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Antiplatelet drugs

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Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; AMP, adenosine monophosphate; COX, cyclooxygenase; GP, glycoprotein; PG, prostaglandin; PGI_2, prostacyclin; PTCA, percutaneous transluminal coronary angioplasty; TP, thromboxane receptor; TX, thromboxane; TXM, thromboxane metabolite

Background

Antiplatelet drugs are intended to prevent and/or reverse platelet aggregation in arterial thrombosis, most prominently in myocardial infarction and ischaemic stroke. Platelets aggregate as haemostatic plugs at the site of vascular injury, whereby bleeding is limited or arrested in advance of plasma coagulation (Figure 1). This function of platelet aggregation is literally 'vital'. Platelets aggregate intravascularly as arterial thrombi in response to haemorrhage into fissures or ruptures in atherosclerotic plaques (Figures 2 and 3). Pathologically, therefore, platelet aggregation is potentially lethal. Because in both situations, the physiological and the pathological, the aggregation mechanism is the same, separating the therapeutic from the harmful effects of antiplatelet drugs depends, in principle, on exploiting differences in the pathophysiological environment in which aggregation takes place. In practice, a favourable balance between the beneficial and harmful effects of antiplatelet therapy is achieved by treating patients whose thrombotic risk clearly outweighs their risk of bleeding complications (Patrono *et al*., 2004).

The aggregation process is induced by changes in the normal haemodynamic and/or biochemical environment of circulating platelets. Although the intravascular aggregation of platelets was recognized at the time of their discovery by Bizzozero in 1882, elucidation of the phenomenon made little progress until it could be investigated *ex vivo* by the technique of optical aggregometry (Born, 1962a, b; Born & Cross, 1963) (Figures 4 and 5).

This technique was developed for quantifying and analysing platelet reactions *in vitro*. The idea came to one of us (G.B.) after making so-called turbidimetric measurements of ribonuclease activity in *Streptomyces* culture filtrates carried out for the Oxford D. Phil. degree. Adaptations were made appropriate for measuring platelet aggregation in plasma. The new method, first published in 1962 (Born, 1962a, b), is quite banal in its simplicity. However, it brought results quickly and reproducibly, and basic observations were published in the following year (Born & Cross, 1963). Aggregation was characterized with respect to velocity, temperature and pH dependence (Born & Cross, 1963). The relation of aggregate formation to the optical changes was quantified (Born & Hume, 1967; Michal & Born, 1971) and accounted for on classical light-scattering theory (Latimer *et al*., 1977).

Optical aggregometry has ever since been used worldwide in fundamental, clinical and epidemiological investigations. Within a few years, the original *Nature* and *Journal of Physiology* papers became *Citation Classics* and have been cited several thousand times.

The *rapid shape change of platelets* (time constant about 1 s at 37°C), which is the first visual evidence of their activation, was quantified and shown to conform to Michaelis–Menten kinetics (Born, 1970). The results suggested that aggregation agonists such as ADP react with specific membrane receptors leading to structural changes; now the receptors are fully identified. Two essential cofactors of aggregation were discovered, viz. calcium and fibrinogen (Born & Cross, 1964; Cross, 1964). The suggestion that fibrinogen forms 'bridges' linking aggregating platelets (Figure 6; Born, 1965) was later confirmed at the electron microscopic and molecular levels (Figure 7). Nowadays, this bridging function and thereby platelet aggregation can be prevented by various molecules, which inhibit binding of fibrinogen to its receptor, the activated conformer of glycoprotein IIb–IIIa, on the platelet surface.

Optical aggregometry led to the discovery of the *first aggregation inhibitors*, viz. ATP and adenosine (Figure 8), looked at first because of their close chemical relationship to proaggregatory ADP (Born & Cross, 1962; Clayton *et al*., 1963). Soon it was shown that these and other aggregation inhibitors are also effective *in vivo*, by stopping the formation and embolization of platelet thrombi in injured arterioles and venules (Born *et al*., 1964). Regional administration of aggregation inhibitors prevented thrombus formation in artificial organs (Richardson *et al*., 1976). The last paragraph of the 1962 *Nature* paper reads: "If it can be shown that ADP takes part in the aggregation of platelets in blood vessels, it is conceivable that AMP or some other substance could be used to inhibit or reverse platelet aggregation in thrombosis". Inhibition of platelet aggregation has indeed turned out to be the most exciting and far-reaching discovery because it

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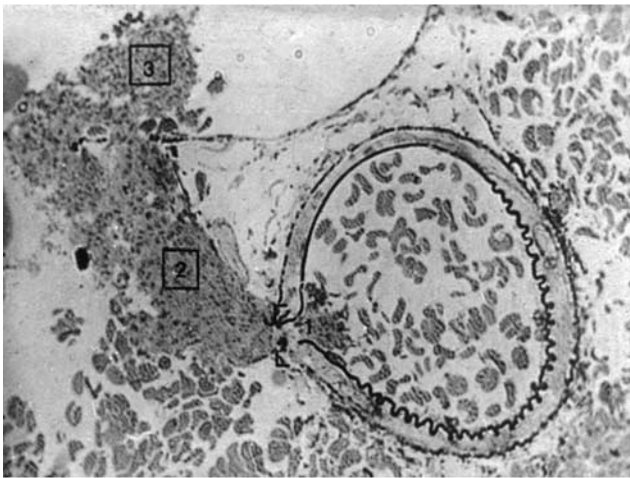

Figure 1 Haemostatic platelet plug forming in a cut arteriole (electron micrograph ×650).

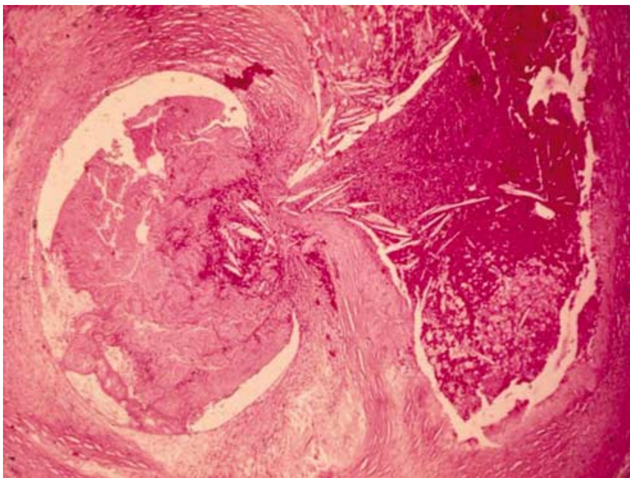

Figure 2 Massive platelet aggregates on an atheromatous plaque fissure blocking a human coronary artery (from Dr Paris Constantinides in the 1950s).

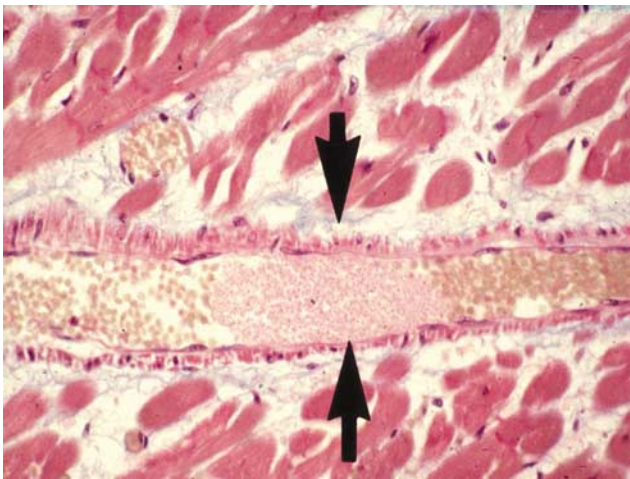

Figure 3 Platelet embolus blocking an intramyocardial arteriole (from Professor Michael Davies in the 1980s).

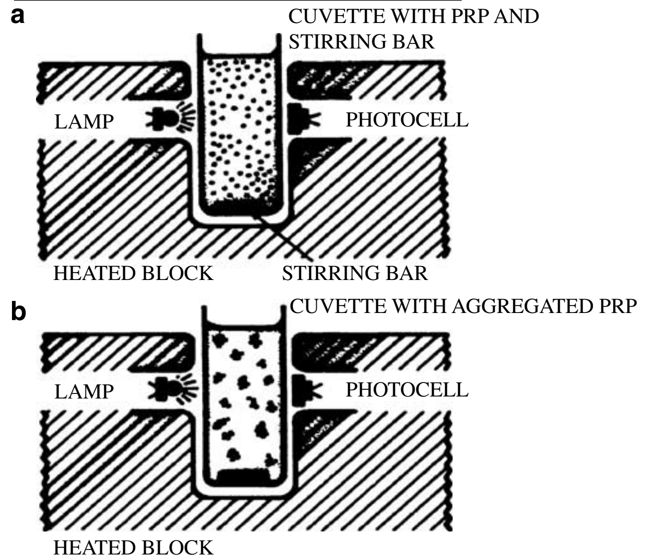

Figure 4 Principle of optical platelet aggregometry: as platelets aggregate in plasma, transmitted light increases.

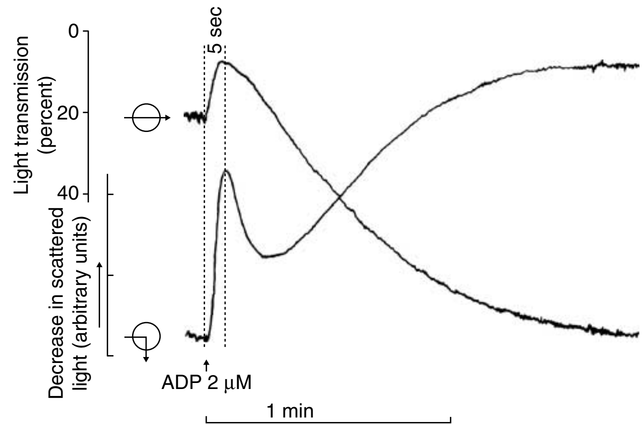

Figure 5 Optical aggregometry records of transmitted (upper trace) and scattered (lower trace) light. The initial deflections are due to the rapid shape change of platelets.

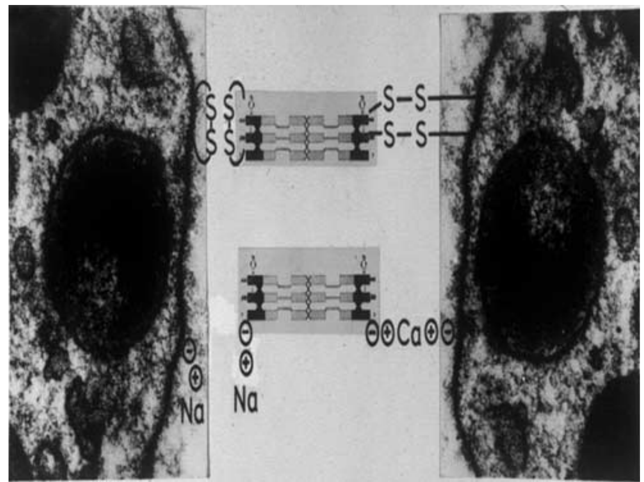

Figure 6 Postulated fibrinogen 'bridges' between neighbouring platelets during aggregation (Born, 1965).

established the therapeutic possibility of preventing arterial thrombosis by means of antiplatelet drugs and initiated the era of their use for the prevention of myocardial infarction and stroke.

Much effort was devoted to elucidating inhibitory mechanisms. ATP was shown to inhibit competitively whereas adenosine did not, but was significantly more effective (Born & Cross, 1962). More effective still were 2-substituted adenosine derivatives, particularly 2-chloroadenosine, out of many related substances (Born, 1964). In volunteer experiments (on Gustav Born and coworkers), which could conceivably have been harmful and which would now be strictly forbidden, it was shown that the relative effectiveness of adenosine analogues as aggregation inhibitors and as arterial vasodilators was the same (Born *et al*., 1965). This has since been explained by the similarity of the cyclic AMP mechanism in platelets and in vascular smooth muscle. Correlation of optical aggregometry with quantitative electron microscopy showed that ATP, as a competitive inhibitor of aggregation by ADP, is able to reverse the rapid shape change of platelets, bringing them back to their normal disc-like appearance (Born *et al*., 1978).

Optical aggregometry made possible the discovery by Macmillan and Oliver of the *second phase of aggregation* (Macmillan & Oliver, 1965); Born and Cross had failed to notice or to think about small anomalous deformations in the manually plotted aggregometer tracings (Figure 9) (this was before the availability of continuous recording), which turned out to be the optical manifestation of the *platelet release reaction*. In this reaction, enough ADP is released to account for the positive feedback mechanism of aggregate growth proposed earlier (Born, 1965; Mills *et al*., 1968). It is this second phase of aggregation which, as discovered in 1967 by Fraser Mustard and co-workers (Evans *et al*., 1968) and by Weiss & Aledort (1967), is inhibited by aspirin (Figure 10). Therefore, optical aggregometry is at the beginning of this very important medical story (see below).

By 1965 the foundations had therefore been laid for the development of antiplatelet drugs as a new class of therapeutic agents, but it took more than two decades before the basic principles and methodologies were sufficiently understood for the introduction of aspirin as the first antiplatelet drug to prevent coronary and cerebral thrombosis.

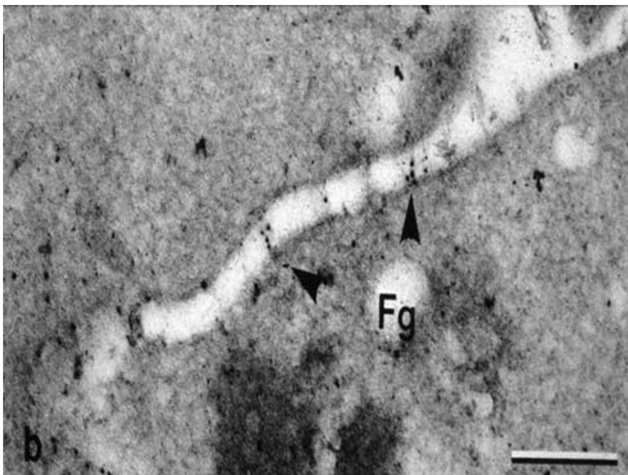

Figure 7 Electron-microscopic image of labelled fibrinogen molecules forming 'bridges' between platelets (from Dr Alan Nurden in the 1980s).

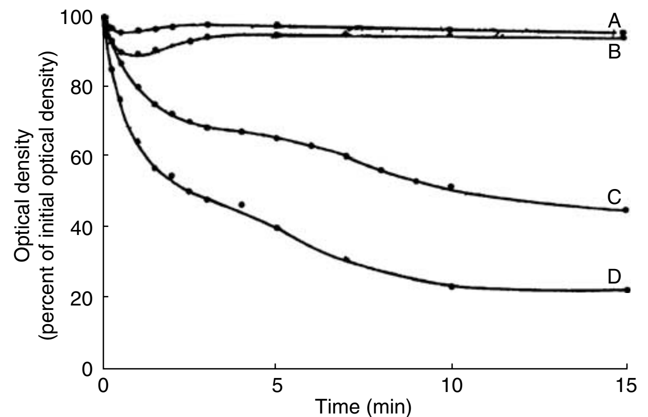

Figure 9 Recording of platelet aggregation by increasing concentrations of ADP: note the anomalous rises in the lower tracings (unpublished record made at the time of Born & Cross, 1962).

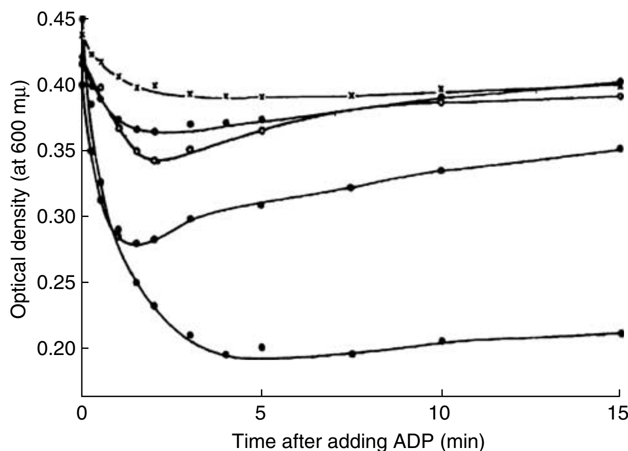

Figure 8 First recording of platelet aggregation inhibition by adenosine (Born & Cross, 1962).

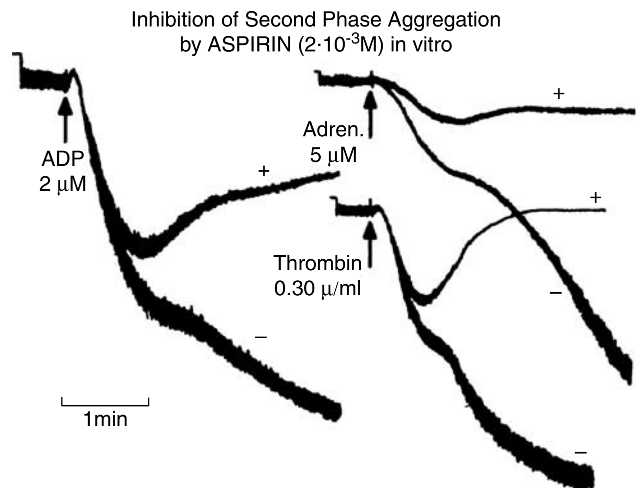

Figure 10 Inhibition by aspirin of second phase platelet aggregation induced by ADP, adrenaline, or thrombin (from Drs David Mills and Gordon Roberts in the late 1960s).

Aspirin and platelets

By the time one of us (C.P.) graduated in Medicine in 1968, Quick had shown that a small oral dose of aspirin, but not sodium salicylate, may prolong the bleeding time even in normal subjects. Moreover, several investigators had shown that aspirin, but not sodium salicylate, when taken orally or added to platelet-rich plasma inhibited platelet aggregation and blocked the release of ADP from these cells (O'Brien, 1968; Weiss *et al*., 1968). It was suggested at the time (Hawkins *et al*., 1969) that the unique capacity of aspirin to form acetyl derivatives of human proteins might well play a role in these phenomena, but no mechanism of action had been proposed.

In 1971, two graduate students working in the Department of Pharmacology of the Royal College of Surgeons of England in London, Bryan Smith working with Gustav Born and Jim Willis working with the late Sir John Vane, reported that aspirin added *in vitro* to human platelet-rich plasma or administered orally to three healthy volunteers selectively inhibited the release of prostaglandin (PG)E_2-like bioactivity from platelets, under experimental conditions, in which the release reaction was unimpaired, and suggested that one action of aspirin on platelets is inhibition of the conversion of arachidonic acid into PGs (Smith & Willis, 1971). In concert with the two accompanying papers (Ferreira *et al*., 1971; Vane, 1971), the paper of Smith and Willis suggested that "the clinical effectiveness of aspirin and indomethacin as anti-inflammatory agents could be explained by the inhibition of the production of prostaglandins". It is perhaps not surprising that no mechanistic link between PG synthesis and platelet function was established at the time of this fundamental discovery, inasmuch as the only two PGs then known, that is PGE_2 and $PGF_{2\alpha}$, had no obvious effects on platelet aggregation.

The fundamental work of Nobel Laureate Bengt Samuelsson and his associates at the Karolinska Institutet in Stockholm elucidated platelet arachidonic acid metabolism with the discovery of unstable biosynthetic intermediates, the cyclic endoperoxides PGG_2 and PGH_2, and with the trapping of an extremely potent but evanescent platelet-aggregating prostanoid named thromboxane (TX)A_2 (Hamberg *et al*., 1975) (Figure 11). The discovery of this potent mediator of platelet aggregation, the synthesis and release of which was activated by a variety of platelet agonists and inhibited by aspirin, identified the missing biochemical link between inhibition of platelet arachidonic acid metabolism and impaired platelet function.

Further insight into the molecular mechanism of action of aspirin was provided by Gerry Roth and Phil Majerus at the Washington University in St Louis, who used aspirin labelled with ^3H at the acetyl group to demonstrate acetylation of PG-synthase and its irreversible inactivation by the drug (Roth & Majerus, 1975; Roth *et al*., 1975). The structural basis of the enzymic inactivation, inferred from the crystal structure of inactivated PGH-synthase, is the blockade of the cyclooxygenase (COX) channel in consequence of the acetylation by aspirin of a strategically located serine residue (Ser-529 in human COX-1 and Ser-516 in human COX-2), which prevents access of the substrate to the catalytic site of the enzyme (Figure 12).

The discovery, 1 year later, of prostacyclin (PGI_2) (Moncada *et al*., 1976), an unstable vasoactive and platelet-inhibiting prostanoid produced by the vessel walls, the synthesis of which is also inhibited by aspirin, created the so-called 'aspirin dilemma', that is the concomitant inhibition of two major modulators of vascular homeostasis with opposing biological effects. Although there was no evidence that aspirin would be thrombogenic at high doses, except under heroic experimental circumstances, it was thought that inhibition of vascular PGI_2 might limit the potential antithrombotic effects of aspirin resulting from inhibition of platelet TXA_2 production. These considerations were responsible for triggering a worldwide search for the lowest effective dose of aspirin to inhibit platelet function, culminating in the clinical development of low-dose aspirin as an antithrombotic agent. Although the importance

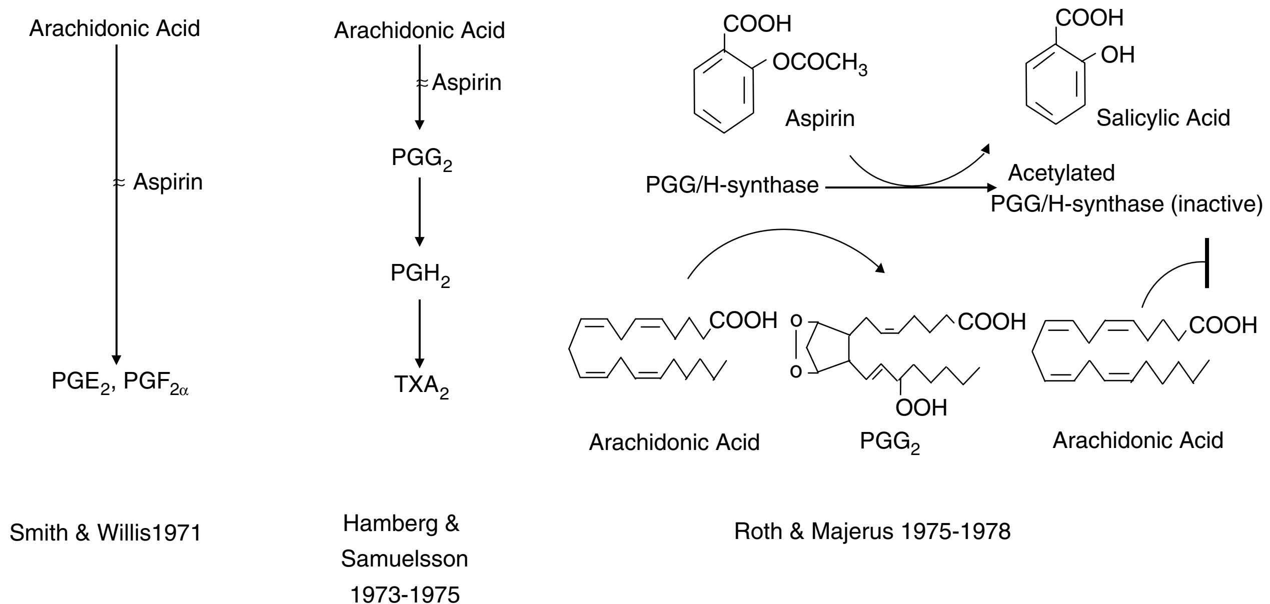

Figure 11 Main historical steps in understanding aspirin's mechanism of action in inhibiting platelet biochemistry and function. PG, prostaglandin; TX, thromboxane.

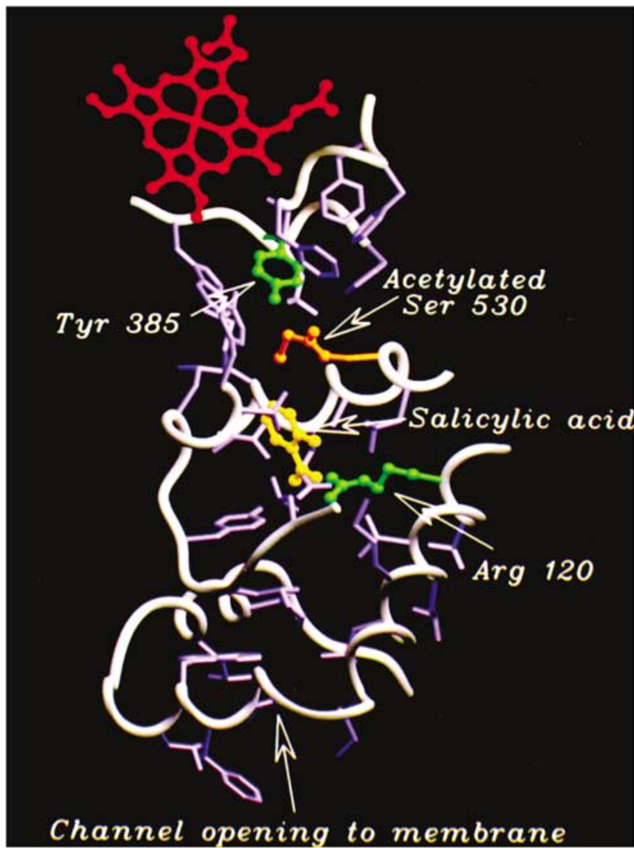

Figure 12 Crystal structure of the cyclooxygenase channel of PGH-synthase 1 and its modification by acetylsalicylic acid. Courtesy of Dr Patrick Loll.

of the thromboxane/prostacyclin balance in cardiovascular homeostasis (Moncada & Vane, 1979) remained unsubstantiated over the following 25 years or so, this concept has been substantially validated by the recent discovery of cardiovascular toxicity associated with COX-2 inhibitors (FitzGerald, 2004).

It is important to realize that the clinical development of low-dose aspirin was orchestrated by the medical-scientific community (though in the absence of an officially appointed 'conductor') and brought to completion mainly with public funding. The negative aspects of such an unusual drug development was its long duration of more than 20 years (no pressure on timing because of no patent issues nor marketing concerns), as well as the 50-fold range of daily doses (from 30 to 1500 mg) evaluated in more than 50 randomized clinical trials. The positive aspects are the very large and solid database, which now supports the efficacy and safety of low-dose aspirin in a variety of clinical indications, including rare diseases such as polycythaemia vera (Landolfi *et al*., 2004).

Although Born's optical platelet aggregometry had demonstrated the ability of aspirin to inhibit platelet function at low-doses, it was not until the discovery of TXA_2 and the development of mechanism-based biochemical end points that the human pharmacology of platelet inhibition by aspirin could be properly elucidated. In particular, two analytical approaches were developed independently to quantify TXA_2 biosynthesis *ex vivo* (serum TXB_2) and *in vivo* (urinary TX metabolites). One of us (C.P.) who had trained with the pioneers of radioimmunoassay, Nobel Laureate Rosalyn Yalow and the late Solomon Berson, replaced the smooth muscle strips that Sir John Vane had used to quantify the release of unstable prostanoids (e.g. 'rabbit aorta contracting substance') with a soluble antibody against TXB_2, the stable hydrolysis product of TXA_2, to determine the synthesis and release of platelet TXA_2 triggered by endogenously formed thrombin during whole blood clotting in a glass test tube at 37°C (Patrono *et al*., 1980). At about the same time, Jack Roberts at the Division of Clinical Pharmacology of Vanderbilt University in Nashville demonstrated the conversion of systemically infused TXB_2 into 20 enzymic metabolites, which were characterized in human urine by gas chromatography/mass spectrometry (Roberts *et al*., 1981). The discovery of 2,3-dinor-TXB_2 as a major enzymic metabolite of TXB_2 paved the way for investigating TXA_2 biosynthesis *in vivo* and its pharmacological reduction by aspirin.

By then a young Irish pharmacologist, Garret FitzGerald, had joined the Vanderbilt Prostaglandin Group of John Oates after training in Clinical Pharmacology with Sir Colin Dollery at the Royal Postgraduate Medical School in London. FitzGerald elucidated the fate of PGI_2 in the human circulation, demonstrating that this prostanoid – like all others – is not a circulating hormone and characterising its major urinary metabolite, 2,3-dinor-6-keto-$PGF_{1\alpha}$ (FitzGerald *et al*., 1981). He joined the race in search of a platelet-selective regimen of aspirin that would spare PGI_2 biosynthesis in vessel walls. Patrono's group at the Catholic University in Rome showed that it was possible to dissociate the effect of low-dose aspirin (30 mg daily) on serum TXB_2 (almost exclusively a product of platelet COX-1) from the effect on urinary 6-keto-$PGF_{1\alpha}$ (mainly a product of renal COX-2) by exploiting the cumulative nature of platelet COX-1 inactivation on repeated daily dosing (Patrignani *et al*., 1982) (Figure 13). Platelet COX-1 activity was almost abolished after approximately 1 week of daily dosing with 30 mg aspirin, while furosemide-induced renal PGI_2 biosynthesis was not significantly diminished (Patrignani *et al*., 1982). An intriguing finding of FitzGerald a year later was that platelet aggregation was maximally inhibited by daily doses of aspirin of approximately 40–80 mg but tended to return to baseline values upon chronic dosing with higher doses up to 2600 mg daily, despite profound suppression of TXM excretion (FitzGerald *et al*., 1983) – possibly the first description of so-called 'aspirin resistance' (Figure 14).

FitzGerald made other important contributions to platelet pharmacology, including the presystemic nature of platelet inactivation by low-dose aspirin (Pedersen & FitzGerald, 1984); the development of a controlled release formulation of aspirin, which achieves substantial sparing of vascular PGI_2 biosynthesis through its negligible systemic bioavailability (Clarke *et al*., 1991); and the demonstration of a nonlinear relationship between inhibition of serum TXB_2 and reduced TXM excretion (Reilly & FitzGerald, 1987) (Figure 15).

The concordant findings of Majerus, Patrono and FitzGerald in demonstrating immediate and virtually complete acetylation of platelet COX-1, suppression of platelet TXA_2 production and negligible TXM excretion produced by 160 mg of aspirin led the Clinical Trial Service Unit at Oxford University, directed by Sir Richard Peto, to test this daily dose of aspirin in the first large-scale, placebo-controlled randomized trial for efficacy and safety in the short-term treatment

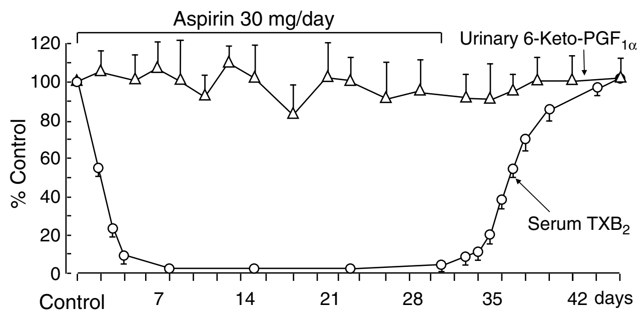

Figure 13 Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. Redrawn from Patrignani *et al.* (1982).

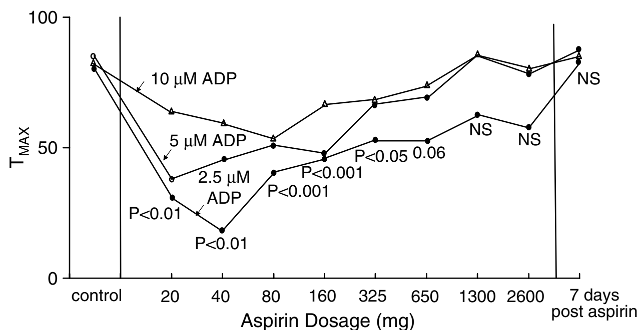

Figure 14 The change in optical density (T_{max}) of the primary phase of the platelet aggregation response induced by ADP (2.5, 5.0, and 10.0 μM) *ex vivo* in five volunteers before receiving aspirin (control) on the 7th day of each dosage period and 7 days postdosage. Doses of 20, 40, 80, 160, 325, 650, 1300, and 2600 mg were administered, each dose for 7 days in sequential weeks. Differences are expressed from predosing control values. Redrawn from FitzGerald *et al.* (1983).

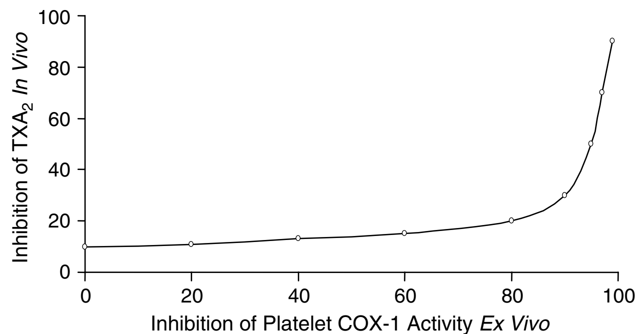

Figure 15 Nonlinear relationship between pharmacologic inhibition of platelet COX-1 activity, as measured *ex vivo*, and inhibition of platelet activation *in vivo*, as reflected by thromboxane metabolite excretion. COX, cyclooxygenase; TX, thromboxane. Redrawn from Reilly & FitzGerald (1987).

of patients with acute myocardial infarction (ISIS-2 Collaborative Group, 1988) (Figure 16). This marked the transition from descriptive phenomenology and empirical trials to molecular understanding of platelet pharmacology, dose-finding studies relying on mechanism-based biochemical end points, and the rational design of randomized clinical trials.

FitzGerald and Patrono together demonstrated the extremely low rate of TXA_2 biosynthesis in humans under physiological conditions (Patrono *et al.*, 1986) and character-

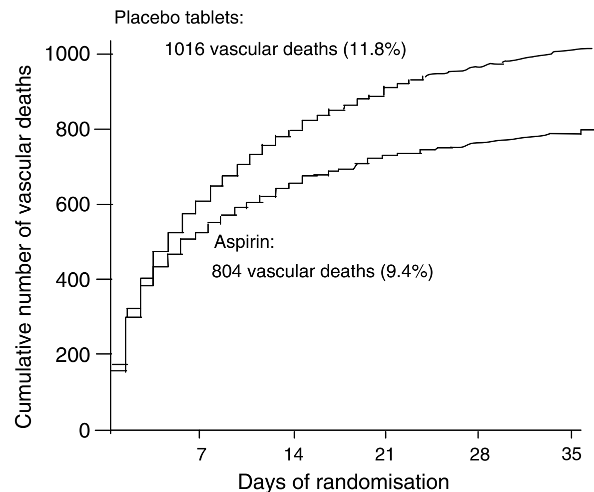

Figure 16 Low-dose aspirin (160 mg daily) reduces vascular mortality vs placebo in patients with an acute myocardial infarction treated within 24 h of the onset of symptoms. Redrawn from ISIS Collaborative Group (1988).

ized episodic increases in TXA_2 biosynthesis in acute coronary and cerebrovascular syndromes, as well as persistent platelet activation in the presence of major cardiovascular risk factors and myeloproliferative disorders. These findings provided a rationale for exploring the efficacy and safety of low-dose aspirin in these settings (see Patrono *et al.*, 2004).

Two important lessons from the platelet pharmacology of low-dose aspirin are noteworthy: (i) The nonlinear relationship between inhibition of the maximal biosynthetic capacity (as reflected by serum TXB_2 measurements) and inhibition of platelet activation *in vivo* (as reflected by urinary TXM measurements), whereby profound ($\geqslant$95%) and persistent inhibition of the former is required to produce a measurable effect on the latter (Figure 15; and (ii)) the 'hit-and-run' paradigm of platelet inhibition with a short half-life moiety (intact acetylsalicylic acid) permanently inactivating a platelet protein that cannot be resynthesized during and beyond the 24-h dosing interval.

Following the successful clinical development of low-dose aspirin as an antithrombotic agent, several drug companies have attempted to make more effective and/or safer antiplatelet agents targeting TXA_2 synthesis or action. These include reversible COX-1 inhibitors, like indobufen and triflusal, inhibitors of TX-synthase and TP-antagonists. None of these compounds has been shown convincingly to be more effective or safer than low-dose aspirin. However, it should be emphasized that most of them have been compared with aspirin in clinical trials with inadequate sample sizes and questionable clinical end points.

Dipyridamole

Dipyridamole was synthesized half a century ago and introduced clinically in the early 1960s as a coronary vasodilator. Dipyridamole was shown to inhibit platelet adhesiveness to glass *ex vivo* in patients with coronary artery disease and to reduce thrombus formation in experimental models. These findings led Boehringer Ingelheim to develop dipyridamole as an antithrombotic agent.

Although the clinical efficacy of dipyridamole, alone or in combination with aspirin, has been questioned on the basis of earlier randomized trials, the issue has been reopened by the reformulation of the drug to improve its relatively low bioavailability and the positive results with the new preparation of the European Stroke Prevention Study-2 (ESPS-2) on 6602 patients with cerebrovascular disease (see Patrono *et al.*, 2004). Unexpectedly, dipyridamole did not increase the bleeding complications in these patients, raising the possibility that other properties of the drug may have contributed to its beneficial effects on stroke prevention. Steve Prescott at the University of Utah has recently reported that dipyridamole inhibits inflammatory gene expression in human platelet–monocyte interactions, which may be involved in atherosclerosis and in its thrombotic complications (Weyrich *et al.*, 2005).

The inhibitory action of adenosine on platelet aggregation (Born & Cross, 1962; Clayton *et al.*, 1963) is potentiated by dipyridamole, which inhibits the uptake of adenosine by platelets (as well as by red cells: Bunag *et al.*, 1964), increasing the concentration of adenosine in the plasma (Born & Mills, 1969). The observation that dipyridamole inhibits adenosine uptake by platelets led to an unexpected but important development in an entirely different field. It is known that the trypanosome parasite responsible for African sleeping sickness is incapable of synthesising nucleotides *de novo* but needs to take up nucleosides like adenosine from surrounding tissue fluids. This raised the idea of the development of trypanocidal drugs acting as inhibitors of the uptake of adenosine by these organisms. The demonstration of a weak effect of this kind on *Trypanosoma brucei* by dipyridamole (James & Born, 1980) initiated extensive research on purine acquisition by these organisms. This has shown that dipyridamole and similar compounds are unlikely to block nucleoside uptake by the parasites, which rely on salvage pathways for purines. The trypanosome genome project has revealed a large number of transporters with different substrate specificities. The intention is to exploit differences between mammalian and trypanosome transporters, either by finding drugs capable of blocking the latter or by using them to introduce novel drugs into the cells (for a review, see El Kouni, 2003). These advances can therefore be traced to work on platelet aggregation inhibitors.

Thienopyridines

Ticlopidine was synthesized in 1972 as a potential substitute for another thienopyridine, tinoridine, the anti-inflammatory properties of which were published in 1970 by a Japanese team. As with most compounds synthesized at that time, ticlopidine was submitted to a battery of phenotypic tests in animals. Ticlopidine had no anti-inflammatory activity but was found to inhibit aggregation of rat platelets induced by ADP *ex vivo*. According to Jean-Pierre Maffrand, the Sanofi scientist responsible for synthesising the molecule, the Company's subsequent good fortune was due to their ability to test the molecule *in vivo* where an unstable metabolite is responsible for its activity, ticlopidine being inactive *in vitro*. Equally fortunate was the use of ADP to induce aggregation, because it was shown later that the active metabolite blocks an ADP receptor on platelets. The Company's 'clairvoyance' lay in their very early interest in antiplatelet agents as potential antithrombotics. Thus, Ticlopidine and later the structurally related Clopidogrel were discovered by *in vivo* phenotypic screening. It took more than 20 years to identify the active metabolites of ticlopidine and clopidogrel and to clone their platelet receptor viz. $P2Y_{12}$.

The active metabolite of clopidogrel is pharmacodynamically similar to aspirin in causing cumulative inhibition of platelet function by repeated daily administration of low doses. As with aspirin, platelet function returns to normal 7 days after the last dose of clopidogrel. Both the cumulative nature of the inhibitory effects and the slow rate of recovery of platelet function are consistent with the active moieties of aspirin (acetylsalicylic acid) and clopidogrel (active metabolite) causing permanent defects in platelet proteins, which cannot be repaired during the 24-h dosing interval and can only be replaced as a function of platelet turnover. This justifies the once daily regimen for both drugs despite their short half-lives in the human circulation. However, the main determinants of interindividual variability in the antiplatelet effects of the two drugs are substantially different (see Patrono *et al.*, 2004).

The role of ticlopidine in the present therapeutic armamentarium is uncertain because serious bone marrow toxicity is less with clopidogrel than with ticlopidine. Clopidogrel may be marginally more effective than aspirin in certain high-risk groups. However, there is evidence that adding a second antithrombotic agent, either an antiplatelet or an anticoagulant, to low-dose aspirin results in much greater risk reduction than replacing aspirin with another drug. Clopidogrel and extended-release dipyridamole produce additive beneficial effects when combined with low-dose aspirin in patients with acute coronary syndromes and those with ischaemic cerebrovascular disease, respectively (see Patrono *et al.*, 2004).

The $P2Y_{12}$ receptor that was identified *post hoc* as the molecular target of ticlopidine and clopidogrel has become the target for novel reversible antagonists that are being developed by several drug companies.

Integrin αIIbβ3 (GPIIb/IIIa) receptor antagonists

Given the redundance of discrete pathways leading to platelet aggregation, it is not surprising that the clinical efficacy of aspirin, ticlopidine, and clopidogrel is only partial. These drugs, while inhibiting TXA_2-mediated or ADP-mediated platelet aggregation, leave the activity of other platelet agonists such as thrombin largely unaffected. Following recognition that, regardless of the initiating stimulus, the expression of functionally active integrin αIIbβ3 (GPIIb/IIIa) on the platelet surface is the final common pathway of platelet aggregation, this glycoprotein became the target of novel antiplatelet drugs (Coller, 1995).

The choice of the GPIIb/IIIa receptor as therapeutic target rests on extensive basic and clinical research on several seemingly disparate topics, including platelet–fibrinogen interactions, the rare platelet disorder called Glanzmann thrombasthenia, platelet membrane glycoproteins, integrin receptors, coronary artery atherogenesis, and experimental thrombosis (Coller, 1995). Glanzmann thrombasthenia was first noted in 1918, but its modern description as a hereditary disorder

causing mucocutaneous haemorrhage, marked prolongation of the bleeding time, and abnormal clot retraction dates from the mid-1960s. At that time it was shown that the patients' platelets failed to aggregate in response to all of the agonists believed to operate *in vivo* including ADP, adrenaline, serotonin, collagen, and thrombin. This profound defect in platelet aggregability contrasted with the lesser inhibition of platelet aggregation produced by aspirin, which was discovered to be an antiplatelet agent about the same time. In the mid-1970s, Nurden and Caen in France and Phillips *et al.* in the U.S.A. independently identified deficiencies of two different platelet membrane glycoproteins in several kindreds with Glanzmann thrombasthenia. These glycoproteins were designated glycoproteins GPIIb and GPIIIa based on their electrophoretic mobilities in sodium dodecyl sulphate–polyacrylamide gel electrophoresis. Soon thereafter it was established that these two glycoproteins exist as a calcium-dependent complex (GPIIb/IIIa) on the platelet surface. Although the essential role of fibrinogen in platelet aggregation had been discovered earlier (Born & Cross, 1964; Cross, 1964), it took until the late 1970s to establish that the binding of fibrinogen to the platelet surface is necessary for platelet aggregation induced by all of the agonists presumed to operate *in vivo* (Coller, 1995).

The technology for preparing monoclonal antibodies using murine hybridomas was developed in the late 1970s and several groups began to produce such antibodies against platelet glycoproteins. Barry Coller at the State University of New York restricted his search to antibodies that interfered with the interaction between platelets and immobilized fibrinogen. He was able to produce such antibodies, and showed that one of them (10E5) abolished aggregation of normal platelets (Figure 17), blocked platelet–fibrinogen interaction and inhibited clot retraction; that is, it could induce a functional thrombasthenic phenotype (Coller *et al.*, 1983). This antibody and others with similar properties prepared elsewhere, immunoprecipitated both GPIIb and GPIIIa, providing crucial confirmatory evidence that these glycoproteins exist as a complex and are involved in fibrinogen binding. Approximately 40,000 antibody molecules bind to the surface of each platelet, indicating that there are probably 40,000–80,000 GPIIb/IIIa receptors per platelet depending on whether the antibodies bind bivalently or monovalently. Thus, it appears that GPIIb/IIIa is the most dense adhesion/aggregation receptor present on any cell type. The aggregation mechanism presumably reflects the need for a rapid and effective response to haemorrhage, explaining the high density of GPIIb/IIIa on the surface of platelets and the high

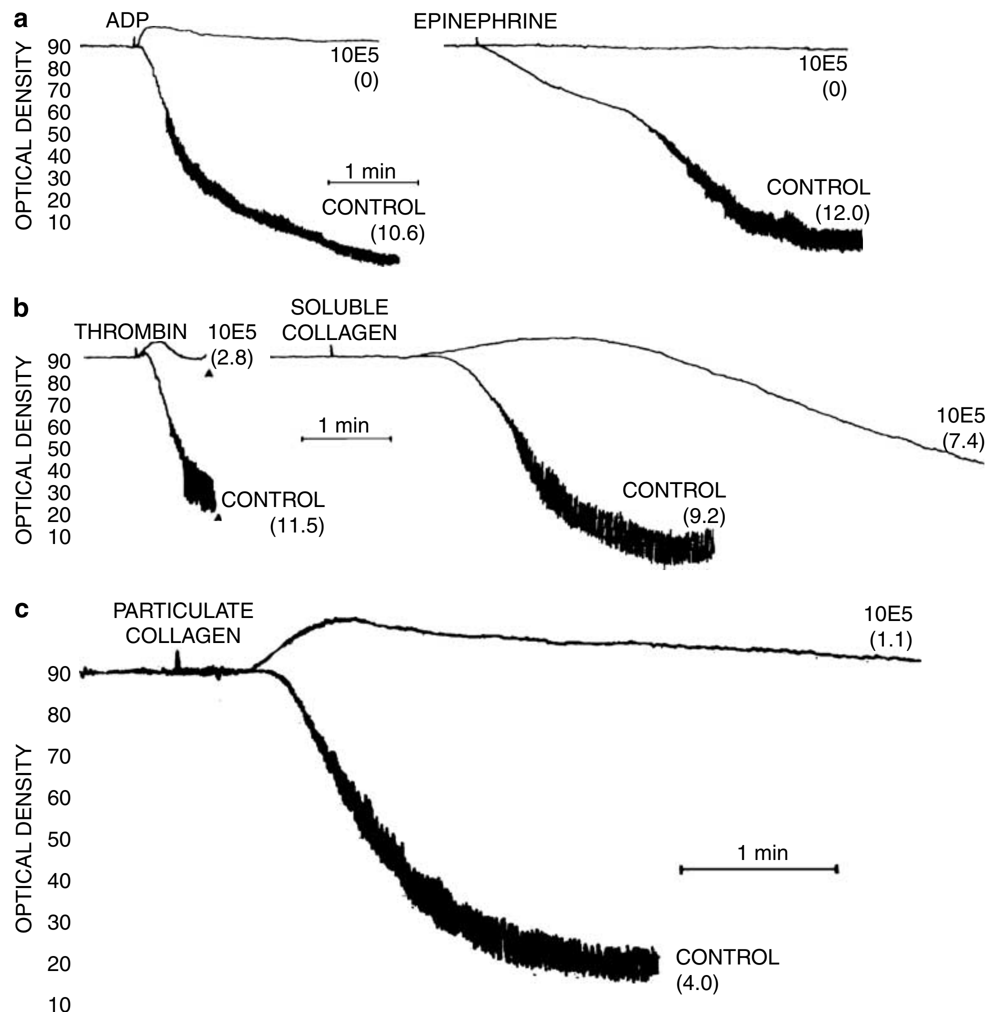

Figure 17 Inhibition of platelet aggregation and release of ATP by 10E5. Reproduced from Coller *et al.* (1983).

concentration of circulating fibrinogen (Coller, 1995). The final common effector for platelet aggregation, regardless of agonist, is the conformational change in the GPIIb/IIIa receptor that results in its developing high affinity for the adhesive glycoprotein ligands.

Correlation of all these findings suggested that blockade of GPIIb/IIIa receptors could be a particularly desirable therapeutic strategy because, first, the monoclonal antibodies to GPIIb/IIIa are more potent inhibitors of platelet function than aspirin; secondly, GPIIb/IIIa is platelet specific; thirdly, inhibition of GPIIb/IIIa still leaves platelet adhesion largely intact, contributing to haemostasis without causing thrombotic damage; and fourthly, the haemorrhagic diathesis produced by the inherited deficiency of GPIIb/IIIa receptors in Glanzmann thrombasthenia only rarely produces spontaneous brain haemorrhage, the most feared complication of anticoagulant and antiplatelet therapy (Coller, 1995).

Owing to concerns about the immunogenicity of the original 7E3 antibody, a mouse/human chimeric 7E3 Fab antibody abciximab was created for clinical development. Pharmacokinetic data on abciximab indicate that following i.v. bolus administration, free plasma concentrations decrease rapidly (initial half-life about 30 min) as a result of rapid binding to platelet GPIIb/IIIa receptors, with approximately 65% of the injected antibody becoming attached to platelets in the circulation and the spleen. After a bolus injection of abciximab, a dose-dependent inhibition of ADP-induced platelet aggregation was recorded in patients who were judged to be at a moderate to high risk of ischaemic complications associated with percutaneous transluminal coronary angioplasty (PTCA). A bolus dose of $0.25\,mg\,kg^{-1}$ resulted in blockade of $>80\%$ platelet receptors and reduced platelet aggregation in response to $20\,\mu$mol ADP to $<20\%$ of baseline value. The dose–response curve was steep. Effects on receptor blockade, platelet aggregation and bleeding time were maximal at the first sampling time, 2 h after bolus administration of $0.25\,mg\,kg^{-1}$. Thereafter platelet function gradually recovered, bleeding times returning to near-normal values by 12 h. Platelet aggregation induced by $20\,\mu$mol ADP returned to $\geqslant 50\%$ of baseline values within 24 h in most patients and within 48 h in almost all patients. Small amounts of abciximab can be detected on circulating platelets as late as 14 days after administration, presumably as a result of antibody redistribution from platelet to platelet (Patrono *et al.*, 2004).

Receptor blockade, inhibition of platelet aggregation and prolongation of bleeding time produced by a $0.25\,mg\,kg^{-1}$ bolus dose of abciximab could be maintained for 12 h by infusing abciximab at $10\,\mu g\,min^{-1}$ for the whole of that period. This regimen was chosen for a phase III trial (the Evaluation of 7E3 for the Prevention of Ischaemic Complications [EPIC] trial), which demonstrated the clinical efficacy of abciximab when added to conventional antithrombotic therapy in reducing the incidence of ischaemic events in patients undergoing PTCA and led to regulatory approval of the drug for this indication in 1994 (see Patrono *et al.*, 2004). This is an interesting example of academic translational research, whereby timely technology transfer from academia to industry has resulted in the rapid clinical development of a novel antiplatelet agent.

The success of short-term, high-efficacy blockade of platelet GPIIb/IIIa with intravenous agents (abciximab, eptifibatide, tirofiban) has led to the development of several oral GPIIb/IIIa antagonists in the hope of extending the benefit to the long-term management of patients with acute coronary syndromes. To date, five large-scale clinical trials have been completed (viz., Evaluation of Oral Xemilofiban in Controlling Thrombotic Events trial, Orbofiban in Patients with Unstable Coronary Syndromes trial, Sibrafiban *versus* Aspirin to Yield Maximum Protection from Ischaemic Heart Events Post-Acute Coronary Syndromes trial 1 and 2, and Blockade of the GP IIb/IIIa Receptor to Avoid Vascular Occlusion trial) (see Patrono *et al.*, 2004). The consistent findings involving over 40,000 patients is that therapy with oral GPIIb/IIIa antagonists (viz., xemilofiban, orbofiban, sibrafiban, and lotrafiban) is no more effective than aspirin therapy, nor when combined with aspirin superior to placebo and may in fact increase mortality (Figure 18). To explain these results, several mechanisms have been put forward. One suggests that the low oral bioavailability of these compounds and the target of approximately 50% inhibition of platelet aggregation resulted in little antiplatelet activity in many patients. This would explain a lack of clinical response but not an increase in mortality. Overall, there was an increase in the incidence of bleeding and a decrease in the requirement for urgent revascularization, suggesting some degree of limited clinical efficacy (see Patrono *et al.*, 2004).

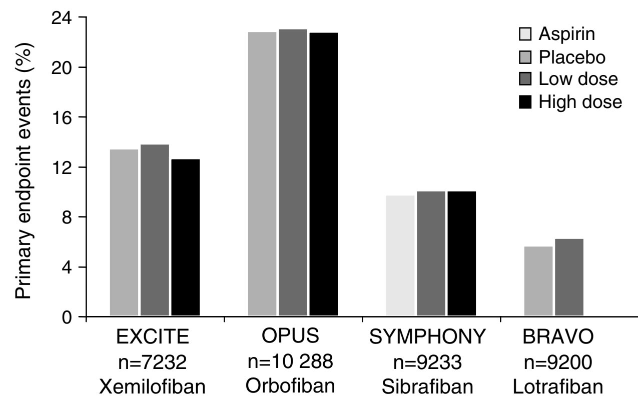

Figure 18 The oral GPIIb/IIIa antagonists, xemilofiban, orbofiban, sibrafiban and lotrafiban, do not reduce the rate of myocardial infarction or death when compared to aspirin or placebo in patients with acute coronary syndromes. EXCITE, Evaluation of oral Xemilofiban in Controlling Thrombotic Events; OPUS, Orbofiban in Patients with Unstable coronary Syndromes; SYMPHONY, Sibrafiban vs aspirin to Yield Maximum Protection from ischaemic Heart events pOst-acute coroNary sYndromes; BRAVO, Blockade of the GPIIb/IIIa Receptor to Avoid Vascular Occlusion.

An alternative explanation is that GPIIb/IIIa antagonists can activate platelets, at least in some individuals. GPIIb/IIIa is not a passive receptor; instead, like other integrins, it responds to ligand binding by activating the cell. Indeed, several studies have suggested that ligands designed to bind to the receptor and prevent platelet aggregation may paradoxically activate the receptor so that it can bind ligand or perhaps trigger some activating signals directly.

It is important to emphasize that relatively limited phase II dose-finding studies were done with these oral agents before beginning large phase III clinical trials, a fact that may have limited the ability to find out the most appropriate dosing regimens for long-term treatment. Issues such as predictable pharmacokinetics, shallow dose–response relationship and perhaps new methods of monitoring GPIIb/IIIa blockade are

likely to be fundamental to any reconsideration of these agents (see Patrono *et al*., 2004).

Conclusion

One of us (G.B.) wrote in 1976 'The haemostatic function of platelets is literally 'vital', that is, essential to survival; and their pathological role is potentially lethal. This clinical significance has led to a remarkable increase in platelet research from every conceivable aspect. This research seems likely to result, for the first time, in the discovery of drugs for preventing arterial thromboses such as 'acute coronaries', which are the scourge of this and other developed countries.' The successful development of antiplatelet drugs based on the understanding of their pharmacological mechanisms in the past 30 years has been summarized in this article. The effectiveness of these drugs in reducing the risks of the main cardiovascular killers is therefore a gratifying contribution to current medical practice, an example of how very great progress can come from understanding very small cells.

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