

8th Seah Cheng Siang Memorial Lecture: New Antithrombotic Agents

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Abstract

For the long-term prevention of thromboembolic events in patients with atherosclerotic vascular disease, aspirin is the preferred antiplatelet drug. Only clopidogrel was shown to be more effective and at least as safe than medium-dose aspirin in direct comparative large-scale trials. Aspirin inhibits the cyclooxygenase dependent pathway of platelet aggregation while ticlopidine and clopidogrel selectively bind to adenosine diphosphate (ADP) receptors on the platelet surface. Compounds which inhibit the synthesis of thromboxane synthase, block the thromboxane receptor or have the dual activity were effective in experimental thrombosis models in animals but not predictive of results in humans.

Activation of the platelet glycoprotein (GPIIb/IIIa) receptor on the platelet surface is the final pathway of platelet aggregation, regardless of the initiating stimulus. Inhibitors of GPIIb/IIIa receptors include monoclonal antibodies (abciximab) against this receptor and peptidic as well as non-peptidic synthetic specific receptor blockers. Abciximab exchanges between and binds to platelets for as long as two weeks whereas synthetic GPIIb/IIIa inhibitors inhibit ex vivo platelet aggregation for only a few hours after the end of infusion but have the advantage of being also orally active.

In the secondary prevention of atherothrombosis, large scale trials were successfully conducted with aspirin, dipyridamole and clopidogrel. In the first large-scale trials with GPIIb/IIIa inhibitors with abciximab was investigated. In aggregate, this class of platelet inhibitors, combined with aspirin and heparin, was shown to reduce ischaemic events in patients with high- and low-risk coronary intervention, stents, unstable angina and non-Q-wave infarction with long-term preservation of the initial benefit. With synthetic GPIIb/IIIa inhibitors there is no suppression of clinical evident restenosis 6 months after the end of treatment. With the doses presently used, bleeding occurs more often with the synthetic GPIIb/IIIa inhibitors (used for 3 days) than with abciximab (used for 12 hours) but there are no direct comparisons between these drugs.

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Introduction

The tremendous interest in finding new anti-aggregating agents stems from the preeminent role that platelets play, not only in haemostasis and thrombosis, but also in atherogenesis and, be it less definitive, in arterial spasm. Compelling data from large-scale trials have established the role of platelet inhibitors in reducing coronary events in patients with coronary and cerebral syndromes and in peripheral arterial occlusive disorders. The remarkable but limited effectiveness of aspirin and ticlopidine in the secondary prevention of cardiovascular disorders has spurred the development of more potent antiplatelet drugs.

Inhibitors of Platelet Adhesion

Platelets do not adhere to normal endothelial cells; this property of non-adhesion is the result of a series of active

processes requiring normally functioning endothelial cells. Disruption of the endothelial lining permits non-activated platelets to adhere to components of the sub-endothelial extracellular matrix through multiple interactive sites. At low shear rate, the platelet glycoprotein (GP)Ib/IIa binds to subendothelial and GPIb/IX to immobilized von Willebrand factor in the subendothelium; this adhesion is a very early event in thrombus initiation soon followed by binding of the dominant GPIIb/IIIa to fibrinogen.' At high shear rates; such as those found in arteries, the aggregation of platelets is more dependent on binding of both GPIb/IX and GPIIb/IIIa receptors to von Willebrand factor than to fibrinogen. Other subendothelial ligands such as fibronectin and vitronectin may also bind to platelet receptors. Another feature of GPIb is its ability to bind thrombin at low concentrations producing platelet activation, secre-

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tion and aggregation.

Monoclonal antibodies raised against GPIb are effective in experimental thrombosis in various animal models.* Similarly, antibodies against multimeric von Willebrand factor also have antithrombotic properties.^{3,4} A recombinant peptide corresponding to monomeric fragment amino acids Leu504-Lys788 of von Willebrand factor blocks the GPIb receptor.⁵ Also, inhibition of the multimerisation of von Willebrand factor with high molecular weight aurointricarboxylic acid, a triphenylmethyl dye compound, results in the prevention of thrombus formation and suppression of neointima lesions.⁶

Patients lacking GPIb (Bernard Soulier syndrome) have a serious bleeding disorder. Whether complete long-term blocking of the platelet GPIb receptors will be safe remains to be investigated.

Inhibitors of Platelet Aggregation

Cyclooxygenase Inhibitors: Aspirin

Several pathways lead to platelet aggregation (Fig.1). Aspirin and other non-steroidal anti-inflammatory drugs

inhibit very selectively thromboxane (TXA₂) formation and this is its principal antithrombotic mechanism. Aspirin only partially impedes platelet aggregation induced by ADP, collagen and low concentrations of thrombin. Aspirin does not inhibit adherence of the initial layer of platelets to the subendothelium or atherosclerotic plaques, the release of granule contents and platelet-derived serotonin and does not oppose initial fibrinogen binding.⁷ Thus the effects of platelet-derived growth factors and other mitogens on smooth muscle cells are not inhibited⁸ and could reduce the antithrombotic efficacy of the drug (Table I).

The ideal dose of aspirin for the primary or secondary prevention of cardiovascular disease is not determined. Doses as low as 1 and 2 mg/kg daily produce virtually complete inhibition of cyclooxygenase dependent platelet aggregation in healthy volunteers^{9,10} and atherosclerotic patients.¹¹ A low daily dose of 75 mg reduced major cardiovascular events by 15% (P = 0.03) and fatal and non-fatal myocardial infarction by 36% (P = 0.002) in patients with hypertension.¹² Slow release aspirins are associated with few gastrointestinal side effects, particularly when an enteric-coated preparation is used.

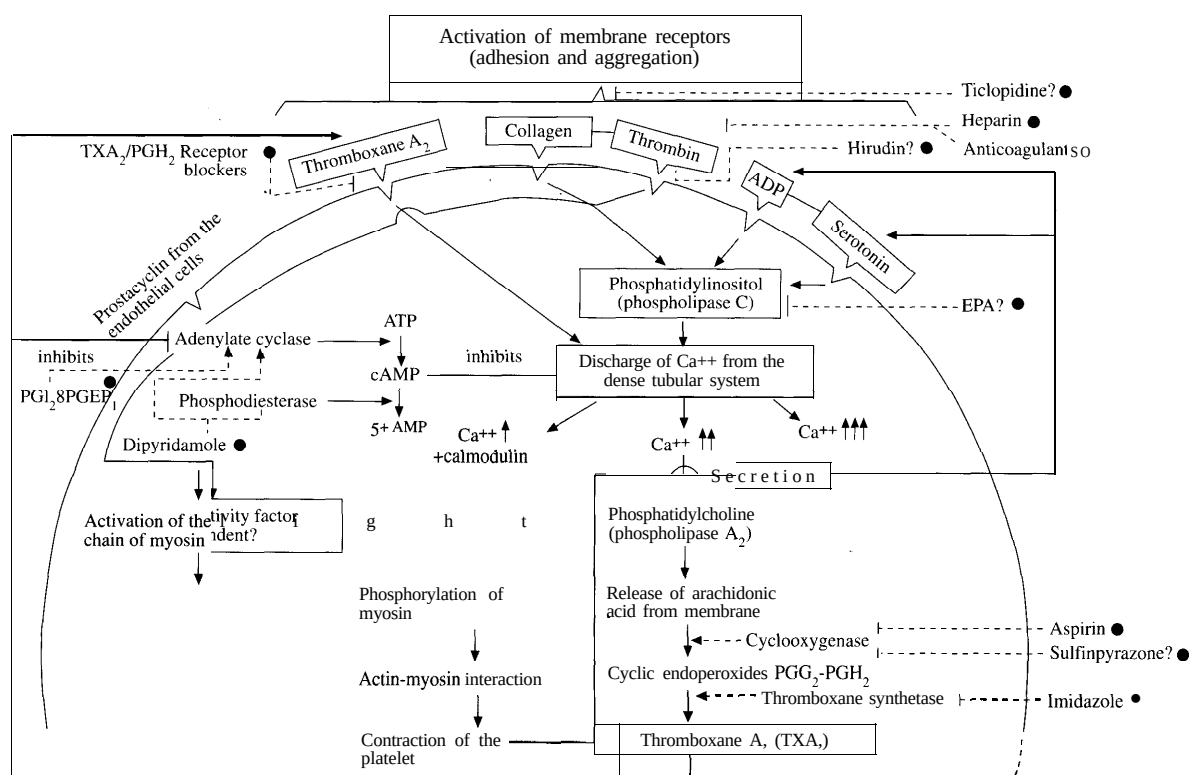


Fig. 1. Mechanism of platelet activation and presumed sites of action of various platelet inhibitor agents. Platelet agonists lead to the mobilization of calcium (Ca^{++}), which functions as a mediator of platelet activation through metabolic pathways dependent on adenosine diphosphate (ADP), thromboxane A_2 (TXA₂), thrombin, and collagen. Cyclic adenosine monophosphate (cAMP) inhibits calcium mobilization from the dense tubular system. Note that thrombin and collagen may independently activate platelets by means of platelet activating factor. • : a platelet inhibitor; Dashed line: a presumed site of drug action; ATP: adenosine triphosphate; EPA: eicosapentaenoic acid; PGE₁: prostaglandin E₁; PGE₂: prostaglandin E₂; PGI₂: prostaglandin I₂ or prostacyclin. (From: Verstraete M, Vermeylen J. Thrombosis. Pergamon Press, 1984:11. Modified: Stein B et al. Platelet inhibitor agents in cardiovascular disease: an update. Reprinted with permission from: The American College of Cardiology--J Am Coll Cardiol 1989; 14:813)

TABLE 1: ASPIRIN IS NOT AN IDEAL PLATELET INHIBITOR

Inhibits only TXA ₂ pathway, much less inhibition of platelet activation by thrombin, ADP, collagen and not by platelet-activating factor (PAF)
No inhibition of platelet adhesion
No effect on the alpha-granule and serotonin release of stimulated platelets
Initial fibrinogen binding is not opposed
No effect on smooth muscle cell proliferation
Prolongs the bleeding time
Gastrointestinal problems
Can induce allergy (rare)

Notwithstanding the uncertainties in the principal mode of action and lowest dose, aspirin is the benchmark for new agent comparisons in the secondary prevention of thrombotic vascular disorders as it provides a 15% reduction in vascular mortality and a 30% reduction in non-fatal stroke or myocardial infarction in a normotensive population.¹³

Of note, PGF₂-like compounds (F₂-isoprostanes) are produced *in vivo* in humans by a non-cyclooxygenase mechanism involving free radical catalysed lipid peroxidation¹⁴ and are thus not inhibited by aspirin. Moreover, there are two prostaglandin G/H synthase genes encoding for different isoforms of the cyclooxygenase enzyme (constitutive PGHS-1, or cox-1, inducible PGHS-2 or cox-2).¹⁵ Cox-1 is constitutively expressed in virtually all cells of the human body, including platelets. In contrast, cox-2 is undetectable in most mammalian tissues, but the expression can be induced rapidly in monocytes, endothelial cells and other specialised cells in response to various inflammatory and mitogenic mediators.^{15,16} Aspirin inhibits only cox-1 in platelets, endothelium, stomach and kidney by acetylation of a single serine residue at position 529 within the polypeptide chain of human platelet PGHS-1.

Inhibition of Adenosine-diphosphate-evoked Signal Transduction: Ticlopidine and Clopidogrel

These two thienopyridine derivatives can be considered as bioprecursors since they are inactive *in vitro* but are potent antiaggregating agents *in vivo*, indicating the importance of at least one active transient metabolite which, however, has not yet been defined. The metabolic activation takes place in the liver as a porto-jugular shunt abolishes the antiaggregating effect. Ticlopidine and its more potent chemical analogue clopidogrel are non-competitive but selective antagonists of ADP-induced platelet aggregation. Since the two compounds are chemically related, their mechanism of action is considered similar. *Ex vivo* studies indicate that the antiaggregating effect is concentration-dependent; the rate of recovery is linked to platelet survival, suggesting

a permanent effect on platelets.¹⁷⁻²⁰ The two compounds irreversibly inhibit platelet aggregation by selective binding to adenylate cyclase ADP receptors on the platelet surface. Both drugs reduce responses to other agonists which require feedback amplification by ADP released from internal storage sites during granule secretion.^{18,21} The binding of fibrinogen to the GPIIb/IIIa complex, triggered by ADP, is dramatically inhibited; this inhibition is not due to a direct modification of the glycoprotein complex.²² Ticlopidine and clopidogrel have no effect on phospholipase A activity or thromboxane A₂ and prostacyclin synthesis. They have no direct effect on either cAMP-phosphodiesterase or adenylate cyclase.

Clopidogrel is approximately 40 to 100 times as active as ticlopidine in inhibiting ADP-induced platelet aggregation in animal models but circa 6 times as potent as ticlopidine in the inhibition of ADP-induced aggregation of human platelets.

Ticlopidine and clopidogrel have been tested in several animal models of platelet-dependent arterial or venous thrombosis and found to be more effective than sulfapyrazole, dipyridamole and aspirin (reviewed in ^{17,23,24}). Other effects are a reduction in fibrinogen levels and blood viscosity and improvement of decreased erythrocyte deformability.¹⁷

The effectiveness of ticlopidine has been convincingly demonstrated in patients at high risk of arterial thromboembolic events, i.e. those with transient ischaemic cerebral attacks and stroke, peripheral arterial or ischaemic heart disease.²⁵ A trial in more than 3,000 patients has shown that ticlopidine has a more pronounced effect than aspirin on death from all causes or non-fatal stroke.²⁶

The superiority in clinical end points of the combination of ticlopidine plus aspirin over aspirin plus oral anticoagulation (target international normalized ratio 2.5 to 3.0) and aspirin alone in medium and high risk stent patients has been convincingly demonstrated.^{27,28}

The efficacies of clopidogrel and aspirin in preventing ischaemic events have been compared in the randomised multicentre double-blind trial CAPRIE.²⁹ 19,185 patients with atherosclerotic vascular disease (recent ischaemic stroke, recent myocardial infarction or peripheral arterial disease) received clopidogrel 75 mg/day or aspirin 325 mg/day. Mean follow-up was 1.91 years.

Primary outcomes in this study were fatal or non-fatal ischaemic stroke and myocardial infarction and other vascular death. Of 939 events in clopidogrel-treated patients and 1021 in patients who received aspirin, 32.8% and 31.4%, respectively, were fatal. Overall, patients who received clopidogrel (n = 9599) had an annual 5.3% risk of primary ischaemic events compared with 5.8% in patients who received aspirin (n = 9586). This equates to a relative-risk reduction of 8.7% in favour of clopidogrel (P = 0.043). A favourable trend was noted for

the effect of clopidogrel on each component of the primary end point (myocardial infarction, stroke, vascular death), with the benefit being greatest for myocardial infarction (event rate 1.56% versus 1.90% per year in clopidogrel and aspirin recipients, respectively). The overall risk of death from any cause was not significantly different between the 2 groups (event rate 3.05% versus 3.11% per year). Most of the benefit was found in patients that entered the trial with peripheral arterial disease or stroke. A relative risk increase of -3.7% (95% CI -22.1 to 12) was even observed in patients entering the trial because of an acute myocardial infarction.

The CAPRIE study was not adequately powered to allow subgroup analyses. Nevertheless, clopidogrel significantly reduced the relative risk for primary ischaemic events in patients with peripheral arterial disease (3.71% versus 4.86%; relative-risk reduction 23.8%; $P = 0.0028$). Relative risk for primary ischaemic events was not significantly different between clopidogrel- and aspirin-treated patients with either recent stroke (7.15% and 7.71%, respectively; relative-risk reduction 7.3%, $P = 0.26$) or recent myocardial infarction (5.03% and 4.84%; relative-risk increased 3.7%, $P = 0.66$). A test for heterogeneity of these treatment effects was positive ($P = 0.042$), suggesting that the benefit of clopidogrel may not be identical across the 3 subgroups.

The most common adverse effects associated with ticlopidine are gastrointestinal. Diarrhoea is the most frequently reported, affecting about 20% of treated patients. Other effects are skin reactions (urticaria, pruritus, erythema), haemorrhagic disorders (epistaxis, ecchymoses, menorrhagia) and thrombotic thrombocytopenic purpura. These effects are generally not severe and resolve after discontinuation of ticlopidine. The most potentially serious problem is bone marrow depression (leukopenia, thrombocytopenia, pancytopenia); close monitoring is therefore essential for at least the first 12 weeks of ticlopidine therapy.²⁴ Ticlopidine has also been associated with an increase in total cholesterol levels by 9%.²⁴ Clopidogrel was developed because this compound was not toxic to bone marrow pluripotent stem cells in the mouse (Till and McCulloch test).

Also, in phase II studies adverse events with clopidogrel were proportionally less frequent than with ticlopidine and not related to the dose. During clopidogrel therapy in the CAPRIE trial, the most frequent adverse events were gastrointestinal upset (indigestion, nausea, vomiting) (15.0% of patients versus 17.6% with aspirin; $P < 0.05$), bleeding disorders (9.3% for each treatment), rash (6.0% versus 4.6%; $P < 0.05$) and diarrhoea (4.5% versus 3.4%; $P < 0.05$).²⁵ Abnormal liver function and gastrointestinal haemorrhage occurred more frequently in aspirin than clopidogrel recipients (Table II).

In CAPRIE there were no clinically significant changes in laboratory parameters during clopidogrel treatment.³⁰ Blinded haematological review revealed platelet counts below $100 \times 10^9/L$ in equal proportions of clopidogrel- and aspirin-treated patients (0.26%). Low neutrophil counts ($< 1.2 \times 10^9/L$) were detected in 0.10% and 0.17% of patients, respectively.

Thromboxane Synthase Inhibitors

Thromboxane synthase inhibitors have been developed with the expectation of not only suppressing TXA_2 biosynthesis, but also sparing or even enhancing the formation of prostacyclin by the vascular endothelium. Thromboxane synthase inhibition offers the advantage over aspirin-type cyclooxygenase inhibitors of reorienting the arachidonic cascade towards an overproduction of inhibitory prostanoids (PGI_2 , PGD_2) and a reduction of TXA_2 formation. In a recent study in rats the thromboxane synthase inhibitors dazoxiben and camonagrel increased prostacyclin synthesis by 78% and 154%, respectively.³¹ However, specific inhibition of TXA_2 synthase produces an accumulation of cyclic prostaglandin endoperoxides, which occupy and activate TXA_2 and endoperoxide receptors on platelets and endothelium and, thus, attenuate the inhibitory effect of PGI_2 and PGD_2 .^{32,33}

Most thromboxane synthase inhibitors have moderate potency, short duration of action, and do not result in a sufficiently sustained inhibition of TXA_2 production to be clinically effective. Moreover, some individuals are poor responders to this type of drugs. The increased

TABLE II: ADVERSE EXPERIENCES (NUMBER AND PERCENTAGES OF PATIENTS)

Adverse experience	Patients ever reporting		Severe	
	Clopidogrel	Aspirin	Clopidogrel	Aspirin
Rash	578 (6.02%)	442 (4.61%)*	25 (0.26%)	10 (0.10%)*
Diarrhoea	428 (4.46%)	322 (3.36%)*	22 (0.23%)	11 (0.11%)
Indigestion/nausea/vomiting	1441 (15.01%)	1686 (17.59%)*	93 (0.97%)	118 (1.23%)
Any bleeding disorder	890 (9.27%)	890 (9.28%)	132 (1.38%)	149 (1.55%)
Intracranial haemorrhage	34 (0.35%)	47 (0.49%)	30 (0.31%)	41 (0.34%)
Gastrointestinal haemorrhage	191 (1.99%)	255 (2.66%)*	47 (0.49%)	68 (0.71%)*
Abnormal liver function	285 (2.97%)	302 (3.15%)*	11 (0.11%)	9 (0.09%)

*statistically significant, $P < 0.05$

generation of endoperoxides, which share the same receptors as TXA₂, is another problem, which will not be solved by more potent and long-acting drugs of this class.³³ Despite the efficacy of thromboxane synthase inhibitors in short-term animal models of thrombosis, limited phase II clinical trials in patients with coronary artery disease have been largely disappointing.

Thromboxane Receptor Blockers

The more recently developed thromboxane receptor blockers specifically impede the action of both TXA₂ and endoperoxides on their presumed common receptors on platelets and prevent vasoconstriction induced by TXA₂. These agents leave the normal pattern of thromboxane and prostacyclin formation unaltered. Thromboxane receptor antagonists prolong bleeding time more than thromboxane synthase inhibitors.³⁴ As expected, TXA₂ synthesis is not inhibited and PGI₂ generation is not augmented by specific thromboxane/endoperoxide receptor antagonists. The potential advantages that thromboxane receptor blockers may have versus low-dose aspirin are related to the discovery of aspirin-insensitive agonists of the platelet receptor such as TXA₂ derived from monocyte cox-2 and the F₂-isoprostene 8-epi-PGF₂₋₁, a product of free radical catalysed peroxidation of arachidonic acid.

Clinical research with thromboxane receptor antagonists has been slow because the first compounds described had antagonistic effects on platelets but were agonistic on the vessel wall or vice versa.^{35,36} Several of the thromboxane/endoperoxide receptor blockers are also relatively short-acting and the magnitude of their blockade is modest. This is particularly the case for daltroban but not so for vapiprost³⁷ and BMS 180291-1³⁸ which are potent TXA₂ receptor blockers with a long duration of action. Unfortunately, the initial clinical studies with vapiprost have been disappointing. The long plasma elimination half-life of BMS 180291-A is most interesting as are its anti-ischaemic effects in canine models of pacing-induced ischaemia. However, no therapeutic studies with this compound have been published and it remains to be demonstrated that such agents will offer a relevant advantage over aspirin.

Thromboxane Synthase Inhibitors and Receptor Blockers

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Some compounds have a dual activity. Ridogrel is a potent TXA₂ synthase inhibitor with modest additional TXA₂/prostaglandin endoperoxide receptor antagonist properties (at least 100-fold less).³⁹ Although the animal pharmacology was very promising, it was not predictive of results in humans, including the clinical use of ridogrel versus aspirin as adjunct to thrombolysis in patients with acute myocardial infarction.⁴⁰ Picotamide is a rather weak thromboxane synthase inhibitor and

receptor blocker.⁴¹ Terbogrel is a potent thromboxane synthase inhibitor and, in contrast to ridogrel, also provides a high endoperoxide receptor affinity.⁴²

Specific Thrombin Receptors

With the recognition of the central role of thrombin in the pathogenesis of arterial and venous thrombosis, much attention has been given in the last years to unfractionated and low-molecular weight heparins and to direct specific thrombin inhibitors. The latter group does not require antithrombin III or cofactor II for their thrombin neutralising function. All thrombin inhibitors impede thrombin-induced platelet aggregation, but have no effect on aggregation induced by other agonists.

A natural specific thrombin inhibitor is hirudin which is now obtained by DNA recombinant technology. Hirulog is a bifunctional 20-amino acid peptide designed on the structure of hirudin. Argatroban (NovastanTM) is an arginine-derivative which specifically inhibits thrombin activity. This is also the case for other synthetic, potent and selective thrombin inhibitors as efegatran, inogatran, megalogatran and nap-sagatran.^{43,44}

Blockers of the Platelet Glycoprotein IIb/IIIa Receptor

Platelets have 50,000 to 80,000 GPIIb/IIIa receptors on their surface. Exposure of these receptors is the final common end point of all pathways leading to platelet aggregation (Fig. 2). All ligands for the GPIIb/IIIa receptor such as fibrinogen, von Willebrand factor, endothelium-derived factors, vitronectin and thrombospondin bind through their arginine-glycine-aspartic acid (RGD) recognition site; a larger recognition sequence (lysine-glycine-alanine-glycine-aspartic acid-valine or KQAGDV) is specific for the gammachain of fibrinogen. With the exception of some tumour cells, the

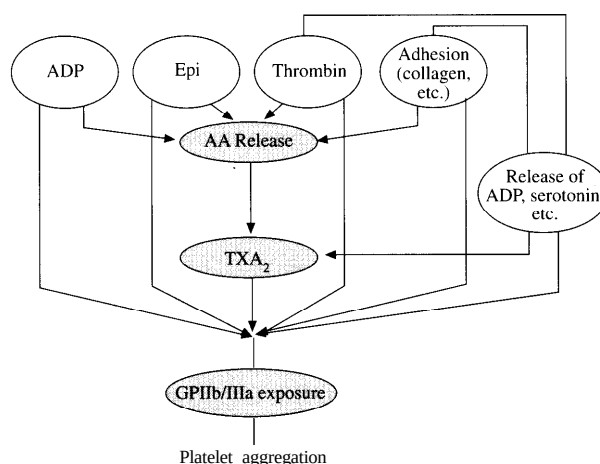


Fig. 2. Pathways of platelet activation. Exposure of GPIIb/IIIa receptors at the platelet surface is the final common endpoint of all pathways. AA: arachidonic acid; ADP: adenosine diphosphate; Epi: epinephrine; TXA₂: thromboxane A₂.

GPIIb/IIIa receptor is confined to cells of the megakaryocyte/platelet lineage. The possibility of a partial agonist effect on receptor blockade is a problem which may surface with RGD mimetics.

Abciximab: The first platelet GPIIb/IIIa antagonists to be developed were murine monoclonal antibodies.^{45,46} *In vitro*, these antibodies completely inhibit platelet aggregation and, in animal models of angioplasty injury and thrombolysis, prevent thrombosis and augment the activity of thrombolytic agents. Because of concerns about their immunogenicity, the derivative product chimeric monoclonal 7E3Fab (c7E3Fab) was created via genetic recombination. This new molecule (abciximab, ReoPro™, Centocor, Eli Lilly Co) consists of the mouse-derived variable regions from the original molecule linked to the constant region derived from human immunoglobulin IgG. It is a well-characterised homogeneous protein of approximately 47,600 daltons. The plasma half-life of abciximab is short (5 min) but its biological effect much longer. Six hours after a bolus dose occupying >80% of the GPIIb/IIIa receptors are still inhibiting platelet aggregation by 60%. Data from a dose-escalation study⁴⁷ and a pilot therapeutic trial⁴⁸ revealed that a bolus injection of 0.25 mg/kg caused an 87% blockade of the platelet GPIIb/IIIa receptor, an 88% inhibition of ADP-induced aggregation and a >30 minute prolongation of the bleeding time. Fifty per cent inhibition of platelet aggregation in whole blood occur at concentrations of approximately 1 µg/ml of abciximab. The binding of abciximab to GPIIb/IIIa, which is reversible, requires the intact, calcium dependent heterodimer structure of the receptor.

Abciximab was evaluated in patients with high-risk PTCA (EPIC Trial).⁴⁹ As compared with placebo, an abciximab bolus of 0.25 mg/kg followed by an infusion of 10 µg/min for 12 hours in 2,099 patients; almost all on aspirin and high-dose intravenous heparin, resulted in a 35% significant reduction at 1 month of the primary composite end points (death, non-fatal myocardial infarction, unplanned surgical revascularisation, unplanned repeat PTCA, stent or balloon pump for refractory ischaemia). There were two cases of haemorrhagic strokes in each treatment group, however, major bleeding episodes and transfusions were more frequent in patients treated with abciximab (14% versus 7% in the placebo group). At 6 months there was a 23% and at 1 year a 19% reduction in the composite end point which persisted even at 3 years (13% reduction: 41% versus 47%, $P = 0.009$). Although abciximab was developed to bind the IIb/IIIa ($\alpha_{IIb}\beta_3$) integrin, there is full cross-reactivity with the $\alpha_v\beta_3$ integrin (vitronectin receptor) and this could explain the persistence of initial benefit. The latter integrin is highly expressed on the surface of activated endothelial cells and smooth muscle cells but also on platelets. Smooth muscle cell migration

is strongly inhibited by vitronectin receptor blockade.

The EPIC trial was the first large trial conducted in high-risk PTCA patients in which the effect of abciximab was superimposed to the antithrombotic combined regimen of aspirin and a standard high-dose intravenous heparin, not weight-adapted. In the pilot study—PROLOG-103 PTCA patients received the same dose of aspirin and abciximab as in the EPIC trial, but patients were randomised to a standard weight-adjusted heparin dose (100 IU/kg bolus) followed by additional doses to prolong the activated clotting time (ACT) >300 s or to low-dose weight-adjusted heparin (70 IU/kg) bolus without further adjustment of the activated clotting time.⁵⁰ The results of this pilot study suggested that excessive bleeding could be minimised by low-dose weight-adjusted heparin in combination with early removal of the arterial sheath.

The EPILOG trial examined the use of weight-adjusted abciximab with standard dose, weight-adjusted heparin (initial bolus of 100 IU/kg), abciximab with low-dose, weight-adjusted heparin (initial bolus of 70 IU/kg) or placebo with standard-dose, weight-adjusted heparin in a broad population of 2792 low-risk patients undergoing PTCA.⁵¹ All patients received aspirin. The incidence of the composite end point of death, myocardial infarction, or urgent revascularisation at 30 days was as 11.7% in the placebo group and 5.2% and 5.4% in the low-dose and standard-dose heparin groups, respectively (56% and 54% reduction). Importantly, there were no significant differences among the treatment groups in the risk of major bleeding. Patients receiving abciximab with standard-dose heparin, but not those receiving abciximab with low-dose heparin, had significantly more minor bleeding events than patients receiving placebo.

The results of the CAPTURE trial could widen the indication of abciximab to include a pre-treatment period surrounding angioplasty for refractory angina.⁵² This study was conducted in 1265 high-risk patients with angina resistant to heparin, nitrates and aspirin. After angiography, patients received a randomly assigned infusion of abciximab (0.25 mg/kg bolus followed by a continuous infusion of 10 µg/min) or placebo for 18 to 24 hours. By 30 days, the primary end point (composite of death, MI, urgent revascularisation, stent or intraaortic balloon for recurrent ischaemia) had occurred in 11.3% of patients who received abciximab and in 15.9% of placebo recipients. This 29% reduction is significant ($P = 0.012$). Major bleeding occurred more often with abciximab than with placebo (3.8% versus 1.9%, $P = 0.0433$). At 6 months, death or myocardial infarction had occurred in 9% and 10.9%, respectively. Study agent was continued for only one hour after PTCA which may contribute importantly to the differences seen in long-term results between CAPTURE and

other abciximab trials.

A randomised, placebo-controlled trial (RAPPORT) of the platelet glycoprotein IIb/IIIa antagonist abciximab was conducted in 483 patients undergoing primary PTCA for acute MI.⁵³ Patients were randomised to abciximab or placebo and received standard doses of heparin before intervention. The primary end point of the study was the combined incidence of death, reinfarction, or any (urgent or elective) target vessel revascularisation at 6 months and occurred in 28% in the two study groups. On an intention-to-treat basis, there was a significant difference favouring abciximab in a protocol defined acute-phase end point (death, reinfarction, or urgent target vessel revascularisation at 7 and 30 days and at 6 months). When only patients who actually received study drug and PTCA were considered (excluding patients who did not receive drug or PTCA as prespecified in the protocol), a statistically significant reduction in the incidence of death or reinfarction was obtained at 7 days and 6 months. There was a significant increase in the bleeding complications with abciximab in this trial in which relatively high doses of heparin were used and the vascular sheaths left in place for prolonged periods of time.

Combination therapy involving administration of abciximab with thrombolytic therapy in patients with myocardial infarction was studied in the TIMI 14 trial.⁵⁴ This is an open label trial. The dose-finding and dose-confirmation stages for the alteplase/streptokinase portion have now been completed. The dose-finding stage evaluated full-dose alteplase (100 mg); abciximab (0.25 mg/kg, 0.125 µg/kg/min infusion) in combination with 20, 35, 50 and 65 mg alteplase; abciximab (0.30 mg/kg, 0.125 µg/kg/min infusion) in combination with 50 mg alteplase; and abciximab (0.25 mg/kg, 0.125 µg/kg/min infusion) in combination with 0.5, 0.75, 1.25 and 1.5 MU streptokinase. Low-dose heparin (60 U/kg bolus, 7 U/kg/h infusion) was given with all abciximab / thrombolytic combinations with the exception of the abciximab / 1.5 MU streptokinase combination (with which no heparin was given) and the 0.30 mg bolus abciximab / 50 mg (with which very-low-dose heparin, 30 U/kg bolus plus 4 U/kg/h infusion was given). The full dose streptokinase plus abciximab was prematurely stopped after 5 patients because of serious bleeding. The subsequent dose-confirmation stage compared full-dose alteplase versus 50 mg alteplase (15 mg bolus, 35 mg infusion over 1 hour) and abciximab (0.25 mg/kg bolus, 0.125 µg/kg/min infusion) + low-dose heparin (60 U/kg bolus, 7 U/kg/h infusion). Additionally, a group of patients was evaluated with abciximab (0.25 mg/kg, 0.125 µg/kg/min infusion) + 50 mg alteplase + very-low-dose heparin (30 U/kg bolus, 4 U/kg/h infusion) to determine if safety could be enhanced with very-low-dose heparin. Within 90 minutes of administering the treatment regimens, coronary angiography was performed on each

patient to determine the grade of blood flow. This was done prior to any percutaneous intervention, such as angioplasty. Outcome measures included the occurrence of death or recurrent MI, the need for urgent revascularisation procedures, and adverse events, as assessed at day 30. Major bleeding was evaluated at hospital discharge or at day 14, whichever was earlier. Interim results on 440 patients have been presented.⁵⁴ About 33% of patients who receive streptokinase (1.5 MU) achieve TIMI grade 3 blood flow at 90 minutes, according to historical control data based on findings from the GUSTO trial. With this in mind, the results from TIMI 14 show that a similar effect on blood flow (TIMI-3:31%) is achieved after treatment with abciximab alone. Additional increases in blood flow were seen when either alteplase (20 mg or 35 mg) or streptokinase (0.5, 0.75 or 1.25 MU) were combined with abciximab. The combination with alteplase 50 mg and abciximab gave a TIMI-3 patency rate of 77% which is higher than with 100 mg alteplase alone (60%). The TIMI-14 trial is being pursued with increasing doses of abciximab as a bolus and adjunctive doses of alteplase (65 mg) or streptokinase (1.5 MU). As the bolus dose of abciximab is increased, 50% less heparin is now being administered. Recently the streptokinase arm has been stopped.

In the EPISTENT study, patients undergoing elective or urgent coronary revascularisation were randomised to receive abciximab plus a coronary stent (n = 794), placebo plus a coronary stent (n = 809) or abciximab plus balloon angioplasty (n = 796).⁵⁵ Abciximab recipients received 0.25 mg/kg for up to 60 minutes before the procedure, followed by an infusion of 0.125 µg/kg/min for 12 hours. All patients received oral aspirin 325 mg/day commencing ≥2 hours prior to the procedure, and heparin during the procedure. Patients with a coronary stent also received ticlopidine at the discretion of the investigator. The percentage of patients who reached the primary end point of death, MI or need for urgent revascularisation within 30 days was significantly lower among abciximab (5.3% and 6.9%) compared with placebo recipients (10.8%). The incidence of major bleeding was slightly higher among placebo patients.

Another randomised trial is looking at the effects of abciximab on in-stent restenosis, as measured by intravascular ultrasound quantitative volumetric reconstruction.

The strong evidence of efficacy of abciximab used in conjunction with aspirin and heparin in patients undergoing percutaneous coronary interventions has in the initial trials been overshadowed by an excess of bleeding complications. With reduced heparin dose on a weight-adjusted basis, more recent trials have not shown an excess of major and even minor bleeding complications. Also cerebral bleeding could be avoided possibly because during treatment with abciximab platelet adhe-

sion is preserved. In pilot trials the combined use of abciximab with thrombolytic drugs (streptokinase, alteplase or reteplase) allowed to considerably decrease the dose of the latter. This may reduce the risk of cerebral bleeding, a point still to be proven in large trials,

Lamifiban: Lamifiban (Ro 44-9883, Hoffmann-La Roche) is a synthetic non-peptide compound of low molecular weight (0.468 kD).⁵⁶ The concentration required for a 50% inhibition of ADP induced platelet aggregation is 25 nmol and the plasma half-life is circa 90 min.

In a double-blind, placebo-controlled Canadian study, intravenous lamifiban (1, 2, 4 and 5 µg/min) protected 365 patients with unstable angina from severe ischaemic events during a 3- to 5-day infusion (3.3% versus 8.1%, $P < 0.05$) and reduced the incidence of death and myocardial infarction at 1 month (3.7% versus 8.1%).⁵⁷ No clear dose-related incremental clinical benefit could be documented in this small study but more major bleeding (2.9% versus 0.8%) and significantly more minor bleeding (11.1% versus 1.6%, $P = 0.002$) were noted in high-dose groups with bleeding time prolongation over more than 25 minutes. The lack of a dose-related correlation to clinical benefit and higher bleeding incidence in the highest dose lamifiban patients were confirmed in the PARAGON-A trial.⁵⁸ In total 2282 unstable angina and non-Q-wave myocardial infarction patients were randomly assigned to lamifiban in a 2 x 2 factorial design: low-dose (1 µg/min) with and without heparin versus high-dose (5 µg/min) with and without heparin or to placebo and heparin. All patients received aspirin. The relative benefit for reduced death or myocardial infarction at 30 days was lowest for those assigned to low-dose lamifiban plus heparin (relative reduction 12%) compared with control. At 6 months this composite end point was again lowest in the low-dose lamifiban plus heparin group ($P = 0.025$; relative reduction 30%) and intermediate for those assigned to high-dose lamifiban with or without heparin ($P = 0.45$) compared with control (12.6%, 16.4% and 17.9%, respectively). The relationship between the clinical outcome at 30 days and the measured plasma levels of lamifiban revealed an unexpected U-shaped curve.⁵⁹ The combination of high-dose lamifiban and heparin resulted in more major or intermediate bleeding (12.1% versus 5.5%, $P = 0.002$) and at 6 months a similar rate of death or myocardial infarction (18%) compared with control (18%). Conversely, low-dose lamifiban and heparin yielded similar bleeding rates as in the control group but had less ischaemic events (12.6% versus 17.9%) at 6 months. Durability and incremental rate benefit have also been observed in the EPIC trials evaluating monoclonal antibodies against GPIIb/IIIa in angioplasty patients. The term passivation has been used to describe this long-term clinical effect of GPIIb/IIIa inhibitors which may be due to the limiting production of platelet-derived vasoconstriction in the

short term and of growth factors in the long term. An optimal therapeutic range is being tested in an ongoing phase III study (PARAGON B).

The PARADIGM trial enrolled 345 patients treated with either t-PA or streptokinase at full doses and concomitant infusion of lamifiban (at 3 different doses) or placebo with little difference in clinical reinfarction or recurrent ischaemia between groups except for recovery of ST segment elevation during Holter monitoring. At higher doses of lamifiban (400 µg bolus and 2.0 µg/min infusion), there was excess transfusion requirement.⁵⁹

Tirofiban: Tirofiban (MK-383, L-700,462 Aggrastat™, Merck and Co) is a tyrosine derivative with a molecular weight of 495 kD with a highly specific inhibitory activity against the fibrinogenbinding to GPIIb/IIIa (4 orders of magnitude more selective than for binding to the vitronectin receptor).⁶⁰ The compound is well tolerated and inhibits platelet aggregation and prolongs the bleeding time in a dose-dependent manner in human volunteers. These effects are 55% to 89% lower about 3 hours after stopping the infusion. The plasma half-life is approximately 90 min.

The dosage regimen of tirofiban was studied in patients with angina pectoris at rest, recurrent angina or complex coronary lesion morphology who were undergoing coronary angioplasty.⁶¹ They were randomised to receive placebo or intravenous tirofiban 5 µg/kg bolus then 0.05 µg/kg/min, 10 µg/kg bolus then 0.1 µg/kg/min or 10 µg/kg bolus then 0.15 µg/kg/min for 36 hours. All 93 patients also received oral aspirin (325 mg) before angioplasty and intravenous heparin (dosage adjusted to maintain a target activated partial thromboplastin time of 60 to 80 seconds). Platelet aggregation was inhibited by 72% to 96% 5 minutes after starting tirofiban administration. There were too few adverse ischaemic events in the study to evaluate efficacy: 5% and 2.7% of placebo and tirofiban recipients, respectively, required repeat angioplasty during the infusion period, and there were no deaths, myocardial infarctions or emergency coronary bypass operations. Bleeding events occurred in 4.8%, 33% and 13.6% of patients receiving the lowest, middle and highest dosages of tirofiban, respectively, compared with 5% of placebo recipients.

The RESTORE trial was a randomised, double-blind, placebo-controlled trial of tirofiban in patients undergoing coronary interventions within 72 hours of presentation with unstable angina pectoris or acute myocardial infarction.⁶² All patients received aspirin and intravenous heparin and were randomised to tirofiban (bolus of 10 mg/kg followed by a 36 hours infusion of 0.15 µg/kg/min or placebo). Two days after angioplasty, the tirofiban group had a 38% relative reduction in the composite end point ($P \leq 0.05$), and at 7 days, there was a 27% relative reduction ($P = 0.022$), largely because of a

reduction in non-fatal myocardial infarction and the need for repeat angioplasty. The primary composite end point at 30 days was reduced from 12.2% in the placebo group to 10.3% in the tirofiban group, a 16% relative reduction ($P = 0.160$). The bleeding observed with tirofiban was not statistically different from that observed with placebo.

In the PRISM study, 3232 patients with unstable angina and non-Q-wave myocardial infarction were treated with a 48-hour infusion of tirofiban or heparin.⁶³ All patients received aspirin. The composite end point of refractory unstable angina, myocardial infarction or death was 32% lower at 48 hours (3.8% in the tirofiban group versus 5.6% in the heparin group ($P = 0.01$)). At 30 days, the frequency of the composite end point was similar in the two groups (15.9% versus 17.1%, $P = 0.34$). However, mortality was significantly reduced in patients receiving tirofiban. Major bleeding occurred in 0.4% of the patients in both groups and reversible thrombocytopenia in 1.1% and 0.4% ($P = 0.04$) in the tirofiban and control groups, respectively. In the PRISM-PLUS trial 1,915 patients with acute coronary syndromes, all on aspirin, were randomised to tirofiban plus heparin or either drug. Study drugs were given for minimum 48 hours prior and 48 to 96 hours after PTCA.⁶⁴ The study arm of tirofiban without heparin was stopped prematurely because of excess mortality at 7 days. There was a significant reduction in death, myocardial infarction and refractory cardiac ischaemia in the tirofiban plus heparin treated patients at 7 days, 30 days and 6 months. The angiographic substudy in PRISM-PLUS also indicated that tirofiban treated patients had significantly less angio-detected intracoronary thrombi 65 ± 17 hours after randomisation and improved blood-flow in the culprit coronary artery. The primary end point of ischaemic events assessed at 7 days was significantly reduced from 18% in the heparin arm to 13% in the heparin plus tirofiban group ($P = 0.004$). The benefit at 30 days was still significant.

Eptifibatide: Eptifibatide (Integrilin™, Cor Therapeutics, Key Pharmaceuticals) is a disulphide linked heptapeptide highly specific antagonist of platelet GPIIb/IIIa which displays antithrombotic effects in numerous animal models of thrombosis. Its cyclic structure increases the affinity to GPIIb/IIIa receptors.⁶⁵ Complete inhibition of aggregation in healthy volunteers is observed at infusion rates between 1.0 and 1.5 $\mu\text{g/kg/min}$. The half-life is approximately 2.5 hours. Inhibition of platelet aggregation and prolongation of the bleeding time are dose-dependent and return towards baseline values within 30 min after cessation of infusion.⁶⁶ A higher dissociation constant for integrilin compared to abciximab results in an almost linear relationship between the plasma and the platelet bound drug pool.

Eptifibatide affords rapid, competitive and reversible

platelet inhibition when administered with concomitant aspirin and heparin in patients undergoing elective percutaneous coronary intervention. Fifty-four patients undergoing elective PTCA were randomised in a pilot trial to receive placebo or an intravenous bolus of eptifibatide 90 to 180 $\mu\text{g/kg}$ plus an 18 to 24-hour infusion of 0.5 to 1 $\mu\text{g/kg/min}$. The procedure was successful in 94% and 96% of patients, respectively.⁶⁷ A subsequent larger study IMPACT-I in 150 patients undergoing elective coronary intervention supported the potential efficacy of eptifibatide (12.2% in the placebo group versus 4.1% end point events, $P = 0.13$) administered as a 90 $\mu\text{g/kg}$ bolus followed by a 1.0 $\mu\text{g/kg/min}$ for 12 hours.⁶⁸ This study was however associated with an increase incidence of bleeding; twice that of placebo treated patients. Moreover, 3 patients developed thrombocytopenia. IMPACT-II is a large trial in 4010 low- and high-risk patients undergoing angioplasty or other coronary procedures under cover of aspirin and heparin.⁶⁹ The purpose was to compare the additional effect of two infusion regimens of eptifibatide (bolus 135 $\mu\text{g/kg}$ followed by an infusion of 0.5 $\mu\text{g/kg/min}$ or 0.75 $\mu\text{g/kg/min}$ for 20 to 24 hours) and placebo in a composite primary end point, which included mortality, myocardial infarction, and need for urgent revascularisation at 30 days after drug administration. The results show a significant effect of both drug regimens at 24 hours and a statistically significant effect of the low dose (19% reduction, $P = 0.035$), but not the high dose (13% reduction) regimen at 30 days after drug administration. Treatment with eptifibatide did not result in any increase in major bleeding versus placebo notwithstanding aspirin and heparin administration in all patients and an infusion of eptifibatide twice as long as in IMPACT-I. The absolute difference in event rate between treatment arms was maintained at 6 months without evidence of suppression of clinically evident restenosis, and a comparison angiographic substudy found no difference in minimal luminal diameter.

In a trial comparing aspirin, low-dose (45 $\mu\text{g/kg}$ bolus and 0.5 $\mu\text{g/kg/min}$) and high-dose (90 $\mu\text{g/kg}$ bolus and 1.0 $\mu\text{g/kg/min}$) eptifibatide for 24 to 72 hours for treatment of unstable angina, 227 patients receiving high-dose eptifibatide had fewer and shorter episodes of angina than other patient groups.⁷⁰ All 227 patients completing the research protocol received standard medical therapy for unstable angina including intravenous heparin in addition to the test drug, but they received aspirin only after termination of study drug. The number and duration of ischaemic events was measured over the first 24 hours of treatment by Holter monitoring. Patients on high-dose eptifibatide had a mean of $0.24 (\pm 0.11)$ ischaemic events per 24 hours, whereas patients on aspirin had 1.0 ± 0.33 events ($P < 0.05$), and patients on low-dose eptifibatide had 0.83 ± 0.32

events. The high-dose eptifibatide group also had shorter ischaemic episodes than the aspirin group 8.4 ± 5.3 min versus 26.23 ± 9.8 min ($P = 0.01$). Bleeding complications were few, mostly ecchymoses at sites of intravenous lines.

The large trial PURSUIT study was conducted to evaluate the efficacy of eptifibatide in preventing death or myocardial infarction at 30 days in nearly 11,000 patients hospitalised with unstable angina or non-Q-wave myocardial infarction.⁷¹ The doses were higher than those used in IMPACT-II because the goal was to achieve 80% platelet inhibition, and *in vitro* data suggested that this was not achieved with either the low or the high doses used in IMPACT-II. In PURSUIT, patients were randomised to 1 of 3 groups: placebo, eptifibatide low dose (180 µg/kg bolus and 1.3 µg/kg/min infusion) or high dose (180 µg/kg bolus and 2 µg/kg/min) for up to 72 hours, or up to 96 hours if percutaneous coronary intervention (stent and/or percutaneous transluminal coronary angioplasty) was performed. At a prespecified review after 3218 patients were recruited, the low-dose arm was stopped given the safety of the high-dose arm. The primary end point was death or reinfarction at 30 days. The composite primary efficacy end point was reached in 15.7% of the 4739 patients in the placebo group and in 14.2% of patients in the eptifibatide group ($P = 0.042$).

Eptifibatide was associated with a moderate increase in major and minor bleeding. The early benefit was maintained without attenuation through 6 months. Irrespective of which treatment was given, age was an independent predictor of adverse outcomes, as for each lo-year increment in age, the adjusted odds ratio of death was 1.97.⁷² Eptifibatide was also shown in a small study of 180 patients with acute myocardial infarction to enhance the incidence (TIMI-3 at 90 min: 66% versus 39%, $P = 0.006$) and speed of reperfusion when combined with accelerated alteplase, aspirin and intravenous heparin without excess bleeding (IMPACT-AMI).⁷³

Xemilofiban: Xemilofiban (SC-5468A, Searle) is a prodrug of a non-peptide, mimetic antagonist of the RGD binding site on the GPIIb/IIIa receptor. The active metabolite SC-54701A is a potent inhibitor of GPIIb/IIIa and exhibits specificity for the GPIIb/IIIa receptor with respect to other integrins. In dogs, more than 50% of the orally administered prodrug is absorbed and half that amount is converted to the active agent.⁷⁴ Platelet aggregation is completely inhibited for more than 8 hours after a single oral dose of 2.5 mg/kg. After intravenous administration, the half-life of the B-phase elimination of the active moiety is 6.5 hours in dogs and the total plasma clearance is 0.3 L/h/kg. The results of a dose-ranging study show that oral administration of the prodrug to dogs gives a dose-dependent inhibition of platelet aggregation, which is maintained during a 14-

day administration period without adverse effects.⁷⁴ At a dose inhibiting collagen-induced aggregation by 80%, bleeding time was increased 2.5-fold.

A dose finding study (5 to 20 mg xemilofiban orally bid) after coronary stent deployment in 170 patients, all on aspirin, produced more than 50% platelet inhibition at a dose of ≥ 10 mg pd bid. In this study no episodes of major bleeding occurred.⁷⁵

Thirty patients with unstable angina who were undergoing percutaneous coronary interventions were randomised to placebo or xemilofiban 35 mg orally before and 20 to 25 mg tid for 30 days after angioplasty. All patients also received aspirin and heparin. More profound inhibition of platelet aggregation for a period up to 3 days was obtained compared to aspirin alone. At all doses tested, acute major bleeding was encountered.⁷⁶

Following PTCA, 549 patients, all on aspirin, were randomised to placebo, 15 mg or 20 mg xemilofiban (first 2 weeks tid, following 2 weeks bid). (ORBIT: Oral glycoproteinIIb/IIIa receptor blockade to inhibit thrombosis). At 3 months cardiac events occurred in 10% of the placebo group, 8% in the low dose and 5% in the high dose xemilofiban group ($P = 0.06$).⁷⁷

EXCITE was an on-going, blinded, placebo-controlled trial to enrol 7200 patients at 450 sites in 17 countries. It evaluated the effectiveness of oral xemilofiban plus aspirin compared with aspirin alone in reducing complications during percutaneous coronary revascularisation procedures and in preventing recurrent events for up to six months. The trial did not indicate effectiveness of xemilofiban and the development of the compound was stopped.

Orbofiban: Orbofiban (SC-57099B, Searle Co) was designed to mimic the arginine-glycine-aspartic acid-phenylalanine (RGDF) sequence thought to be the primary peptide recognition signal on the fibrinogen molecule. It is an ethyl-ester pro-drug with an RGD-containing peptide sequence that is recognised by the platelet GPIIb/IIIa receptor. The active molecule SC-57101B has a terminal elimination half-life of 16 to 18 hours. Renal excretion is the major metabolic route of elimination.⁷⁷ Patients with stabilised ischaemic syndromes, all on aspirin, were given orally 30 mg to 50 mg orbofiban bid in the SOAR trial. They were randomized to study drug treatment within 120 hours after the index event (unstable angina, Q-wave and non-Q-wave MI). A good dose response in terms of *ex vivo* ADP-induced platelet aggregation was obtained.⁷⁸ With the highest dose, 2 of 45 patients (4%) stopped the study drug because of bleeding.⁷⁹

The primary objective of the OPUS trial (TIM1 16) is to test the hypothesis that oral orbofiban (two dosing strategies) plus aspirin will prevent major cardiovascular events (death, subsequent myocardial infarction, recur-

rent ischaemia requiring rehospitalisation, urgent revascularisation, or stroke) compared with -placebo plus aspirin during long-term treatment in 12,000 patients with unstable coronary syndromes. One year after the start of the trial, further recruitment was stopped as no significant difference in composite endpoint between the trial groups was obtained. As the death rate in the two orbofiban groups was almost significantly higher than in the placebo group, further 'development of orbofiban was halted.

Sibrafiban: Sibrafiban (Ro 48-3657, Hoffmann-Laroche, G7333 Genentech) is an orally active, non-peptidic, selective antagonist of GP IIb/IIIa receptors. It is a double prodrug, which is converted in two enzymatic steps in the active compound Ro 44-3888.⁸⁰ In studies in normal volunteers, sibrafiban had a 39% bioavailability and in patients with coronary syndrome, the half-life of the active compound was 11 hours. In the TIMI-12 trial, several oral dosing regimens were tested for 28 days in patients with acute coronary syndrome. A clear dose response relationship was seen with higher drug levels correlating with higher levels of platelet inhibition and higher levels of bleeding complications.⁸¹ The peak blood level was achieved at circa 6 hours after ingestion. With the twice-daily dosing, the degree of inhibition was sustained, with 70% inhibition of ADP-induced platelet aggregation at 24 hours. Similar to inhibition of platelet aggregation, there was a dose response demonstrated for prolongation of the bleeding time. A clinically significant major or minor bleeding rate occurred at the doses that achieved 70% to 80% platelet inhibition (5 to 10 mg twice daily). SYMPHONY is a double-blind, aspirin-controlled study enrolling 9000 patients in 40 countries. It will evaluate the effectiveness of oral sibrafiban versus aspirin in achieving secondary prevention. Average treatment is expected to last 90 days and end points will be measured at 3, 6 and 12 months.

Fradafiban: Fradafiban (BIBU-52 ZW, Boehringer Ingelheim) binds with high affinity and selectivity to human GPIIb/IIIa complex and potently inhibits human platelet aggregation. Fradafiban has only very limited oral activity probably due to its high polarity and thus poor absorption after oral ingestion. Esterification of the carboxyl group and acylation of the amino group of fradafiban led to a far less polar pro-drug lefradafiban (BIBU-104-XX), which does not interact with GPIIb/IIIa receptors and has to be converted metabolically to fradafiban for platelet inhibition. Esterases, but not cytochrome P450-dependent enzymes, are involved in the conversion of lefradafiban to fradafiban *in vivo*.⁸²

The activity and plasma levels of fradafiban and lefradafiban were evaluated in 130 healthy male subjects. One to 15 mg fradafiban continuously infused over 30 minutes reversibly inhibited platelet aggregation in

platelet-rich plasma *ex vivo* in response to ADP and collagen.⁸³ Single oral doses of lefradafiban inhibited ADP-induced aggregation after 50 mg by 59%, after 100 mg by 90% and after 150 mg by 99% 8 hours after administration. Correlations between activity and fradafiban plasma levels were identical after fradafiban and lefradafiban treatment. After day 1, oral tid lefradafiban treatment for 7 days inhibited aggregation by $\geq 31\%$ (25 mg tid), 53% (50 mg tid) and 88 % (75 mg tid) just before the next dose. A similar correlation between the activity and fradafiban plasma levels was observed at days 1, 2 and 7. Thus oral administration of lefradafiban maintains the potent platelet GPIIb/IIIa antagonism of fradafiban during treatment of healthy subjects for 1 week without signs of loss of the antiplatelet activity.

In order to test the dose-response in patients undergoing PTCA, lefradafiban was administered orally for 2 days starting 1 hour before the procedure.⁸⁴ At 30 mg tid platelet fibrinogen receptor occupancy varied between 60% and 80%, and at 45 mg tid between 70% and 90%. For 60 mg tid almost all values exceeded 80%. Doses up to 45 mg tid were well tolerated. At the highest dose (60 mg tid) frequent minor but no major bleedings were observed.

L-738,167: L-738,167 (Merck Research Laboratories) is a highly potent and intrinsically active non-peptide antagonist of GPIIb/IIIa. Dog liver preparations and *in vivo* pharmacokinetic studies in dogs have characterised L-738,167 as a metabolically stable, high-clearance, high-volume-of-distribution compound with biliary excretion as the major route of elimination.⁸⁵ The terminal plasma half-life after intravenous administration to dogs was estimated to be circa 4 days, suggesting the potential for sustained duration of action.

The prolonged retention of L-738,167 in blood despite a relatively short release time from the receptor has been proposed to result from the rapid recapture of L-738,167 by the high concentration of platelet GPIIb/IIIa receptors before it is cleared from the plasma.⁸⁶ The specificity of L-738,167 for GPIIb/IIIa over other integrin receptors has been demonstrated in HUVEC attachment assays, in which IC_{50} for inhibition of HUVEC attachment to fibrinogen-, vitronectin-, and fibronectin-coated surfaces by L-738,167 all exceeded 300 $\mu\text{mol/L}$.

Low-dose oral administration of L-738,167 was highly effective in preventing occlusive coronary artery thrombosis and myocardial infarction at 24 hours after the initiation of electrolytic injury in dogs.⁸⁷ Inhibition of *ex vivo* platelet aggregation after single oral doses of 30 and 100 $\mu\text{g/kg}$ L-738,167 in dogs was dose dependent and sustained in duration. Complete prevention of coronary artery thrombosis was achieved with 100 $\mu\text{g/kg}$ oral L-738,167 in the conscious dog efficacy model and associated with 290% inhibitions of ADP- and collagen-induced *ex vivo* platelet aggregation for most of the

24-hour postdosing interval.

Risk of excessive bleeding is the principal concern in the development of novel antithrombotic agents. In the present studies, template bleeding time was elevated five-fold to six-fold or more after the 100 µg/kg oral dose of L-738,167, which afforded complete prevention of coronary artery thrombosis in the conscious dog model. The lower, 30 µg/kg oral L-738,167 dose, which significantly reduced the incidence of occlusive thrombosis and/or reduced thrombus mass was associated with a sustained but modest two-fold to three-fold increase in template bleeding time.

Roxifiban: DMP-754 (DuPont Merck Pharmaceutical Co) is an isoxazolinylacetamide and an ester prodrug which, upon conversion to its active form XV459 (Roxifiban), appears to be a competitive, high affinity and selective inhibitor of GPIIb/IIIa.⁸⁸ Roxifiban blocks platelet aggregation and clots retraction with IC₅₀ values <50 nM, but has no significant effects on the kinetics of coagulation. In dogs, maximal antiplatelet efficacy was demonstrated with roxifiban at 0.1 mg/kg i.v. and had submaximal antiplatelet efficacy (60% to 80% inhibition of ex vivo platelet aggregation) at 0.4 mg/kg p.o., as compared with the maximal antiplatelet effect of DMP 754 at 0.3 mg/kg p.o., suggesting an improved bioavailability with the prodrug form, as compared with its free acid form. Neither DMP 754 nor roxifiban affected platelet counts in whole blood. When administered intravenously to dogs at 1.0 mg/kg, both DMP 754 and roxifiban significantly ($P < 0.001$) extended bleeding time to >30 min, as compared with a basal bleeding time of 3 to 4 min. Template bleeding time increased to 6 to 8 min after administration of 0.1 mg/kg p.o. of DMP 754, extended to 12 to 15 min after 0.4 mg/kg p.o. of roxifiban, and extended to 15 to 20 min after 0.3 mg/kg p.o. of DMP 754. The effect on the bleeding time is reversible while maximal inhibition of platelet aggregation is maintained.⁸⁹

In the Folts model in dogs, DMP 754 prevented the incidence of cyclic flow reduction with an ED₉₀₋₁₀₀ of <0.1 mg/kg i.v. or p.o. Additionally, DMP 754 (0.1 mg/kg i.v. or 0.3 to 0.5 mg/kg p.o.) demonstrated maximal antithrombotic efficacy in preventing electrically-induced carotid and coronary artery thrombosis. DMP 754 demonstrated significant antithrombotic efficacy ($P < 0.001$) when administered intravenously or orally at relatively low doses in different settings or arterial thrombosis in canine.⁸⁹ After bolus administration of roxifiban in dogs, the plasma concentration declined poly-exponentially with a terminal half-life of 12 hours. DMP754 at the same oral dose had 24 hours of antiplatelet effect in baboon as compared to 8 to 12 hours in dogs.⁹⁰

Klerval: Klerval (RPR-109891, Rhône-Poulenc Rorer) is a competitive inhibitor of platelet GPIIb/IIIa available in both intravenous and oral formulations. In the

ongoing ORATOR trial in stent patients, it was found that at least 50% occupancy of the surface GPIIb-IIIa receptors by Klerval are necessary to have measurable inhibition of platelet aggregation induced by 20 µM ADP.⁹¹

The response to the intravenous formulation was investigated in 62 patients with acute coronary syndromes (TIMI 15A).⁹² Three drug regimens were investigated (bolus 30, 45 or 60 µg/kg) followed by 0.4, 0.6 or 0.8 µg/kg/min for 24 to 96 hours. Inhibition of platelet aggregation at the end of infusion was 85%, 90% and 95% for the low, medium and high dose, respectively. No bleeding problems were noted in this dose finding pilot trial. In another study, 320 patients with acute myocardial infarction were randomized to Klerval or placebo for 8 weeks. Clinically significant minor bleeding occurred in 7.9% of the patients. A decrease in platelet count <100.10⁹/L occurred in 13.7% and 5% of the Klerval and placebo patients, respectively.⁹³ Further development of Klerval was stopped.

Lotrafiban: SB-214857 (SmithKline Beecham) is a non-peptide selective antagonist of the human platelet fibrinogen receptor GPIIb/IIIa. The binding is saturable, of high affinity ($K_d = 2.5$ nM) and reversible. The affinity of SB-214857 is more than 1,000-fold greater for GPIIb/IIIa integrin than for other adhesive integrins such as $\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$. Aggregation of human, monkey and dog platelets is inhibited with an IC₅₀ of 54 nM in response to diverse stimuli.

In the dog, an oral dose of 0.1 mg/kg provides minimal inhibition, while 1.0 mg/kg completely inhibits ex vivo platelet aggregation for approximately 5 hours. In the canine Folts model intravenous infusion of SB-214857 at doses ranging from 0.1 to 0.8 µg/kg/min given over 60 min, produced dose-related inhibition of both platelet aggregation and platelet-dependent cyclic blood flow reductions in stenosed coronary arteries. The same dose was effective in the canine coronary artery wire model and there was a dose-related inhibition of coronary artery thrombosis. At 0.4 µg/kg/min (over 60 min) SB-214857 was at least as effective as aspirin (5 mg/kg IV) in reducing coronary occlusion and thrombus mass.

In a dose-finding study (APLAUD) atherosclerotic patients, all on aspirin, were randomized to 5, 20, 50 or 100 mg SB-214857 or oral placebo twice daily for 12 weeks.⁹⁴ Platelet aggregation was dose-dependently inhibited, 87% after 50 mg and 100% after 100 mg. Major bleeding was noted in 12% of patients on 100 mg oral dose and in 2.9% after 50 mg versus 2.1% in placebo-treated patients. Thrombocytopenia occurred in 0.6% of the 349 patients exposed for 12 weeks to SB-214857.

A large scale placebo-controlled double-blind two-arm trial (BRAVO) in atherosclerotic patients is planned. Patients will initiate therapy either on 30 mg or 50 mg of lotrafiban versus placebo b.i.d., depending on their age

or renal function. All patients will be on aspirin and followed for 2 years (3500 per treatment group).

SX-122566: SR-121566 (Sanofi Research) is a non-peptide antiplatelet agent with high affinity and specificity for the GPIIb/IIIa receptor that exhibited potent *in vitro* antiaggregating activity in rabbit platelets.⁹⁵ SR-121566 and its orally active prodrug SR-121787 revealed antithrombotic efficacy in several thrombosis models in rabbits and baboons.⁹⁶

GPIIb/IIIa Receptor Blockers in Perspective

Clinical trials on the intra- and post-procedure short-term administration of abciximab have conclusively documented the efficacy of this drug in preventing death, myocardial infarction and urgent interventions associated with coronary angioplasty or atherectomy, both in the early days following intervention and with preservation of the initial benefit for up to 3 years. A marked reduction in the composite rate of death and myocardial infarction was seen among refractory angina patients, with and without diabetes, when percutaneous coronary intervention is planned; an 18- to 24-hour pre-treatment with abciximab provides significant protection against ischaemic events within 30 days which was lost at 6 months. It would appear that a 12-hour post-procedure infusion is important for achieving more sustained clinical benefit. While abciximab is not itself a thrombolytic drug, by blocking the progression of thrombosis, it appears to markedly enhance the efficacy of streptokinase and alteplase. The high incidence of bleeding observed in the first trials with abciximab could be significantly reduced by using a lower heparin dose and weight-adjusted abciximab.

To provide protection against ischaemic cardiac events in acute coronary syndromes, more than 80% blockade of the GPIIb/IIIa receptors are required. It is unknown which receptor inhibition is needed to prevent long-term ischaemic events. The dose of the drug should be possibly titrated to a target degree of platelet inhibition in patients with a very high thrombotic risk and in those with a decreased renal function. Another possibility to be investigated is to use a dosing adjusted on body weight initially but a lower dose if the patient experiences bleeding and also for prolonged secondary prevention.

Whether GPIIb/IIIa inhibitors are effective in acute coronary syndromes as stand-alone therapy has still to be demonstrated. This is important as haemorrhagic complications occurred with GPIIb/IIIa inhibitors when used superimposed on aspirin and/or heparin.

One surprising finding is that abciximab has an independent effect of prolonging the activated coagulation times (ACTs) and produces dose-dependent inhibition of tissue factor-induced thrombin generation.⁹⁷

The optimal duration of therapy with abciximab is still to be defined. In most trials, a 12-hour infusion was given but there is no experience with longer infusions, although it is known that platelet deposition at the site of endothelial injury can persist for over 120 hours. Despite only a 12-hour infusion, the delayed, sustained, and in some trials incremental benefit over time after abciximab was not fully anticipated. This findings raised the hypothesis that arterial passivation was obtained, such that the therapy was capable of transforming atherosclerotic vessel wall surface from one that supports platelet-thrombus deposition to one that cannot do so. This late benefit could possibly be related to the fact that abciximab binds with great affinity to the GPIIb/IIIa receptor but also with equivalent affinity to the closely related vitronectin receptor ($\alpha v \beta 3$) which is present on human platelets in small numbers but well represented in vascular endothelium and smooth muscle cells.⁹⁸

The vitronectin receptor has been implicated in endothelial cell migration and proliferation which can lead to restenosis of injuries in arteries. Abciximab also binds to the β_2 integrin, Mac-1, although with lower affinity than to the above mentioned receptors. Mac-1 is present on monocytes and binds fibrinogen.

Critical thrombocytopenia following abciximab has been estimated to be less than 1%.⁹⁹ The same adverse effect has also been observed with several synthetic GPIIb/IIIa inhibitors and appears to be a class effect.

Abciximab is a chimeric antibody with some mouse-derived sequences. This raises a safety issue in case of retreatment due to the potential for allergic reactions, decreased therapeutic efficacy and enhanced risk of thrombocytopenia. A clinical trial on retreatment with abciximab is in progress.

At least a dozen GPIIb/IIIa inhibitors have been synthesised. Cyclic compounds are about 10 times more potent and more stable than linear analogues, although not orally active. They also exhibit a higher selectivity for GPIIb/IIIa receptors compared to the vitronectin receptor.

Intravenous GPIIb/IIIa inhibitors are desirable when rapid and reliable platelet inhibition is requested but oral inhibitors lend themselves to chronic administration and for the secondary prevention following interventional procedures. Thus orally active synthetic GPIIb/IIIa inhibitors vastly expand the potential area of application of this class of platelet inhibitors. It remains to be seen, however, whether long-term inhibition of GPIIb/IIIa receptors rendering patients similar to those with congenital Glanzmann thrombasthenia disorder, would not be associated with the bleeding problems encountered by the latter group.

The remarkable long-term benefit obtained with abciximab has not yet been replicated by specific small-

molecule inhibitors of GPIIb/IIIa. Efforts are underway to better understand the mechanisms by which abciximab may exert long-term inhibition of "clinical stenosis".

APPENDIX-Selected Achronyms

BRAVO	= Blockade of the receptor to avoid vascular occlusion
CAPTURE	= Chimeric 7E3 anti-platelet in unstable refractory angina
EPIC	= Evaluation of IIb/IIIa platelet receptor antagonist 7E3 in preventing ischaemic complications trial
EPILOG	= Evaluation in PTCA to improve long-term outcome with abciximab GPIIb/IIIa receptor blockade
EPISTENT	= Evaluation of platelet IIb/IIIa inhibitor in stenting
EXCITE	= Evaluation of oral xemilofiban in controlling thrombotic events
IMPACT	= Integrilin to manage platelet aggregation to combat thrombosis trial
OPUS	= Orbotiban in patients with unstable coronary syndrome
ORATOR	= Oral glycoprotein IIb/IIIa receptor antagonist versus ticlopidine on coronary stent revascularization
ORBIT	= Oral glycoprotein IIb/IIIa receptor blockade to inhibit thrombosis
PARADIGM	= Platelet aggregation receptor antagonist dose investigation for reperfusion gain in myocardial infarction
PARAGON	= Platelet IIb/IIIa antagonist for the reduction of acute coronary syndrome events in Global Organisation Network
PRISM	= Platelet receptor inhibition for ischaemic syndrome management study
PRISM PLUS	= Platelet receptor inhibition for ischaemic syndrome management study plus
PROLOG	= Precursor to EPILOG study
PURSUIT	= Platelet IIb/IIIa in unstable angina: receptor suppression using Integrilin Ischemia Trial
RAPPORT	= Reopro and Primary PTCA Organisation and Randomised Trial
RESTORE	= Randomised efficacy study of tirofiban for outcomes and restenosis trial
SOAR	= Safety of orofiban in acute coronary research
SYMPHONY	= Sibratiban vs aspirin to yield maximum protection from ischaemic heart events post acute coronary syndromes
TIM1	= Thrombolysis in myocardial infarction

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