



Přehledový článek | Review article

Antiplatelet therapy – a pharmacologist's perspective

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INFORMACE O ČLÁNKU

Historie článku:

Došel do redakce: 29. 1. 2013

Přepřacován: 5. 3. 2013

Přijat: 6. 3. 2013

Dostupný online: 13. 3. 2013

Keywords:

ADP receptor P2Y₁₂ inhibitors

Antiplatelet agents

GP IIb/IIIa receptor inhibitors

5-HT receptor inhibitors

PAR-1 inhibitors

TXA₂ synthesis inhibitors

SUMMARY

There are only few areas of cardiology that have witnessed such dramatic innovations as that occurring in the treatment and prophylaxis of thrombotic events. Antithrombotic (i.e., antiplatelet and anticoagulation) therapy plays a pivotal role in the prophylaxis of the pandemic of cardiovascular disease. Given the host of triggers activating primary hemostasis, various therapeutic strategies are currently available. The current approach, monotherapy or dual therapy or, possibly, combination therapy with antiplatelet and anticoagulant agents is selected based on the risk of a thrombotic event, dominant disease, and the risk of bleeding. The main problem associated with the current therapeutic strategy was not an insufficient effect, but major inter-individual variability of effect resulting in therapy failure or an unacceptable risk of bleeding documented in a non-negligible proportion of patients. Hence the drive for devising new antiplatelet strategies and innovation in (already) established classes of drugs.

Milestones in the evolution of antiplatelet therapy included the advent of new, more effective and/or safer antiplatelet agents inhibiting platelet activation. The new irreversible P2Y₁₂ receptor antagonist prasugrel and reversible P2Y₁₂ receptor antagonist ticagrelor have been approved for clinical use. There has also been major progress in the development of thrombin protease-activated receptor 1 (PAR-1) antagonists (vorapaxar, atopaxar) or serotonin receptor blockers (sarpogrelat). Another promising therapeutic strategy is targeted at platelet stabilization through increased cyclic adenosine monophosphate (cAMP) activity by platelet phosphodiesterase-3 inhibition (cilostazol). Further, new insights into the bioavailability of acetylsalicylic acid under specific conditions have been reported regarding the class of agents inhibiting thromboxane A₂-mediated activation.

SOUHRN

Málokterá oblast kardiologie zaznamenala tak významné inovace jako léčba a profylaxe aterosklerotických příhod. Antitrombotická, tj. protideštičková a antikoagulační léčba hraje v profylaxi pandemie kardiovaskulárních chorob zásadní úlohu. Vzhledem k řadě podnětů aktivujících primární hemostázu využíváme různé léčebné strategie. Podle rizika trombotické příhody, dominujícího postižení a rizika krvácení volíme vlastní přístup – monoterapii či duální léčbu, eventuálně kombinaci protideštičkové léčby s antikoagulancii. Dominující problematikou dosavadní léčebné strategie nebyl nedostatečný efekt, ale významná inter-individuální variabilita účinku. U nezanedbatelného procenta nemocných léčba selhávala či se objevovalo neúnosné riziko krvácení. Z těchto důvodů zaznamenáváme významnou aktivitu ve vývoji nových protideštičkových postupů a inovaci ve skupinách již zavedených.

Významným vylepšením léčby je nástup nových účinnějších či bezpečnějších protideštičkových léků inhibujících aktivaci trombocytů. Ze skupiny nových inhibitorů destičkových receptorů P2Y₁₂ byl zaveden ireverzibilní inhibitor receptorů ADP prasugrel a reverzibilní působící ticagrelor. Významně pokročil vývoj trombinových receptorů typu PAR-1 (vorapaxar, atopaxar) nebo serotoninových receptorů (sarpogrelat). Rovněž strategie cílená na stabilizaci trombocytů zvýšením nabídky cAMP (např. cilostazolem) nabízí nové možnosti. Též ve skupině léků tlumících aktivaci zprostředkovanou tromboxanem A₂ máme nové poznatky o změně dostupnosti kyseliny acetylsalicylové za specifických podmínek, které mohou významně ovlivnit výsledky léčby.

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Klíčová slova:

Inhibitory ADP receptorů P2Y₁₂

Inhibitory receptorů GP IIb/IIIa

Inhibitory receptorů 5-HT

Inhibitory receptorů PAR-1

Inhibitory syntézy TXA₂

Protideštičkové léky

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DOI: 10.1016/j.crvasa.2013.03.003

1 Introduction

A crucial role in the etiopathogenesis of cardiovascular events is played by thrombosis. Activation of primary (platelet-based) hemostasis is the key factor in the development of atherothrombotic events in the arterial bed whereas activation of hemocoagulation underlies the development of thrombotic and thromboembolic events in the venous system and cardiac chambers. It clearly follows from the above that, while antiplatelet therapy is crucial in conditions impairing arterial wall integrity (prophylaxis of myocardial infarction, cerebrovascular events, and critical limb ischemia), anticoagulation therapy is instituted in the presence of blood stagnation (in atrial fibrillation or in the venous system at slow flow rates). However, it should be always remembered that the division into primary and secondary hemostasis is arbitrary as both processes are intertwined. An example of this is phospholipid expression on the surface of activated and aggregated platelets whereby an active surface will enormously accelerate hemocoagulation. On the other hand, thrombin, the key enzyme of the coagulation cascade, activates platelets by stimulation of protease-activated receptors (PARs).

In fact, the main reason behind developing new therapeutic strategies was not insufficient efficacy of available treatments; the ambition was to obtain more advantageous pharmacological properties. A major improvement was the *more rapid onset of action* (obtained with prasugrel and ticagrelor or new direct thrombin and factor Xa inhibitors). Another benefit was a *limited duration of action* (with the reversible P2Y₁₂ receptor antagonist ticagrelor). The former characteristic will be appreciated in acute atherothrombotic states, the latter in bleeding. The third goal was to obtain a *more reliable effect* in all patients regardless of their pharmacogenetic makeup, that is, to minimize inter-individual differences in their response to therapy. It was just this characteristic that has actually contributed to the superiority of prasugrel and ticagrelor over clopidogrel in terms of therapeutic efficacy. As suggested by analysis of Kaplan-Meier curves, the benefit of the more rapid onset of effect was smaller in this context. Similarly, the more reliable anticoagulant action combined with small variability in terms of an insufficient or excess effect has projected into enhanced efficacy and safety profiles of new direct thrombin and factor Xa inhibitors compared with warfarin. Finally, the last advantage enhancing the image of novel an-

Table 1 – Overview of binding, activating and stabilizing platelet receptors, their ligands, and inhibitors.

Platelet receptors		
Surface binding receptors	Ligand	Inhibitor
Collagen, Ib-IX-V receptors	vWF, collagen	ARC1779, ARC15105, ALX-0081 (clinical evaluation)
Collagen, GPVI receptors	Collagen	JAQ1 (clinical evaluation)
Activating receptors	Inductor	Inhibitor
Thromboxane/prostaglandin TPβ receptors	TXA ₂ , PGH ₂ , PGF _{2α}	TXA ₂ synthesis blockade (ASA, triflusal), TXA ₂ and TPβ rec. synthesis blockade (picotamide), TPβ rec. blockade (terutroban, sulotroban – development discontinued)
ADP, P2Y ₁₂ receptors	ADP	Reversible (ticagrelor, cangrelor) and irreversible (clopidogrel, ticlopidine and prasugrel) inhibitors
ADP, P2Y ₁ receptors	ADP	MRS2500 (clinical evaluation)
Thrombin, PAR-1 receptors	Thrombin	Vorapaxar, atopaxar (clinical evaluation)
Thrombin, PAR-4 receptors	Thrombin	Not known
Serotonin (5-HT _{2A/2B}) receptors	Serotonin	Sarpogrelat (clinical evaluation), naftidrofuryl
α-adrenergic receptors	Noradrenaline	α ₁ -adrenergic receptor inhibitors
PAF receptors	PAF (platelet-activating factor)	Rupatadine, lexipafant (antiallergic indication)
Stabilizing receptors	Inductor	Inhibitor
Adenosine, A ₂ receptors	Adenosine	Methylxanthines (caffeine, aminophylline, etc.)
β-adrenergic, β ₂ receptors	Adrenaline	Non-selective beta-blockers
Thromboxane/prostaglandin TPα receptors	Prostacyclin + PGE ₂ with analogs (iloprost, treprostinil, epoprostenol, alprostadil, limaprost)	Not known

ADP – adenosine diphosphate; 5-HT – 5 hydroxy tryptamine (serotonine); PAF – platelet-activating factor; PAR – protease-activated receptor; PGE₂ – prostaglandine E₂; PGF_{2α} – prostaglandin F_{2α}; PGH₂ – prostaglandin H₂; TP rec. – thromboxane-prostanoid receptor; TXA₂ – thromboxane A₂; vWF – Von Willebrand factor.

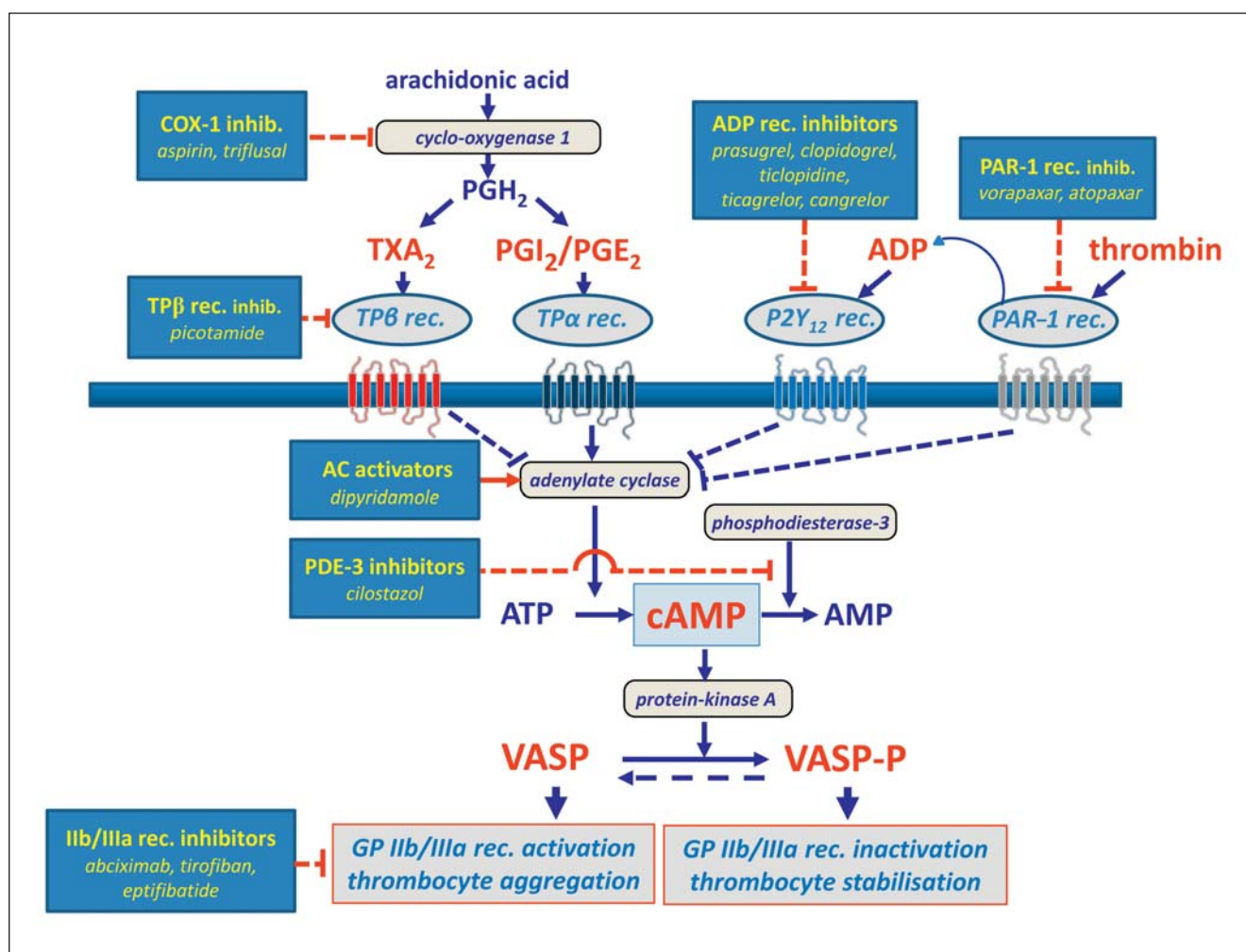


Fig. 1 – Mechanism of action of different antiplatelet drugs. The critical point is the adenylate cyclase activation or inhibition and cAMP production. In case of platelet phosphodiesterase-3 (PDE-3) inhibition by cilostazol, cAMP degradation is suppressed. By modifying cAMP availability, cilostazol and dipyridamole may enhance the effect of ADP receptor blockers and COX-1 inhibitors.

tithrombotics is their reduced propensity for drug interactions. This is true not only when comparing new P2Y₁₂ receptor antagonists with clopidogrel but, also, when comparing new anticoagulants with warfarin.

Antiplatelet agents include several classes of drugs differing in their mode of action (Table 1). The current classification of antiplatelet drugs is used primarily for didactic reasons; in fact, the modes of actions are closely interrelated as the activation of the thromboxane/prostacyclin α receptor (TP α) by the vasodilators prostaglandins I₂ and E₂ will suppress the effect of stimulation of the P2Y₁₂ receptor by adenosine diphosphate (ADP), TP β receptor by TXA₂ and the protease activated receptor 1 (PAR-1) by thrombin. The common place of action of the majority antiplatelet drugs is adenylate cyclase activation (followed by cyclic adenosine monophosphate synthesis and reduction the ratio of phosphorylated to dephosphorylated vasodilator-stimulated phosphoprotein). Ratio VASP/VASP-P determines the actual thrombocyte activation/stabilization and GP IIb/IIIa receptor stereoconformation (Fig. 1).

1.1 Inhibition of TXA₂ synthesis by COX-1 blockade – aspirin and triflusal

The longest, and currently the most widely used strategy is inhibition of thromboxane A₂ (TXA₂)-induced platelet activation. There are two types of thromboxane/prostanoid (TP) receptors on the platelet surface – TP α and TP β . Activation of the receptor α isoform (whose activators include prostacyclin and prostaglandin E₂) results in platelet stabilization whereas stimulation of the receptor β isoform (with TXA₂ as the primary ligand) activates the thrombocyte (Fig. 2).

This class includes *acetylsalicylic acid (aspirin)* and *triflusal*, both inhibiting TXA₂ synthesis through cyclo-oxygenase 1 (COX-1) blockade and the virtually unused TP β receptor inhibitors. The reduced TXA₂ synthesis or inhibition of TP receptors (subtype β) results in the expression of GP IIb/IIIa receptors at the level of platelet and reduced activation and chemotaxis of the surrounding platelets. This is paralleled by stimulation of vasoconstriction in the vascular smooth muscle and endothelial adhesion molecule expression leading to activation

Table 2 – Comparison of the effects of the most important antiplatelet drugs.

Drug	Mechanism of action/type of bond	Route of administration	Metabolic activation	Plasma half-life	Onset of effect	Peak effect achieved	Effect wears off within
Clopidogrel	P2Y ₁₂ rec. inhibition/irreversible	Oral	CYP2C19 (sensitive to CYP2C19 inhibition of type of polymorphism)	≈ 6 h (active metabolite)	1–4 h (loading dose 600 mg)	4–5 h (loading dose 600 mg)	≈ week
Ticlopidine	P2Y ₁₂ rec. inhibition/irreversible	Oral	CYP2C19 (sensitive to CYP2C19 inhibition of type of polymorphism)	7–13 h (active metabolite)	1–2 days	3–4 days	≈ week
Prasugrel	P2Y ₁₂ rec. inhibition/irreversible	Oral	CYP3A4 and 2B6 (resistant to inhibition or CYP isoenzyme polymorphism)	≈ 7 h (2–15) (active metabolite)	30 min	1–2 h in fasting state, 2–3 h post-prandially	≈ week
Ticagrelor	P2Y ₁₂ rec. inhibition/reversible	Oral	Not necessary, active metabolite involved in the effect by 1/4	6–13 h (parent drug and active metabolite)	30–60 min	1–2 h	1–2 days
Cangrelor (being evaluated)	P2Y ₁₂ rec. inhibition/reversible	Parenteral	Not necessary	10–15 min	Minutes	15 min	1 h
Vorapaxar (being evaluated)	PAR-1 rec. inhibitor/reversible	Oral	Not necessary	165–310 h	30 min	2 h	> 8 weeks
Acetylsalicylic acid (non-enterosolvent tablets)	Irreversible COX-1 blockade (inhibition of TXA ₂ synth.)	Oral and parenteral	Not necessary	2–3 h	< 10 min with i.v. admin., 20–30 min with oral route	1 h	≈ week
Dipyridamole (non-extended released form)	Stimulation of cAMP synthesis	Oral	Not necessary	8–15 h	1–2 h	2–4 h	1–2 days
Cilostazol	Inhib. of cAMP degradation (PDE-3 inhib.)	Oral	Not necessary	11–13 h	1–2 h	2–3 h	1–2 days
Abciximab	Inhib. of GP IIb/IIIa rec. (antibody)	Parenteral	Not necessary	8–12 h	Minutes	Minutes	1–2 days
Eptifibatide	Inhib. of GP IIb/IIIa rec. (cyclopeptide)	Parenteral	Not necessary	2–3 h	Minutes	Minutes	6–12 h
Tirofiban	Inhib. of GP IIb/IIIa rec. (non-peptide)	Parenteral	Not necessary	1–2 h	Minutes	Minutes	5–10 h

cAMP – cyclic adenosine monophosphate; COX-1 – cyclo-oxygenase 1; CYP – cytochrome P450; PAR-1 – protease-activated receptors; PDE – phosphodiesterase; PON-1 – paroxonase-1; TXA₂ – thromboxane A₂.

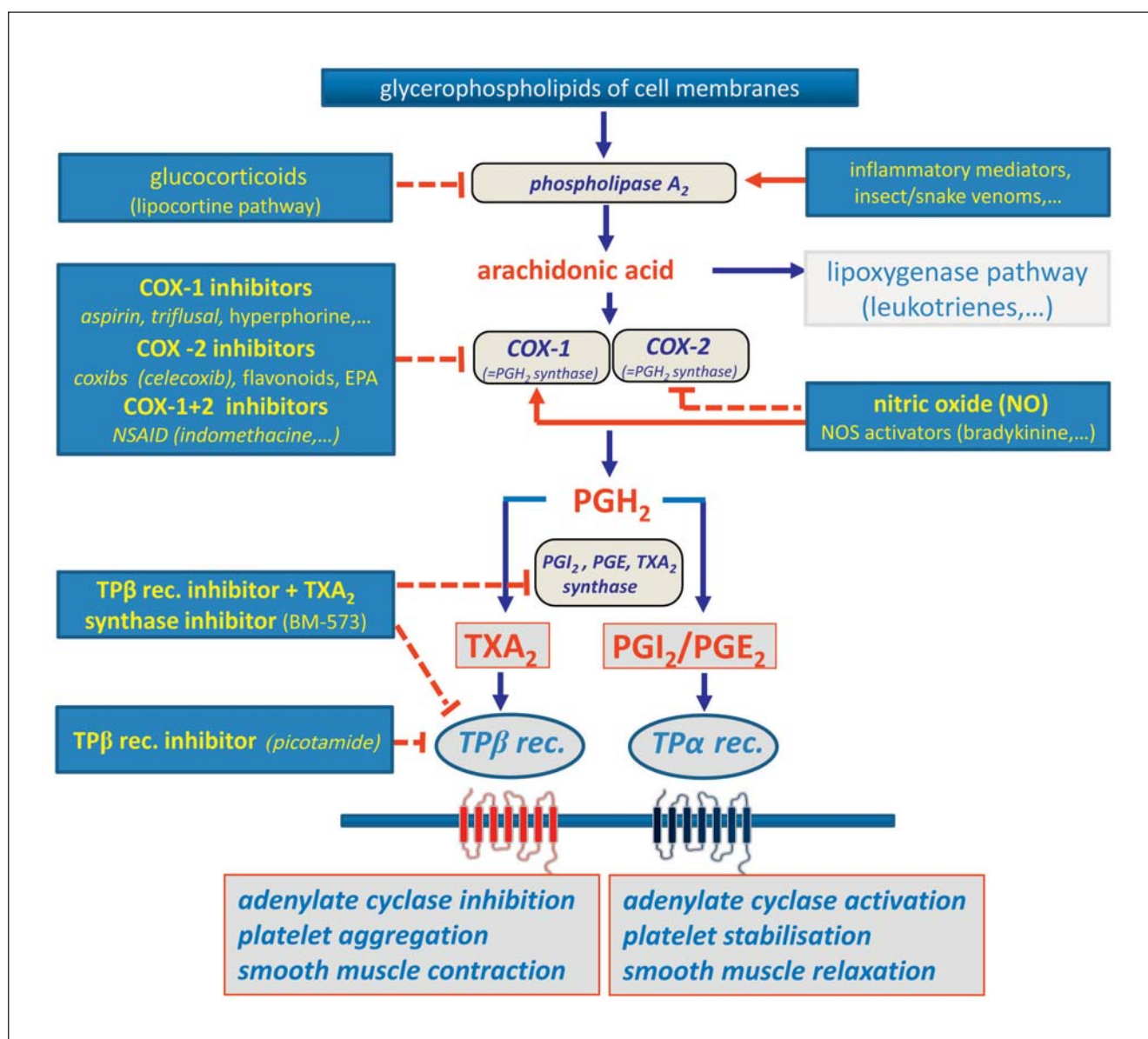


Fig. 2 – Cyclo-oxygenase pathway of platelet activation/inhibition by thromboxane A₂ and prostacyclin or prostaglandin E₂ synthesis. Stimulation of the target TPβ receptor by thromboxane A₂ inhibits adenylate cyclase activity and induces platelet aggregation. The inhibition of TXA₂ activity could be achieved by COX-1 or TXA₂ synthase inhibition or TPβ receptor blockade.

of additional elements of blood, in particular monocytes/macrophages [1,2]. Both aspirin and triflusal inhibit platelet function by suppressing TXA₂ synthesis via preferential COX-1 inhibition; the effect on the isoenzyme COX-2 is small.

As the evidence of the effect of aspirin was obtained under clinical conditions different from the current ones, the risk of potentially very frequent drug interactions or use of specific drug forms should not be disregarded.

The mechanism of the antiplatelet action of aspirin is irreversible inactivation of COX-1 in the platelet via serine acetylation in the region of the enzyme's catalytic center. The result is inhibited conversion of arachidonic acid to TXA₂ precursors (intermediate products – prostaglandins G₂ and H₂).

The critical process in producing the antiplatelet effect of aspirin is *resorption* (Fig. 3). Acetylsalicylic acid (a weak hydrophilic acid) will consistently resorb only in a non-dissociated state (at a pH < 3.5, i.e., in the acidic milieu of the stomach and proximal duodenum). Platelet deactivation through COX-1 acetylation occurs largely in portal blood; in the liver, the bulk of aspirin is degraded to salicylic acid [3,4]. Salicylic acid also reduces the activity of COX-1 and COX-2, but the effects are through actions on nuclear factor κB (NF-κB) and inhibition is transient and is not irreversible. Unless there is resorption in the stomach, aspirin becomes de-acetylated by aspirin esterases in the intestines to salicylic acid which (as a lipophilic molecule) will already absorb well in the intestine. Because of the degradation by esterases and variable resorption

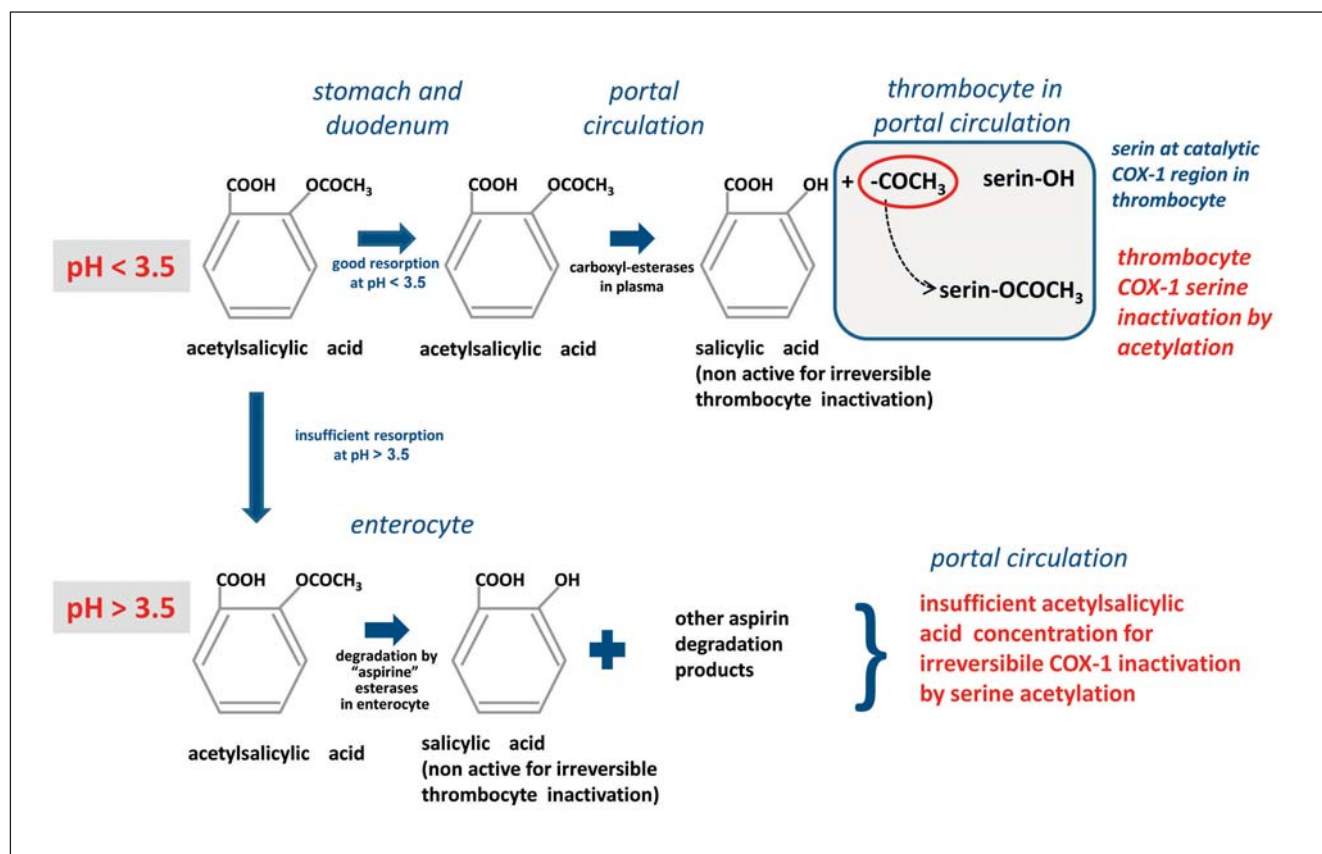


Fig. 3 – Mechanism of irreversible COX-1 thrombocyte inactivation in relation to absorption and degradation of aspirin in the course of different stomach pHs. Acetylsalicylic acid, a weak hydrophilic acid, has an acid dissociation constant pK_s of 3.5. Aspirin will consistently resorb only in a non-dissociated state (at a pH < 3.5), i.e., in the acidic milieu of the stomach and proximal duodenum. Unless there is resorption in the stomach, aspirin becomes de-acetylated by aspirin esterases in the intestines to salicylic acid, a lipophilic molecule, and will already absorb well in the intestine. Salicylic acid also reduces the activity of COX-1 and COX-2, but the inhibition is short-lasting and reversible. Because of the aspirin degradation by esterases and variable resorption rates in the alkaline milieu, the resulting concentration of aspirin in the portal circulation is unpredictable and overall appreciably decreased. Platelet deactivation through COX-1 acetylation occurs largely in portal blood; in the liver, the bulk of aspirin is degraded to salicylic acid.

rates in the alkaline milieu, the resulting concentration of aspirin in the portal circulation is unpredictable and overall appreciably decreased [5,6]. Hence, we are faced with a situation whereby one effect – irreversible inhibition of platelet COX-1 through serine acetylation – occurs presystemically in the portal circulation by the actual parent molecule (acetylsalicylic acid) and by the systemic anti-inflammatory and analgesic action of its metabolite, salicylic acid. Should pH rise above 3.5 – as is the case during co-administration of aspirin with proton pump inhibitors (PPI) or administration of aspirin as enterosolvent tablets – resorption in the stomach is impaired. It has been suggested that aspirin concentrations in the portal circulation remain at subtherapeutic levels. As studies published to date, which documented an effect of aspirin in the prophylaxis of atherothrombotic disease, did not use PPIs or involve the administration of drug presentations solvent in the acidic milieu, PPI co-administration or use of enterosolvent tablets cannot be considered an "evidence-based strategy". Although no prospective controlled trials exploring modulation of the clinical impact of co-medication are available, retrospective studies have

documented an adverse impact. In this context, mention should be made of a Danish registry showing that a combination of aspirin with a PPI increased the incidence of vascular events and cardiovascular mortality by 46% [7].

At the platelet level, the translocation of acetylsalicylic acid to the cytoplasm is controlled by multidrug resistance protein-4 (MRP-4), an efflux pump whose higher activity decreases the intracellular availability of aspirin. In addition to the pump's functionally significant polymorphism (with inter-individual differences), its activity is largely affected intra-individually by drug interactions or pathological conditions (e.g., extracorporeal surgery or diabetes). Indeed, it is just the inhibition of MRP-4 by dipyridamole which may explain why the effect of aspirin is significantly enhanced by dipyridamole. Its clinical relevance is supported by MRP-4 upregulation seen in patients after revascularisation using coronary artery bypass grafting with a several-fold increase in TXA₂ production during aspirin-based therapy and resumed inhibition of platelet synthesis after dipyridamole administration [8].

The onset of effect following oral solvent aspirin occurs quickly, within 20 minutes, and its plasma half-life is

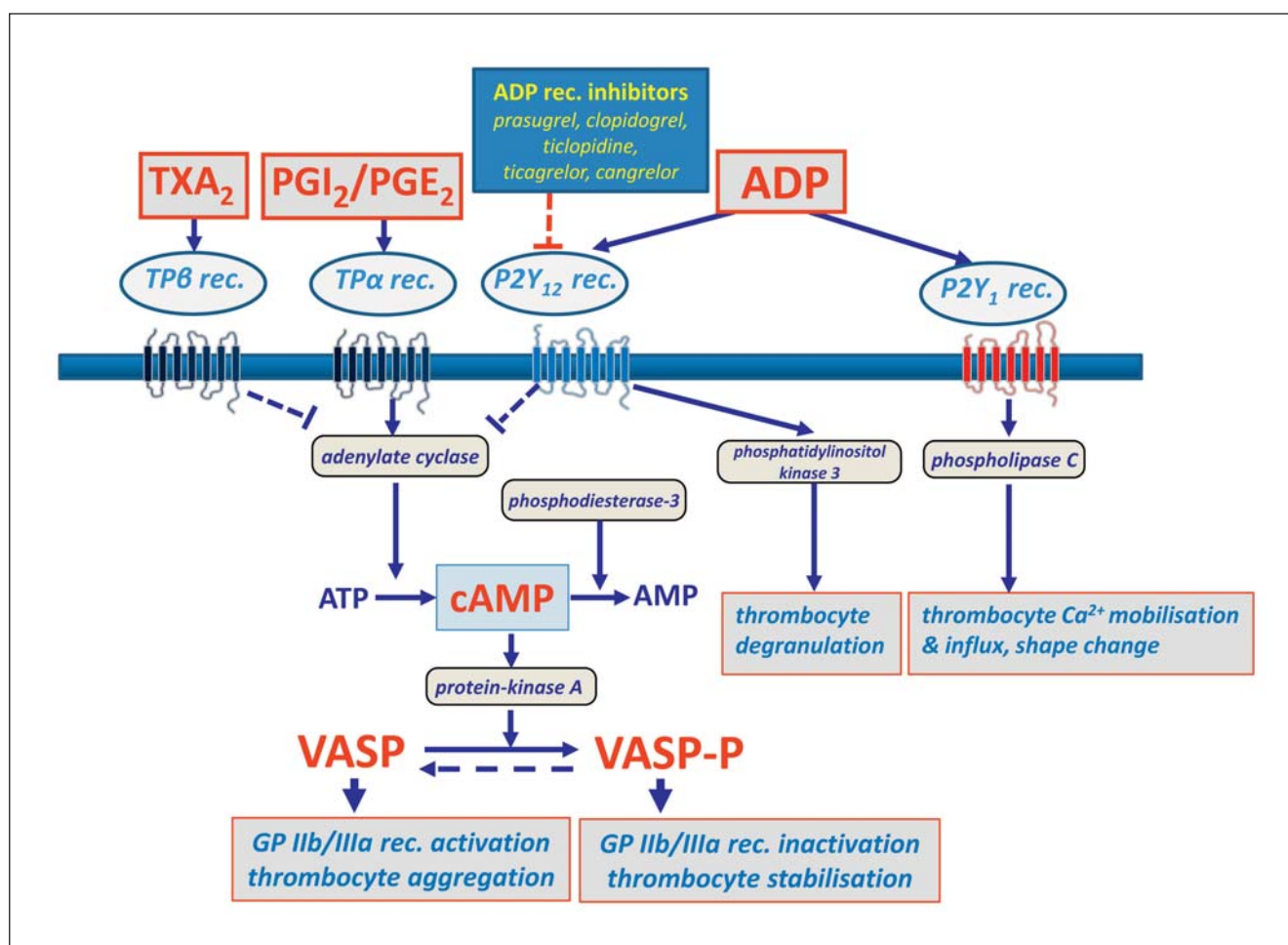


Fig. 4 – Adenosine diphosphate pathway of platelet activation and relation to eicosanoid (TXA₂ and PGI₂/PGE₂) effects. Opposing effects of ADP (or TXA₂) and prostacyclin/PGE₂ on thrombocyte activation. Adenylate cyclase inhibition or stimulation eventually results in a change in the ratio of phosphorylated to dephosphorylated protein VASP controlling actual thrombocyte activation and GP IIb/IIIa receptor stereoconformation. The outcome is then also modified by other nucleotide receptors, specifically the namely P2Y₁ receptor. Stimulation of the P2Y₁ receptor induces calcium platelet mobilization and calcium influx with a change in the thrombocyte shape and short-lasting thrombocyte activation.

insignificant; the effect is predominantly presystemic (i.e. in the portal circulation). The antiplatelet effect persists as long as the platelets remain in the circulation. As the antithrombotic action of antiplatelet agents is believed to be optimal at inhibition of activation of 60–90% platelets and, assuming the full effect lasts over a period of one to two days, complete restoration of primary hemostasis will occur within a week (Table 2).

A similar action is seen with *triflusal*, also a preferential COX-1 inhibitor causing irreversible serine acetylation at the active site of the enzyme. Its advantage over aspirin is increased nitric oxide (NO) availability via NO synthase stimulation [9]. Triflusal, whose effect is likewise supported by a large body of evidence, is in use in a number of European countries and the USA. While equally effective, it is safer than acetylsalicylic acid and there is no problem with bioavailability when co-medicated with PPIs.

Of the drugs approved and recommended in the recent American College of Clinical Pharmacology (ACCP) guidelines, mention should be made of *picotamide*, a combined TXA₂ synthesis inhibitor (through the inhibi-

tion of thromboxane synthase, an enzyme one step below COX) and inhibition of type TPβ receptors [10]. It has not yet been approved for clinical use in Europe. Development of terutroban, a selective antagonist of the TPβ receptor, has been discontinued. In secondary prevention it did not show any superiority over aspirin in preventing ischemic events.

1.2 Adenosine diphosphate (ADP)-induced activation inhibitors (platelet P2Y₁₂ receptor inhibitors)

Another class embraces *inhibitors of adenosine diphosphate (ADP)-induced activation*, that is, platelet P2Y₁₂ receptor inhibitors. Inhibition of the P2Y₁₂ receptor entails lower availability of the vasodilator-stimulated phosphoprotein (VASP) inducing stereoconformation changes in GP IIb/IIIa receptors, which facilitate actual platelet aggregation. Increased production of the phosphorylated VASP form (VASP-P) results in *platelet stabilization* (Fig. 4). This is paralleled, via a VASP-independent pathway, by suppressed *platelet degranulation*. The crucial point in the pathway leading to a change in the phosphorylated-

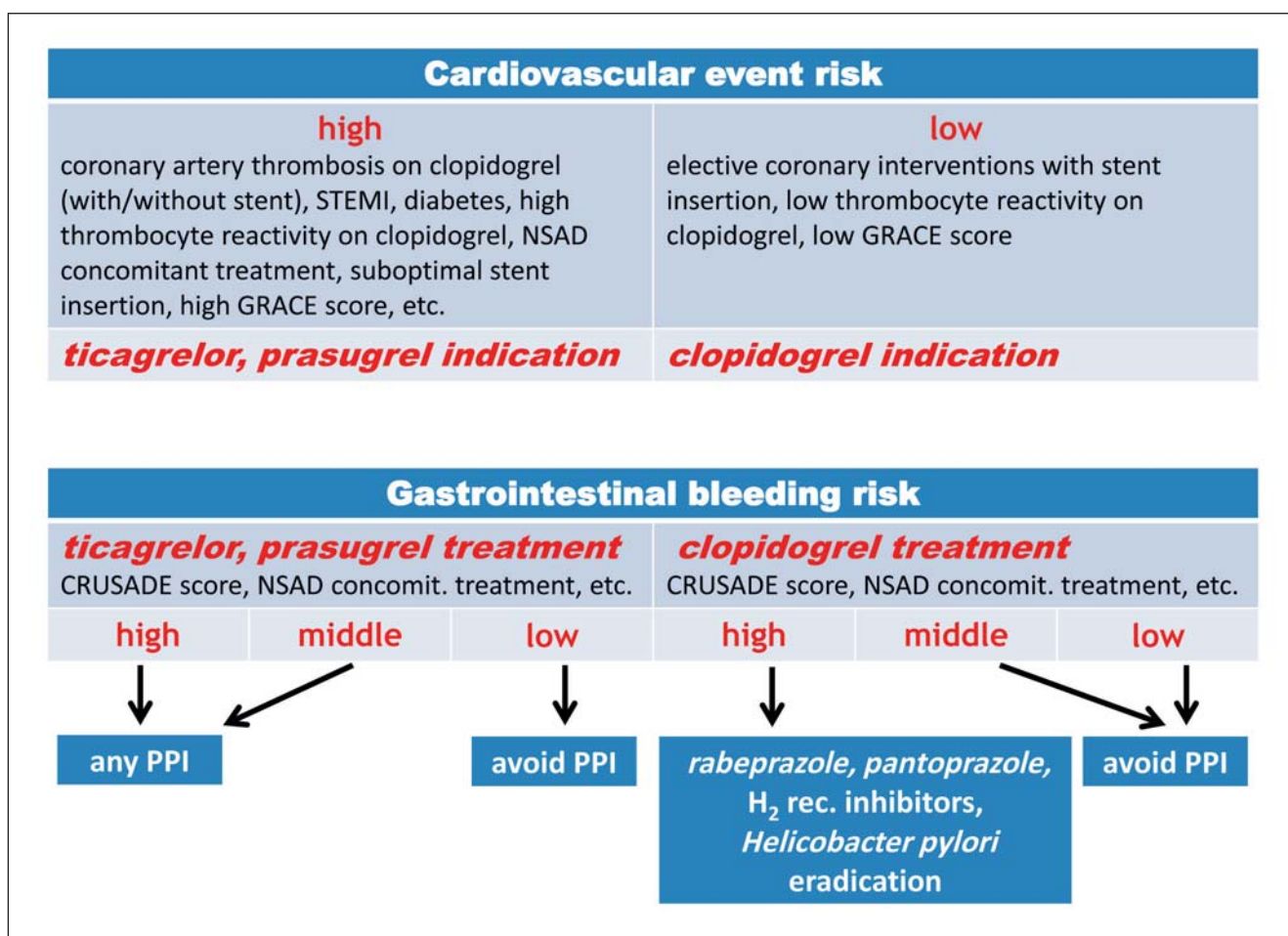


Fig. 5 – Selection of the optimal ADP receptor inhibitor and indication of prophylactic proton pump inhibition according to the risk atherothrombotic event and bleeding risk.

-to-dephosphorylated ratio is adenylate cyclase activation and increased cAMP availability.

Essentially, this class of antiaggregants is divided, based on the type of inhibition, into *irreversible* (prasugrel, clopidogrel, and ticlopidine) and *reversible* (ticagrelor) ones. Irreversible inhibitors bind covalently to the platelet receptor thus deactivating it permanently. Consequently, the platelet is unable to activate ADP as long as it remains in circulation (even once the drug concentration has decreased). By contrast, platelet inhibition by the reversible inhibitor ticagrelor is dependent on the current drug levels, and binding to platelet receptors is short-term (binding and dissociation occur within minutes); hence, platelet function is quickly restored once the levels of the drug have decreased.

Irreversible inhibitors are derived from thienopyridines and should be bioactivated to pharmacologically effective metabolites. The problem with the first- and second-generation thienopyridine ADP receptor inhibitors (*ticlopidine* and *clopidogrel*) is their complicated bioactivation taking hours to occur, even after the administration of the loading dose. Moreover, in the case of clopidogrel, conversion of 2-oxo-clopidogrel to an active thiol metabolite depends on the rate of resorption

by the glycoprotein P (P-gp) efflux pump and the rate of bioconversion by CYP isoenzymes, particularly CYP2C19 oxidase. Both systems are polymorphous, a significant percentage of the population has genotypes with high or, conversely, low activity of both the transport and metabolic systems. In cases where clopidogrel bioavailability at the level of the intestine is low or the bulk of clopidogrel undergoes degradation by esterases, the antiplatelet effect is insufficient. This condition is referred to as "high on-treatment platelet reactivity". Conversely, low P-gp activity or hyperfunctional CYP2C19 oxidase polymorphisms significantly enhance the resultant pharmacological action of clopidogrel. In the Caucasian population, heterozygote genotypes with lower, or homozygote types with zero activity of CYP2C19 (CYP2C19*1/*2, *1/*3 and/or CYP2C19*2/*2, *2/*3, *3/*3) occur in 20–30%, genotypes with higher activity (CYP2C19*1/*17 or CYP2C19*17/*17) are found in about 20%; hyperfunctional or hypofunctional P-gp genotypes (TT and/or CC ABCB1 polymorphism) occur equally in an approximate 25% of the population, and half have a genotype TC (intermediate activity pump). The resultant mosaic of phenotypes affecting clopidogrel resorption and activation is thus most varied [11,12].

Further, given the fact that bioavailability and activation are modified by a variety of drug interactions, e.g., with proton pump inhibitors (PPIs), antidepressants or steroid hormones, the clopidogrel-treated population includes a wide spectrum of patients with a varied response to therapy. In patients receiving concomitant therapy with aspirin (i.e. the substantial proportion of patients), an additional consideration is the variable availability of aspirin due to frequent co-administration of dual antiplatelet therapy with PPIs. It is quite logical then that trials exploring the role of one or two factors fail to report consistent results.

The challenges posed by variable availability, bioactivation, and slow onset of action in the class of irreversible ADP inhibitors have been eliminated by the introduction of *prasugrel*. Its resorption and bioactivation are unproblematic and they are not markedly modulated by pharmacological effects or drug interactions. The onset of action after a saturation dose is rapid, in the order of tens of minutes. The duration of effect is dependent mainly on platelet turnover, with full effect lasting 1 to 2 days, and complete restoration of primary hemostasis occurring within 5 to 7 days (Table 2).

An innovation in the class ADP inhibitors came with the introduction of *reversible P2Y₁₂ receptor inhibitors*, specifically *ticagrelor*. Ticagrelor, an adenosine derivative, is a competitive ADP receptor blocker. The bulk of its action is mediated by the mother molecule, and there is no need for bioactivation. The inter- and intra-individual variability of effect is small. The onset of effect is rapid, in the order of tens of minutes, and does not persist beyond 24 hours (Table 2). The shorter duration of action helps reduce the risk of bleeding in the event of injury or need for surgery; however, ticagrelor use requires increased cooperation on the part of the patient.

Unlike the irreversible P2Y₁₂ receptor inhibitors, ticagrelor has a broader pharmacodynamic effect activating as it does also adenosine receptors. Whether the effect is direct or mediated by a metabolite, or whether ticagrelor stimulates adenosine release from erythrocytes, is yet to be determined [13]. Whatever the case, the effects of adenosine help explain some, ticagrelor-specific adverse effects. Stimulation of A_{2B} adenosine receptors activates the respiratory center and bronchial muscle to contract, possibly resulting in dyspnea experienced over the first days after therapy initiation. Conversely, stimulation of myocardial A₁ and A_{2A} receptors will slow the generation and conduction of impulses, whose rate correlates with asymptomatic pauses on ECG recordings, again typically only encountered within the first days of therapy.

When comparing ADP receptor blockers, the main advantage of clopidogrel are lower direct health care costs while its drawbacks include a slow onset of effect and major inter- and intra-individual variability in its antiplatelet action. By contrast, both prasugrel and ticagrelor have a rapid onset of effect, with prasugrel shown to have a longer duration of effect while the effect of ticagrelor wears off earlier. The former property is of advantage in patients reluctant to cooperate, the latter in those at higher risk of bleeding or requiring surgery. Compared with clopidogrel, both prasugrel and ticagrelor offer a more consistent action and are effective in (up

to a third) patients with a reduced antiplatelet effect of clopidogrel; consequently, a higher risk (again by a third) for bleeding is to be anticipated. When comparing the efficacy and safety profiles of prasugrel and ticagrelor, if indicated in secondary prevention post-MI, their effect on reducing the incidence of atherothrombotic events is equal and comparable with the incidence of bleeding (if disregarding revascularisation-related events). In patients scheduled for emergency surgical revascularisation, ticagrelor is significantly safer. Adverse effects related to adenosine receptor activation by ticagrelor usually occur within the first days of therapy, with dyspnea requiring therapy discontinuation seen in only a small proportion of patients.

1.3 Drugs stabilizing the platelet by increasing cAMP availability – dipyridamole and cilostazol

Increased availability of platelet cyclic adenosine monophosphate (cAMP) blunts the response to various stimuli such as P2Y₁₂ receptor activation inhibiting the effect of the key enzyme adenylate cyclase. Increased cAMP availability can be obtained by two opposite mechanisms, i.e., synthesis stimulation or degradation inhibition (Fig. 1). While the former mechanism, increased cAMP activity, is obtained with *dipyridamole* (together with P2Y₁₂ receptor inhibitors), *cilostazol* is a powerful antagonist of the platelet phosphodiesterase-3 (PDE-3) isoenzyme degrading cAMP. Blockade of PDE-3 reduces the cAMP degradation and increases the availability of this signal molecule. A higher cAMP activity alters the ratio of the vasoactive VASP protein and its phosphorylated form.

The other factor possibly contributing to the ultimate effect of dipyridamole may be increased aspirin availability by inhibition of multidrug resistance protein 1 (MRP-1). The MRP-1 is an efflux pump expressed in thrombocytes. Higher MRP-1 activity (e.g. in post CABG patients or in diabetics) results in decreasing platelet aspirin availability and insufficient COX inhibition [8].

Both drugs – dipyridamole and cilostazol – have been shown to exert a beneficial effect in the secondary prevention of stroke; their pharmacokinetic characteristics are shown in Table 2.

1.4 Thrombin PAR-1 receptor inhibitors and serotonin 5-HT_{2A} and 5-HT_{2B} receptor antagonists

Platelet activation is also mediated by a number of membrane receptors listed (including their activators and inhibitors) in Table 1. The most important ones – in clinical terms – are those that can be effectively modulated.

This class of drugs is exemplified by platelet *protease-activated receptors* (PAR-1 and PAR-4). Their dominant ligand is thrombin; platelet activation by thrombin serves as a link between secondary and primary hemostasis. The significance of the PAR-1 receptor is complex – adenylate cyclase inhibition, platelet intracellular Ca²⁺ mobilization and thrombocyte degranulation with ADP release (Fig. 1, Ref. [14]).

At present, two PAR-1 inhibitors are being evaluated in clinical trials in combination with ADP-induced activation inhibitors or thromboxane-induced activation pathway inhibitors. While *vorapaxar* has been through a se-

ries of phase III trials, clinical trials with *atopaxar* are still ongoing.

Vorapaxar, a selective reversible thrombin PAR-1 antagonist, has been shown to have a rapid onset of action (within an hour) and long duration of effect ($t_{1/2}$ of 160 up to 310 h), allowing for a once-a-day oral dosing regimen (following the administration of the loading dose). Although the receptor blockade is reversible, the effect will reliably last for several weeks (Table 2). This fact may be of critical importance given the current unavailability of an antidote. As vorapaxar elimination depends on CYP3A4 oxidase, significant interaction with inhibitors and inducers of this isoenzyme is likely.

Another PAR-1 receptor inhibitor is *atopaxar* which, besides blocking platelet activation, inhibits the release of endothelial acute phase molecules, in particular of the cytokines interleukin-6, sCD40L, and P-selectin [15]. As no effect of pharmacogenetics on the resultant antiplatelet action has been demonstrated with vorapaxar or *atopaxar*, and their availability was not modified by a change in gastric pH value, there is no risk for interaction with PPIs.

Other receptors we have been able to modulate are *platelet serotonin receptors*, specifically the 5-HT_{2A} and 5-HT_{2B} isoforms. Their inhibition by *sarpogrelat*, an antagonist of both subtypes if indicated as an antithrombotic, has been approved in a number of Asian countries, but not in the EU. The other receptors, specifically PAF or PAR-4, play only a supporting role and are thus not expected to be indicated for primary hemostasis inhibition.

1.5 Platelet GP IIb/IIIa receptor inhibitors

Inhibitors of aggregation as such are represented by *glycoprotein (GP) IIb/IIIa receptor blockers* (*abciximab*, *integrilin* or *tirofiban*). Aggregation occurs by fibrinogen-forming fibrin bridges between platelets following GP IIb/IIIa receptor activation, the sequence of receptors is occupied by bivalent proteins (fibrin, Von Willebrand factor – vWF, vitronectin, and others) resulting in primary thrombus formation.

Agents belonging to this class of drugs inhibit the last phase of primary hemostasis. While the above listed classes suppress the expression or stereoconfiguration of these two membrane-bound glycoprotein subunits, GP IIb/IIIa receptor antagonists prevent platelets from binding together, a process mediated by bivalent proteins containing the specific arginine–glycine–aspartate amino acid sequence. The inhibition may be obtained using either specific antibodies to receptors, which block bivalent protein binding (*abciximab*) or peptides or non-peptide molecules occupying their own domain (*eptifibatide* or *tirofiban*).

Abciximab has been shown to have a strong and long-term affinity for the GP IIb/IIIa receptor, a plasma half-life of 8–12 h; its effect thus wanes gradually lowering the risk for enhanced hemostasis due to the rebound phenomenon. The cyclic heptapeptide *eptifibatide* or the peptidomimetic molecule *tirofiban* show a weaker affinity for the receptor and have a shorter plasma half-life (1–3 h); their action is thus shorter with hemostasis returning to normal soon after infusion has been discontinued. As both *eptifibatide* and *tirofiban* are excreted via the kidney, renal failure will prolong their effect.

Regarding the pharmacological effects, there have been reports of inter-individual variability of the anti-thrombotic response and risks of thrombosis and bleeding associated with some types of GP IIb/IIIa receptor polymorphisms [16]. A number of mutations at individual bases in the control genes or receptors *per se* have been reported. While *in vitro* studies have linked many of these single nucleotide polymorphisms to increased aggregation, other mutations have been associated with decreased aggregation. The body of our knowledge is still not big enough to make monitoring of GP IIb/IIIa receptors clinically relevant and allow for targeted therapy.

In summary, development in the class of antiplatelet drugs has made major advances over the last decade. However, the main problem is not insufficient efficacy of drugs as, if using the optimal dose and combination, hemostasis is inhibited; it is actually bleeding complications which pose a problem. The variable antiplatelet action of clopidogrel was eliminated when third-generation P2Y₁₂ receptor inhibitors had appeared on the scene.

It is thus no exaggeration to claim that, with the introduction of ticagrelor- and prasugrel-based therapy, prophylaxis of thrombotic events no longer resembles an unpredictable roulette game with the physician unable to tell which patient will respond to clopidogrel therapy, and has turned into a sophisticated chess game with strictly defined rules instead.

An issue to be yet solved is the availability of aspirin when co-administered with proton pump inhibitors. The risk of failure of dual antiplatelet therapy involving PPIs rests both with aspirin and clopidogrel. The addition of a PPI to dual antiplatelet therapy as well as the optimal antiplatelet combination should no doubt be considered relative to the risks of thrombosis and bleeding. A potential algorithm according to M.R. Thomas and R.F. Storey is outlined in the schema shown below (Fig. 5, Ref. [17]).

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