopyridine ticagrelor is a non-competitive antagonist of P2Y12 receptor resulting in no receptor activation in spite of increased adenosine diphosphate concentration. [22, Rank 4]

Pharmacological Aspects

Ticagrelor is rapidly absorbed on oral administration with food intake having no appreciable effect on the absorption of ticagrelor. The Tmax of ticagrelor is 1.3–2 h and plasma half life (t1/2) is 7–12 h. It is metabolized in the liver by CYP3A4 enzyme to produce active metabolite AR-C124910XX. This metabolite is as potent as ticagrelor on P2Y12 receptor and is present in the circulation at approximately 1/3 of the concentration of the parent drug.16 as ticagrelor is metabolized by CYP3A4, concomitant administration of CYP3A4 inducers and inhibitors should be avoided. Elimination of ticagrelor and AR-C124910XX occurs primarily via hepatic metabolism and biliary secretion, respectively. Therefore no dose adjustment is required for renal patients. [21, Rank 3]

Clinical Development

Safety and tolerability of ticagrelor was tested in various phase I and phase II trials. In phase I trial ticagrelor was tested in healthy volunteers in dosage of 50–600 mg once daily or 50–300 mg twice daily. Findings of phase I illustrated that the pharmacokinetics of ticagrelor is predictable and is associated with consistent inhibition of platelet activity. Inhibition of platelet aggregation (IPA) with ticagrelor was greater and better sustained at high levels with twice daily ticagrelor than once daily regimens. [17, Rank 4]

Results of phase II DISPERSE study showed that ticagrelor 100 mg and 200 mg bd have more beneficial safety and tolerability profile and therefore these two doses were carried forward for further clinical evaluation. DISPERSE II was a dose confirmation study in NSTEMI patients. Results of DISPERSE II demonstrated that protocol-defined major or minor bleeding at 4 weeks, was not different among the ticagrelor 90 mg bd, ticagrelor 180 mg bd and clopidogrel 75 mg od groups. However ticagrelor 180 mg bd was associated with increase in minor and minimal bleeds. Based on safety and efficacy profile ticagrelor 90 mg bd was selected for phase III study. [19, Rank 3]

Onset–Offset study illustrated that inhibition of platelet aggregation with ticagrelor 180 mg loading dose was greater than clopidogrel 600 mg loading dose at all the time points. Just 30 min post loading, inhibition of platelet aggregation with ticagrelor was 41% versus 8% in clopidogrel group. At the end of 2 h inhibition of platelet aggregation with ticagrelor was 88% versus 38% in clopidogrel group. At 2 h post-loading, 90% patients in ticagrelor group achieved greater than 70% inhibition of platelet aggregration versus 16% in clopidogrel group. Higher level of inhibition of platelet aggregation achieved with ticagrelor was maintained throughout 6 weeks of study period which indicates sustained and consistent antiplatelet action of ticagrelor. After last dose, antiplatelet effect of ticagrelor declined very rapidly as compared to clopidogrel. 24 h after last dose, inhibition of platelet aggregation with ticagrelor was similar to clopidogrel. This means patients who miss 1 dose of ticagrelor will still have inhibition of platelet aggregation at 24 h equivalent to patients on clopidogrel therapy. Inhibition of platelet aggregation at day 3 and 5 with ticagrelor were comparable to IPA at day 5 and 7 with clopidogrel respectively.

Effect of ticagrelor in clopidogrel non-responders was studied in RESPOND

study which showed that ticagrelor treatment result in consistently higher inhibition of platelet aggregation in patients irrespective of responder status. Ticagrelor was found to be effective in overcoming high platelet reactivity below the ischemic cut off points in both responders and non-responders to clopidogrel therapy. This study also showed that switching patients from clopidogrel to ticagrelor result in rapid, higher and consistent IPA. [20, Rank 5]

Potential Effect on ADP-Induced Vasoconstriction

Preclinical studies also suggest that reversible P2Y12 inhibition may be associated with beneficial effects on P2Y12-mediated vasoconstriction, effects that may permit reduction in thrombogenic vasospasm or reduce deficits in myocardial perfusion after thrombosis. P2Y12 receptors are present in vascular smooth muscle cells in concentrations greater than other adenosine diphosphate receptors – for example, P2Y1 and P2Y13– and are active in stimulating vessel contraction. In studies in arterial segments from patients undergoing coronary bypass, P2Y12-mediated vasoconstriction was demonstrated using 2-MeSADP-induced contraction of sub maximally pre contracted vessels. This contraction was blocked by a selective reversible P2Y12antagonist (AR-C67085) and not blocked by a selective P2Y1 antagonist. No inhibition of contraction was observed in vessels from patients pre-treated with clopidogrel.

The ability of ticagrelor to inhibit adenosine diphosphate-induced contractions in vascular smooth muscle was shown in an ex vivo study in denuded mouse aortic rings. In these studies, 2-MeSADP-induced contractions were 59% of maximal values (obtained with potassium-rich buffer) in mice that were not pre-treated and 64% in mice pre-treated with clopidogrel 50 mg/kg [40]; the addition of ticagrelor 10 μM to the vessel segment in tissue bath resulted in significant inhibition of adenosine diphosphate-induced contraction, to 33% of maximal in the untreated group and 32% of maximal in the clopidogrel pre-treated group. These findings suggest that ticagrelor could modulate vasoreactivity mediated by locally increased levels of adenosine diphosphate in vivo; pre-treatment with clopidogrel does not appear to inhibit adenosine diphosphate-induced contractions. The potential effects of such inhibition were shown in a study in a dog thrombosis model, in which treatment with the

selective P2Y12 antagonist AR-C69931MX (4 μg/kg•min) resulted in decreased re-occlusion and cyclic flow variation and improved myocardial flow compared with placebo in animals receiving tissue-type plasminogen activator and heparin after thrombus formation. Another study using the same thrombosis model in dogs compared adjunctive infusion of ticagrelor (75 μg/kg IV bolus, followed by 10 μg/kg•min IV infusion over 2 h) with clopidogrel (10 mg/kg IV bolus over 5 min) and placebo, on top of fibrinolytic therapy (t-PA + heparin). In this study, both clopidogrel and ticagrelor treatment resulted in complete blockade of adenosine diphosphate-induced platelet activation, aggregation, and recruitment, and prevented platelet-mediated thrombosis. However, despite similar antiplatelet effects, animals treated with ticagrelor had significantly lower rates of reocclusion (0% for ticagrelor vs. 30% for clopidogrel), less cyclic flow variation (0% vs. 30%), longer reflow duration (120 ± 0.0 min vs. 96.9 ± 38.9 min) and greater reductions in infarct sizes (final size of 6.31 ± 2.86 cm2 vs. 14.63 ± 4.29 cm2). Taken together, these data suggest that the systemic presence of reversible inhibitors may exert additional benefit, for example, via inhibition of non platelet P2Y12receptors. [24, Rank 2]

Ticagrelor may confer additional benefits in patients with acute coronary syndrome by inhibiting uptake of adenosine by human erythrocytes. In suspensions of washed human erythrocytes, ticagrelor dose-dependently inhibited adenosine uptake by erythrocytes with a pIC50 of 7.0 ± 0.1 (n = 5), which was 10-fold less potent than dipyridamole. In vivo data from a canine model of coronary blood flow regulation confirmed that both ticagrelor (n = 8) and dipyridamole (n = 8) dose-dependently augment the hyperaemic response to temporary occlusion of direct intracoronary adenosine infusion, without affecting measured blood flow in the circumflex artery. In vehicle-treated animals, infusion of adenosine 15 μg/min or 30 μg/min resulted in blood flow in the left anterior descending artery 126.8 or 227.3% of baseline, respectively. Systemic administration of ticagrelor 30 or 100 μg/(kg•min) resulted in blood flow 114 ± 10% or 150 ± 19% of baseline, respectively, following infusion of adenosine 15 μg/min, and 109 ± 10% or 140 ± 13% of baseline, respectively, following infusion of adenosine 30 μg/min. Administration of dipyridamole 0.17 or 0.50 μg/(kg min) resulted in blood flow 160 ± 2% or 193 ± 17% of baseline following adenosine 15 μg/min, and 136 ± 17% or 153 ± 30% following adenosine 30 μg/min. These

results confirm that both ticagrelor and dipyridamole augment the adenosine-induced increase in coronary blood flow in this model and suggest that ticagrelor may deliver additional benefits to patients with acute coronary syndrome by enhancing adenosine-induced coronary blood flow increases. Translation of these results to clinical benefit will need to be confirmed with clinical data. [22, Rank 3]

Conclusion

For reasons of economy and the medical care system, clopidogrel is still the most widely used antiplatelet medicine in acute coronary syndrome in addition to aspirin. A number of individuals experience clopidogrel resistance, which leads to adverse thrombotic events. Although a study shows that the most common cause of clopidogrel's failure in treatment is medication nonadherence, inter-individual variability in response to clopidogrel is also an important reason, which is appreciated in up to 30–40 % of patients who received clopidogrel treatment. As another P2Y12R antagonist, ticagrelor is becoming a strong competitor to clopidogrel. [18, Rank 5]

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