Therapeutic Inhibitors of Platelet Aggregation – from Aspirin to Integrin Blockers

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Key Words

Inhibitors of platelet aggregation · ASS ·
Integrin blockers · COX · GPIIb/IIIa

Summary

A variety of orally and intravenously administered agents have been made available for anti-platelet therapy. Clinical trials in patients with acute manifestations of cardiovascular disease have shown that the combined administration of substances which inhibit different steps of platelet adhesion and aggregation reduce cardiovascular adverse events. However, the risk of bleeding may increase depending on the patient's comorbidity, age and the agents that are used for concomitant anticoagulation. Therefore, ongoing efforts are made to develop highly effective inhibitors of platelet function with an excellent controllability and the lowest acceptable risk of bleeding events. The current pharmacological strategies for the inhibition of platelet function are i) the inhibition of the cyclooxygenase, ii) inhibition of the phosphodiesterase, iii) blockade of the platelet P2Y₁₂ ADP receptor, and iv) blockade of the platelet glycoprotein IIb/IIIa. In the following review, the mechanisms of action and the clinical use of approved anti-platelet agents will be described. Furthermore, substances for the future that are under development are discussed.

Schlüsselwörter

Inhibitoren der Thrombozytenaggregation · ASS ·
Integrin-Blocker · COX · GPIIb/IIIa

Zusammenfassung

Gegenwärtig stehen zahlreiche intravenös applizierbare Substanzen zur Hemmung der Thrombozytenfunktion zur Verfügung. In klinischen Studien bei Patienten mit akuten Manifestationen kardiovaskulärer Erkrankungen konnte nachgewiesen werden, dass die Morbidität und Mortalität durch die kombinierte Applikation von Substanzen, die unterschiedliche Schritte der Thrombozytenadhäsion und -aggregation hemmen, reduziert werden konnte. Dennoch kann das Risiko von Blutungskomplikationen abhängig von den jeweiligen Komorbiditäten und dem Alter der Patienten sowie von den gegebenenfalls gleichzeitig verwendeten Antikoagulantien durch die antithrombozytäre Therapie steigen. Aus diesem Grund werden zur Zeit hochpotente Inhibitoren der Thrombozytenfunktion entwickelt oder bereits evaluiert, die sich zum einen durch eine hervorragende labortechnische Kontrollierbarkeit, zum anderen durch ein möglichst geringes Blutungsrisiko auszeichnen. Die gegenwärtigen pharmakologischen Strategien zur Hemmung der Thrombozytenfunktion umfassen 1) Hemmung der Cyclooxygenase, 2) Hemmung der Phosphodiesterase, 3) Blockade des thrombozytären P2Y₁₂ADP-Rezeptors sowie 4) Blockade des Glykoprotein-IIb/IIIa-Rezeptors der Thrombozyten. In diesem Übersichtsartikel werden die Wirkungsmechanismen und die therapeutischen Einsatzmöglichkeiten dargestellt. Darüber hinaus werden Perspektiven der neuen, noch in der Entwicklung befindlichen anti-thrombozytären Substanzen diskutiert.

Introduction

A number of physiological agonists are able to promote platelet activation. Most of those agonists unfold their activity through specific receptors that are located on the platelet surface. The common final step in the downstream signaling cascade of these receptors is the activation of the platelet integrin $\alpha_{IIb}\beta_3$ (the platelet receptor for fibrinogen, also termed glycoprotein IIb/IIIa (GPIIb/IIIa)). Activation of $\alpha_{IIb}\beta_3$ leads to a global conformational rearrangement that allows binding of soluble fibrinogen or von Willebrand factor. Both fibrinogen and von Willebrand factor bind to activated GPIIb/IIIa and crosslink platelets, therefore leading to platelet aggregation and finally contribute to the formation of a thrombus. To date four different classes of clinically approved anti-platelet substances are available. Three of these classes of agents interfere with different ways of platelet activation (fig. 1, table 1) [1–3], the fourth class blocks the final common step of platelet aggregation (fig. 1, table 1) [4].

Inhibition of Platelet Cyclooxygenase (COX)

Acetylsalicylic Acid (Aspirin®)

Acetylsalicylic acid (ASA) is one of the first discovered and best studied anti-platelet drugs. The substance ASA inhibits the enzymes cyclooxygenase-1 (COX-1; prostaglandin (PG)H synthase 1) and cyclooxygenase-2 (COX-2; PGH synthase 2) in their conversion of arachidonic acid to PGG₂ and PGH₂ in a dose-dependent manner and therefore has dose-dependent side effects. Reduced production of PGG₂ and PGH₂ leads to

reduced formation of thromboxane A₂ (TXA₂), a potent stimulator of platelet activation, thereby reducing platelet secretion and aggregation. TXA₂ is mainly produced by platelets and spontaneously degraded to the biological inactive thromboxane B₂ which can be measured in serum and urine, therefore serving as a marker of TXA₂ production [1, 2]. COX-1 is constitutively expressed in most cell types, whereas COX-2 is detectable after induction in inflammatory, endothelial and other cells. It is generally believed that platelets express only COX-1 although in one study COX-2 was found in platelets too [3]. ASA selectively acetylates the serine residue at position 529 of COX-1, which results in the steric hindrance of arachidonic acid to access the catalytic center and thereby mediates a permanent loss of cyclooxygenase activity. COX-2 is inhibited by the same mechanism, however, higher doses of ASA are needed for inhibition. The product of COX-1 is predominantly metabolized to TXA₂. Through its release and binding to the TXA₂ receptor, TXA₂ represents an amplification system of the platelets that is active after stimulation with divergent primary platelet agonists (ADP, collagen, thrombin, epinephrin, etc.). Since platelets cannot re-synthesize COX-1, the irreversible blockade of COX-1 results in irreversible platelet inhibition. Thus, although ASA is detectable in plasma only for a limited time, platelet inhibition can be demonstrated for about 7–10 days [4].

The pivotal role of platelets in arterial thrombosis has essentially been established by the beneficial effects of ASA in patients with myocardial infarction. In the ISIS-2 trial, the administration of ASA reduced the 5-week mortality of patients with myocardial infarction by 23%. The effect of platelet inhibition equaled and added to the effect of thrombolysis by streptokinase [5]. In the 10-year-follow-up, the original benefit

Fig. 1. Mechanism of action of platelet inhibitors (see also table 1).

Table 1. Therapeutic inhibitors of platelets

Substance	Trade name	Mechanism of action	Common dosage
Acetylsalicylic acid	Aspirin	COX inhibition (1) (irreversible antagonist)	75–100 mg/day (oral)
Cilostazol	Pletal	PDE3 inhibition (2)	2x 50–100 mg/day (oral)
Dipyridamole	Persantine, Aggrenox*	PDE5 inhibition (2)	2x 200 mg/day (oral)
Ticlopidine	Tyklid	P2Y ₁₂ -ADP receptor inhibition (3)	2x 250 mg/day (oral)
Clopidogrel	Iscover, Plavix	P2Y ₁₂ -ADP receptor inhibition (3)	1x 75 mg/day (oral)
(Prasugrel)	in clinical trials phase III	P2Y ₁₂ -ADP receptor inhibition (3)	10 mg/day (oral)
(Cangrelor)	in clinical trials phase III	P2Y ₁₂ -ADP receptor inhibition (3)	(intravenously)
Abciximab	ReoPro	GPIIb/IIIa blockade (4) (irreversible antagonist)	0.25 µg/kg bolus + 0.125 µg/kg/min infusion (intravenously)
Eptifibatide	Integrilin	GPIIb/IIIa blockade (4) (competitive antagonist)	180 µg/kg bolus + 2 µg/kg/min infusion (intravenously)
Tirofiban	Aggrastat	GPIIb/IIIa blockade (4) (competitive antagonist)	10 µg/kg bolus + 0.15 µg/kg/min infusion (intravenously)

*Contains 25 mg acetylsalicylic acid.

of ASA was still present [6]. Unstable angina is also associated with platelet activation [7] and, in fact, ASA does reduce the rate of death and myocardial infarction in patients with unstable angina [8–10]. Coronary angioplasty is linked to platelet activation, and ASA significantly reduces the rate of acute vessel closure [11, 12]. In all these acute clinical situations, the administration of ASA is a therapeutic necessity. Nevertheless, ASA saved most lives in the secondary prevention of cardiovascular events – and as a single drug probably ASA saved most lives of all. In the Antiplatelet Trialists' meta-analysis of approximately 70,000 patients with coronary artery disease, transient ischemic attack, stroke or peripheral arterial vascular disease, ASA showed an odds reduction of 25% for myocardial infarction, stroke or vascular death [13]. Another meta-analysis of 200,000 patients treated with anti-platelet drugs confirmed the benefits and even showed additional advantages of anti-platelet therapy for the secondary prevention of cardiovascular complications in patients with stable angina pectoris, intermittent claudication, and (if oral anticoagulants are unsuitable) atrial fibrillation [14]. In the primary prevention of cardiovascular events, the increase of hemorrhagic strokes – a severe side effect of ASA – seems to counteract the beneficial effects of ASA on the rate of myocardial infarctions [15–17]. In the Physicians' Health Study, the rate of strokes increased by 21% and the rate of myocardial infarctions decreased by 44% [15]. Nevertheless, a reduction of mortality from all cardiovascular causes was not associated with ASA [15]. When extrapolated from the data of ASA treatment in primary and secondary prevention, the cut point at which the benefit in the prevention of ischemic cardiovascular events outweighs the increase of the risk of hemorrhagic stroke has to be determined with caution. A higher cardiovascular risk seems to correlate with a higher benefit conferred

by ASA. The previously published results of the Women's Health Study indicate that at least in women ASA is inefficient for the primary prevention of myocardial infarction, but reduces the risk of ischemic stroke [18]. However, patients with clear cardiovascular risk factors, especially with diabetes mellitus, may indeed profit from ASA treatment in the primary prevention of cardiovascular events [19–21].

For a long time, the dosing of ASA has been debated controversially. There is no doubt that ASA-induced gastrointestinal toxicity is dose-dependent [22]. On the other hand, an increase in the dose does not correlate with an increase in antiplatelet effects of ASA. The results of the 2002 Antithrombotic Trialists' Collaboration's meta-analysis convincingly demonstrate that high daily doses of ASA (500–1,500 mg) are not more effective than medium doses (160–325 mg) or low doses (75–150 mg) [14]. Thus, low-dose ASA seems to exert the full anti-platelet efficiency with the lowest risk of side effects. In clinical settings that require an immediate effect of ASA on platelet aggregation, a loading dose of at least 160 mg should be administered [23].

Inhibition of Phosphodiesterase

The phosphodiesterases (PDEs) are a family of enzymes catalyzing the hydrolysis of cyclic nucleoside monophosphates, namely cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) [24]. At least nine different isoforms termed PDE1 through PDE9 have been identified in mammalian tissues [25]. Platelets have been found to express PDE2, PDE3 and PDE5 isoenzymes. Two substances acting by inhibition of PDE, namely dipyridamole and cilostazol, are clinically approved. Dipyridamole primarily inhibits

the degradation of cGMP [26] by PDE5 inhibition, but it also elevates platelet cAMP levels. The increased platelet cAMP and cGMP concentrations lead to a reduced platelet reactivity by decreasing the cytoplasmic calcium and inhibiting platelet prostaglandin synthesis. Another nonplatelet-specific effect of dipyridamole is the elevation of extracellular adenosine levels by reducing their uptake and metabolism [27]. Dipyridamole also enhances the release and prevents the metabolic degradation of endothelial prostaglandin PGI₂ [28, 29], a potent inhibitor of platelet aggregation.

Cilostazol potentially inhibits the PDE3 and adenosine uptake, thereby increasing platelet and vascular smooth muscle cell cAMP levels, which ultimately helps to inhibit platelet aggregation [30]. Additional beneficial effects of cilostazol have been observed and cannot be explained solely by the elevation of cAMP levels. The lipid metabolism seems to be influenced in a positive way since cilostazol facilitates the removal of triglycerides and increases high-density lipoprotein (HDL) cholesterol levels [31, 32].

Dipyridamole (Persantin®, Aggrenox®)

Since the first introduction of dipyridamole as an anti-anginal medication in 1959, there has been a great controversy about the clinical relevance of dipyridamole as an antithrombotic agent. Two major studies were performed to elucidate the role of dipyridamole in the secondary prevention of myocardial infarction: first, the Persantine-Aspirin Reinfarction Study (PARIS I) [33] and second, the PARIS II study [34]. PARIS I directly compared ASA 324 mg three times daily to the same dose ASA plus pyridamole 75 mg three times daily in a total of 2,026 patients. Primary endpoints were total mortality, coronary mortality, and fatal plus nonfatal myocardial infarction. The average follow-up was 41 months. There was no statistical significant difference between the two treatment groups. In PARIS II, patients were randomized to ASA plus dipyridamole or placebo. A 24% reduction of coronary adverse events was found in the group treated with ASA plus dipyridamole. However, a group receiving ASA only was not included in this trial so that a direct beneficial effect of dipyridamole added to ASA could not be proven.

Furthermore, other clinical trials failed to show consistent benefit of dipyridamole added to ASA in patients having undergone coronary artery bypass surgery [35], in patients with peripheral vascular disease [36], or in patients with prosthetic heart valves [37]. However there is evidence that dipyridamole plays an important part in the prevention of stroke. The European Stroke Prevention Study 2 (ESPS-2) was a randomized placebo-controlled double-blind trial examining ASA 50 mg daily, dipyridamole 400 mg daily, a regimen of both drugs together or of placebo. The combined treatment with ASA 50 mg and dipyridamole 400 mg daily reduced the relative risk of major vascular events by 22% compared to a

treatment with ASA alone [38]. A subgroup analysis of the ESPS-2 study showed that the efficacy of the combination of ASA and dipyridamole compared to ASA alone increases in higher-risk patients [39].

The combination of low-dose ASA and dipyridamole is now recommended for the secondary prevention of ischemic stroke by the German Society for Neurology and the American Heart Association / American Stroke Association [40]. The mechanism by which dipyridamole prevents stroke still needs to be determined. A recent study failed to establish a link between dipyridamole and a permanent reduction of blood pressure. This link would at least partially have explained how strokes are prevented [41]. Enrollment of 20,333 patients in the PROFESS trial, a large clinical trial that compares the combination of ASA (25 mg) and dipyridamole (200 mg) twice daily with clopidogrel (75 mg) once a day for the secondary prevention of ischemic stroke, has just been finished. The results of this trial will further clarify the role of dipyridamole in the secondary prevention of ischemic stroke.

Cilostazol (Pletal®)

The quinolinone derivative cilostazol has been shown to inhibit platelet activation [42] and increase vasodilation [43]. Cilostazol inhibits the proliferation of vascular smooth muscle cells [44]. After oral administration, cilostazol is extensively metabolized by hepatic cytochrome P450 enzymes. Two metabolites of cilostazol are active and account for the pharmacologic effects observed in patients treated with cilostazol 100 mg or 50 mg (when administered together with other antiplatelet drugs) twice daily. The elimination half-lives of cilostazol and its active metabolites amount to 11–13 h. The substance has been extensively studied in patients with peripheral vascular disease and intermittent claudication. A meta-analysis of placebo-controlled trials with cilostazol in the treatment of peripheral artery disease showed that cilostazol therapy (administered for 12–24 weeks) increased the maximal and pain-free walking distance in patients with intermittent claudication by 50 and 67%, respectively [32]. In addition to the enhancement of quality of life, treatment with cilostazol reduced plasma triglyceride levels by 15.8% and increased HDL by 12.8% [32]. Cilostazol is primarily used for the treatment of symptomatic peripheral artery disease. The role of cilostazol in the treatment of other diseases secondary to atherosclerosis needs to be carefully investigated in larger scale placebo-controlled clinical trials.

Blockade of the Platelet P2Y₁₂ ADP Receptor

The thienopyridines ticlopidin and clopidogrel are prodrugs metabolized in the liver by cytochrome P450. The short-lived

active metabolite of clopidogrel was identified as being a thiol derivate of the parent agent clopidogrel [45]. The selectivity of thienopyridines for the P2Y₁₂ ADP receptor is thought to be caused by covalent modification of the four cysteine residues in the P2Y₁₂ receptor via the thiol metabolites [45, 46].

The inhibition of platelet aggregation by the thienopyridine ticlopidine was already reported in 1975 by Thebault et al. [47]. Rather recently, clopidogrel, also belonging to the thienopyridines, replaced ticlopidine in clinical use since ticlopidine showed significant adverse effects such as skin rash, gastrointestinal symptoms and bone marrow toxicity with severe and fatal neutropenia. Treatment with clopidogrel, however, caused none or much fewer of these problems [48–51]. The breakthrough study for the widespread clinical use of clopidogrel was the CAPRIE trial [48]. In this study, clopidogrel proved to be slightly more effective than ASA for the secondary prevention of thromboembolic complications in patients with atherosclerotic disease (prior myocardial infarction, ischemic stroke or peripheral vascular disease). In addition, safety and tolerability of clopidogrel and ASA were similar. In interventional cardiology, the combination therapy of ASA and clopidogrel after stent placement has become a widespread standard [51]. Particularly the risk of a subacute stent thrombosis after stent placement could be substantially reduced by the combination of ASA and thienopyridines [51–54]. Finally, the concept of long-term treatment of coronary syndromes with the combination of ASA and clopidogrel was borne [55].

The specific abundance of the P2Y₁₂ ADP receptor limited to platelets and potentially to the brain makes this receptor an interesting pharmacological target, promising an almost ideal selectivity [46]. Clopidogrel has been shown to block all available P2Y₁₂ receptors on the platelet and thus demonstrates highest efficiency [56]. Moreover, clopidogrel presents an almost ideal safety and tolerability profile [48, 50, 51]. In fact, clopidogrel is a remarkable pharmaceutical agent. Nevertheless, two characteristics of clopidogrel justify the search for other P2Y₁₂ ADP receptor inhibitors. First, even with a loading dose of 300 mg at least 2, but probably up to 12 h are needed for the full anti-platelet effect of clopidogrel to develop [57]. The data of the ISAR REACT study suggest that with a loading dose of 600 mg, a steady state can be reached in less time than with 300 mg, and that the higher dose may lead to a better outcome in the setting of percutaneous coronary interventions (PCI) [58]. Two pharmacokinetic studies confirmed that maximum plasma levels of the active metabolite of clopidogrel can be reached as early as 2 h after administration of a 600 mg loading dose, which also results in a maximum level of inhibition of platelet aggregation [59, 60]. However, in acute myocardial infarction or urgent coronary interventions for example, the anti-platelet effect of clopidogrel might still be not fast enough. Second, the P2Y₁₂ ADP receptor is irreversibly blocked and thus the effect persists for the entire platelet life span [61]. Since there is no antagonist available, which would

be needed for example when urgent operations are performed, bleeding complications may occur. To overcome these problems, a new class of P2Y₁₂ ADP receptor inhibitors has been developed [62], based on the fact that ATP is a competitive antagonist of ADP. Structural homologues were screened for selectivity against the P2Y₁₂ receptor, and there were indeed several agents found, some of them already having been tested in clinical trials. The advantage of these agents consists in their immediate anti-platelet effects after intravenous application that, because of their short half-life, are rapidly reversible [62]. Some of these agents can be used as intravenous, some as oral drugs [62]. One of the intravenously administered substances (cangrelor) has already entered phase III trials and is going to be compared with clopidogrel in a clinical trial in patients with acute coronary syndromes (ACS) in the setting of PCI [63]. Another agent which was developed as an oral drug (prasugrel) has also reached phase III trials and will be examined in the TRITON-TIMI 38 trial in direct comparison to clopidogrel [64].

Blockade of the Platelet GPIIb/IIIa

GPIIb/IIIa, or integrin $\alpha_{IIb}\beta_3$, is the platelet receptor for fibrinogen and mediates the final step in platelet aggregation. As the default state of integrin $\alpha_{IIb}\beta_3$ is a non-activated and resting state, it needs to become activated in order to bind its major ligand, i.e. soluble fibrinogen. This occurs after the activation of platelets by physiological agonists (e.g. thrombin, ADP, collagen), which stimulate intracellular signal pathways and thereby induce conformational changes of integrin $\alpha_{IIb}\beta_3$. This process is termed ‘inside-out’ signaling [65]. It switches the receptor into an activated state with high affinity for fibrinogen and numerous other ligands [66]. As a result of integrin $\alpha_{IIb}\beta_3$ -mediated binding to the bivalent molecule fibrinogen, platelets aggregate and form a thrombus rich in platelets. To date, two binding sites have been well characterized in integrin $\alpha_{IIb}\beta_3$: an Arg-Gly-Asp (RGD) binding site and a Lys-Glu-Ala-Gly-Asp-Val (KQAGDV) binding site [67]. Other binding sites have been described as well, with yet unknown functional properties [68, 69]. Interestingly, fibrinogen binds via the KQAGDV binding site. Agents that bind within the ligand-binding region of integrin $\alpha_{IIb}\beta_3$ and block the binding of its natural ligands have been developed and termed GPIIb/IIIa inhibitors. Of the three clinically approved parenteral GPIIb/IIIa inhibitors, one (abciximab; ReoPro®) is based on an antibody structure, and the two others (eptifibatide; Integrilin® and tirofiban; Aggrastat®) are described as small-molecule GPIIb/IIIa inhibitors. All of them bind to integrin $\alpha_{IIb}\beta_3$ either in the resting or activated conformation and inhibit fibrinogen binding and, consequently, platelet aggregation. The first substance to be developed was a murine monoclonal antibody (mAb) that blocked integrin $\alpha_{IIb}\beta_3$ [70]. The immunogenicity of this mAb could be reduced by its human-

ization, i.e. by the exchange of the constant regions of the mouse antibody with the constant regions of human immunoglobulin IgG₁ to produce a chimeric Fab fragment called abciximab. The other two GPIIb/IIIa inhibitors belong to the group of small molecules and can be subdivided into synthetic peptide inhibitors (eptifibatide) and synthetic non-peptide inhibitors (tirofiban).

Abciximab (ReoPro®)

The Fab fragment abciximab, with a molecular mass of 48 kDa and a reported equilibrium dissociation constant (KD) of 5 nmol/l is a high-affinity GPIIb/IIIa inhibitor for parenteral use only [71]. The high receptor affinity and low KD characterize the antagonist–receptor binding as non-competitive, rendering a low plasma concentration of unbound abciximab that is eliminated through protein metabolism. Besides binding to integrin $\alpha_{IIb}\beta_3$, abciximab has been reported to bind with equal affinity to integrin $\alpha_v\beta_3$ [72] and with lower affinity (KD: 160 nmol/l) to the activated integrin $\alpha_M\beta_2$ (Mac-1) on monocytes and granulocytes [73]. The blockade of Mac-1 by abciximab inhibits leukocyte adhesion and aggregation and thus directly attenuates inflammatory reactions [74]. Furthermore, since abciximab inhibits the binding of factor X and its conversion to Factor Xa, it attenuates the cell-based initiation of the coagulation cascade [74]. However, the clinical relevance of the cross-reactivity of abciximab against integrin $\alpha_v\beta_3$ and Mac-1 remains to be determined. Interestingly, abciximab competes with heparin for binding to Mac-1 [75] and may thereby prolong the activated clotting time. This has been observed in patients treated with unfractionated heparin and abciximab [76, 77]. The cross-reactivity of abciximab with the endothelial cell integrin $\alpha_v\beta_3$ and the leukocyte integrin Mac-1 provides a possible explanation for abciximab-related effects on the coronary microcirculation. Microvascular coronary resistance decreased after abciximab bolus administration in patients with unstable refractory angina pectoris and hemodynamic-relevant coronary artery stenosis [78]. Abciximab is currently approved for the use in patients undergoing PCI. It has also been studied as first-line medical treatment in patients with ACS in the absence of PCI, but for up to 48 h no benefit of abciximab treatment could be reported in this setting [79]. Initial dose-finding trials were performed to identify the dosage leading to over 80% inhibition of platelet aggregation in humans, or to less than 20% of baseline ADP-induced platelet aggregation. The findings led to the current administration as a bolus of 0.25 mg/kg followed by a 12-hour infusion of 0.125 μ g/kg/min [80]. This dosing regimen has been kept constant in several clinical trials with a large number of patients. The trials proved the benefit of abciximab treatment in the setting of acute myocardial infarction and PCI. A recent study using the current dosing regimen showed that although most of the abciximab-treated patients achieved a platelet in-

hibition of 95% or higher 10 min after bolus administration, as assessed with the Ultegra Rapid Platelet Function Assay (RPFA); the level of platelet inhibition 8 h after bolus administration and during continuous infusion of 0.125 μ g/kg/min was only $90 \pm 11\%$. The patients showed a significantly higher rate of major cardiac adverse events if the platelet inhibition was less than 95% 10 min after bolus administration [81]. This result may lead to further dose-adjustment trials, monitoring the platelet function inhibition. However, even in the contemporary setting of combined inhibition of platelet aggregation with ASA and a higher loading dose of clopidogrel, the additional administration of abciximab in patients with non-ST segment elevation myocardial infarction (NSTEMI) reduced the risk cardiac adverse events [82].

The high affinity and the low half-time rate of dissociation from the receptor lead to prolonged platelet inhibition lasting for up to 7 days and distinguish abciximab from the small-molecule inhibitors with a lower affinity and more rapid dissociation [83, 84]. Due to the rapid plasma clearance of unbound abciximab and the high receptor affinity, a dose reduction in patients with renal disease does not seem to be necessary and platelet inhibition can rapidly be reversed by platelet transfusion. A rare adverse event of abciximab administration is the development of acute, severe thrombocytopenia with platelet counts of less than 20,000 platelets/mm³. This condition develops within 24 h in approximately 0.7% of patients [85]. The pathogenesis of this acute thrombocytopenia is unknown. One of the most often discussed theories states that pre-existing antibodies against integrin $\alpha_{IIb}\beta_3$ conformations induced by abciximab or other antagonists can induce thrombocytopenia [86]. There was one patient described who presented with severe thrombocytopenia and in whom abciximab treatment resulted in direct platelet activation. It has been hypothesized that the sequestration of these activated platelets caused thrombocytopenia [87].

Eptifibatide (Integrilin®)

Eptifibatide is a synthetic heptapeptide with a mass of 800 Da, modeled on the active site of barbourin, a peptide from the disintegrin family found in the venom of the southeastern pigmy rattlesnake. Unlike other disintegrins that have an RGD sequence and block all integrins that recognize this sequence, barbourin mediates its high affinity to integrin $\alpha_{IIb}\beta_3$ through a Lys-Gly-Asp (KGD) sequence [88]. Eptifibatide contains a modified KGD sequence and binds with high specificity to integrin $\alpha_{IIb}\beta_3$, but with a lower affinity (KD 120 nmol/l) than abciximab.

Despite its initially proposed specificity for integrin $\alpha_{IIb}\beta_3$, binding of eptifibatide to α_v integrins has been reported [89] and inhibition of integrin $\alpha_v\beta_3$ on different cell types, including smooth muscle and endothelial cells, has been demonstrated [90]. The low affinity of the drug accounts for the high plasma

concentration of unbound eptifibatide, with only 25% of eptifibatide bound to plasma proteins. As eptifibatide is eliminated through the kidney, dose reductions in patients with severe renal disease or renal failure are necessary. Eptifibatide is currently approved for patients with ACS and for patients undergoing PCI. The Integrilin to Minimize Platelet Aggregation and Coronary Thrombosis (IMPACT II) trial [91] showed that eptifibatide, given as a 0.135 µg/kg bolus followed by 0.75 µg/kg/min infusion, reduced the rate of ischemic events during the 24-hour treatment period in patients undergoing elective, urgent or emergency PCI. It was found that the calcium-chelating property of the anticoagulant sodium citrate used for platelet aggregation assays in the IMPACT II trial led to an overestimation of the inhibitory effect of eptifibatide [92]. The dosing regimen was re-evaluated in the Posicor Reduction of Ischemia During Exercise (PRIDE) trial [93], with the direct thrombin inhibitor Phe-Pro-Arg chloromethyl ketone (PPACK) as an anticoagulant for platelet aggregation assays. In the PRIDE trial, the dose of 180 µg/kg bolus followed by a 2.0 µg/kg/min infusion proved to be sufficient to achieve and maintain an over 90% inhibition of the 20 µmol/l ADP-induced platelet aggregation. This dosing regimen was confirmed in the Platelet Glycoprotein IIb/IIIa in Unstable Angina trial (Receptor Suppression Using Integrilin Therapy (PURSUIT) trial). Compared to a treatment with placebo, patients with unstable angina or NSTEMI had a significantly lower incidence of death or myocardial infarction when treated with eptifibatide 180 µg/kg bolus followed by 2.0 µg/kg/min infusion [94]. Data from the recently published PROTECT-TIMI-30 trial demonstrate that the 2.0 µg/kg/min infusion rate of eptifibatide in patients with an estimated creatinine clearance ≤ 50 ml/min by Cockcroft-Gault lead to an significant increase in bleeding events [95]. Based on these data, a reduction of the infusion rate by 50% seems to be a reasonable approach in patients with a creatinine clearance below 50 ml/min.

The plasma elimination half-life of eptifibatide is approximately 2.5 h. The level of platelet aggregation inhibition 4 h after cessation of the infusion is less than 50%, and the bleeding time 6 h after cessation is not remarkably prolonged. As an early peak level with a small decline within 4–6 h after the bolus administration was observable, a double bolus of 180 µg/kg followed by another 180 µg/kg 10 min later was considered safe in patients undergoing PCI in the Enhanced Suppression of the Platelet IIb/IIIa Receptor with Integrilin Therapy (ESPRIT) trial [96]. Data from patients who underwent PCI suggest that the decline in platelet aggregation inhibition could be overcome by the double bolus administration of eptifibatide [97].

Tirofiban (Aggrastat®)

The synthetic non-peptide tirofiban is a small-molecule (500 Da) GPIIb/IIIa inhibitor that mimics the RGD sequence [98].

Tirofiban is a competitive antagonist with a receptor affinity higher than eptifibatide but lower than abciximab (KD 15 mmol/l). To date, no cross-reactivity of tirofiban with other integrins has been reported. The fraction of unbound tirofiban in human plasma is 35%, the plasma elimination half-life of 2 h is comparable with that of eptifibatide. Normal hemostatic function is restored within 4 h after cessation of the drug infusion. The renal clearance of tirofiban accounts for 39–69% of the plasma clearance. Patients with creatinine clearance of less than 30 ml/min present a significantly decreased plasma clearance (>50%) of tirofiban. For these patients, the manufacturer recommends a weight-adjusted reduction of the dose by 50%. Tirofiban is licensed for the treatment of ACS and in the setting of PCI. The dosing regimen for the use in ACS is a bolus infusion of 0.4 µg/kg/min over 30 min, followed by an infusion of 0.1 µg/kg/min. In patients undergoing PCI, a bolus dose of 10 µg/kg given 10 min prior to PCI followed by a 0.15 µg/kg/min infusion for 18 h has been used in the Randomized Efficacy Study of Tirofiban for Outcomes and Restenosis (RESTORE) trial [99]. Sufficient and consistent platelet aggregation inhibition of more than 80% in the response to 20 µmol/l ADP has been reported with either dosing regimen during the time of infusion [100]. However, an inhibition of platelet aggregation exceeding 80% after a bolus of 10 µg/kg (RESTORE regimen) was not achieved in all patients at 15 and 30 min, but could only be observed after 4 h [100]. The same dosing regimen for tirofiban was used in the Tirofiban and ReoPro Give Similar Efficacy Outcomes Trial (TARGET) [101]. This trial, intended to assess the non-inferiority of tirofiban compared to abciximab, was the first direct comparison of the two GPIIb/IIIa inhibitors abciximab and tirofiban in the setting of PCI. The 30-day results demonstrated the superiority of abciximab. The primary endpoint occurred in 7.6% of patients treated with tirofiban versus 6% of patients treated with abciximab. A possible explanation for this phenomenon is the incomplete inhibition of platelet aggregation by tirofiban. Platelet aggregation measurements were not incorporated in the study protocol. Although a recent substudy of the On-TIME clinical trial suggests that there is no correlation between the level of inhibition of platelet microaggregation and clinical outcome [102], this issue has to be addressed by further investigations with at least two different established methods that are used for the determination of platelet aggregation.

Oral GPIIb/IIIa Inhibitors

The rationale behind the development of oral GPIIb/IIIa antagonists was the assumption that long-term inhibition of integrin $\alpha_{IIb}\beta_3$ would provide a new therapeutic approach for the prevention of recurrent ischemic events in patients with cardiovascular disease. Four oral GPIIb/IIIa antagonists (lotrafiban, orbofiban, sibrafiban and xemilofiban), all of which

are non-peptide prodrugs, have been examined in large phase III clinical trials. None of them fulfilled the high expectations. Therapies applying these substances have not proven to be superior to ASA therapy. Furthermore, a meta-analysis of four major phase III trials (Evaluation of Oral Xemilofiban in Controlling Thrombotic Events (EXCITE), Orbofiban Post Unstable Coronary Syndromes (OPUS), Sibrafiban versus Aspirin to Yield Maximum Protection from Ischemic Heart Events Post-Acute Coronary Syndromes (SYMPHONY), and 2nd SYMPHONY) including 33,326 patients showed a statistically significant increase in mortality in patients treated with the oral GPIIb/IIIa antagonists [103]. The short plasma half-life of these drugs that causes undulating plasma levels and pro-aggregatory effects especially at low concentrations of the GPIIb/IIIa blockers is discussed as a potential reason of the disappointing outcomes of these large phase III trials [66].

Conformation-Specific Blockade of GPIIb/IIIa

The currently therapeutically used GPIIb/IIIa blockade is accompanied by several adverse effects: Bleeding complications are frequent with a 1% prevalence of severe thrombocytopenia, which is associated with the expression of immunogenic ligand-induced binding sites (LIBS) [104, 105]. Furthermore, GPIIb/IIIa inhibitors have been described to induce paradox-

ical platelet activation [66]. The use of oral GPIIb/IIIa inhibitors has even been associated with a significant increase in mortality as mentioned above, and this was at least partially due to prothrombotic events [106]. All of these problems are associated with the property of the inhibitors to bind to the GPIIb/IIIa receptor, irrespective of its activation state, and to act partially as ligand mimetics.

In our working group we have taken advantage of the existence of different conformational states of GPIIb/IIIa to develop an alternative, unique strategy that targets only activated platelets. Therefore, we used single-chain antibody phage display. We performed serial rounds of subtractive panning with depletion of non-activated and selection of activated GPIIb/IIIa. In vitro testing of the selected clone scFv MA2 demonstrated differential inhibition only of activated platelets, which are for example present in a coronary thrombosis [107]. Resting platelets could still adhere to the vessel wall, which is important for occlusion of injuries. Moreover, the scFv MA2 is less immunogenic and does not induce immunogenic LIBS. The differential effect could also be demonstrated in vivo: In a murine carotid occlusion model, scFv MA2 demonstrated an effective inhibition of thrombus formation with significant reduction of the bleeding time compared to the currently used GPIIb/IIIa blockers [108]. Altogether, our studies indicate that this novel concept might overcome some of the problems associated with GPIIb/IIIa blockade.

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Erratum

In the article

«Reproductive Immunology – an Update»

(*Transfus Med Hemother* 2006;33:474–485) one author was accidentally left out.

The full author list must read:

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We apologize for this mistake.