Platelet-Active Drugs

The Relationships Among Dose, Effectiveness, and Side Effects

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Abbreviations: ACE trial = Acetylsalicylic Acid and Carotid Endarterectomy; ADP = adenosine diphosphate; AMP = adenosine monophosphate; CAPRIE study = Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events; CI = confidence interval; COX = cyclooxygenase; DVT = deep venous thrombosis; EPISTENT trial = Evaluation of Platelet IIb/IIIa Inhibitors for Stenting; ESPS = European Stroke Prevention Study; EXCITE trial = Evaluation of Xemilofiban in Controlling Thrombotic Events; FDA = Food and Drug Administration; GP = glycoprotein; HOT study = Hypertension Optimal Treatment; INR = international normalized ratio; LMWH = low-molecular-weight heparin; MI = myocardial infarction; NSAID = nonsteroidal anti-inflammatory drug; OPUS trial = Orbofiban in Patients with Unstable Coronary Syndromes; PCI = percutaneous coronary intervention; PE = pulmonary embolism; PEP trial = Pulmonary Embolism Prevention; PG = prostaglandin; PTCA = percutaneous transluminal coronary angioplasty; RGD = Arg-Gly-Asp sequence; RR = relative risk; TIA = transient ischemic attack; TPT = Thrombosis Prevention Trial; TTP = thrombocytopenic purpura; TX = thromboxane; VTE = venous thromboembolism

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S ince our last report on antithrombotic therapy in 1998,[1] new information has been published on the role of aspirin and other platelet-active drugs in the treatment and prevention of atherothrombosis. These new data can be summarized as follows: (1) a large randomized study, the Acetylsalicylic Acid and Carotid Endarterectomy (ACE) trial,[2] has directly compared low-dose and high-dose aspirin for the prevention of ischemic stroke in patients undergoing carotid endarterectomy; (2) the role of aspirin in prophylaxis of venous thromboembolism (VTE) has to be reassessed in the light of the Pulmonary Embolism Prevention (PEP) trial[3]; (3) three nonrandomized studies with historical control subjects[4–6] and one randomized trial[7] have compared the safety of clopidogrel

plus aspirin vs ticlopidine plus aspirin in patients undergoing intracoronary stent implantation; (4) the results of clinical and angiographic follow-up of the patients in the Evaluation of Platelet IIb/IIIa Inhibitors for Stenting (EPISTENT) trial at 6 months[8] and 1 year[9] provide evidence that stent implantation and platelet glycoprotein (GP) IIb/IIIa blockade by abciximab improve the efficacy and safety of percutaneous coronary revascularization; and (5) preliminary reports from three large trials of long-term GPIIb/IIIa blockade (Orbofiban in Patients with Unstable Coronary Syndromes [OPUS]; Evaluation of Xemilofiban in Controlling Thrombotic Events [EXCITE][10]; and sibrafiban vs aspirin to yield maximum protection from ischemic heart events post-acute coronary syndromes [SYMPHONY] (sibrafiban)[11]) in approximately 27,000 patients taking oral drugs for 3 months to 1 year, are largely disappointing in terms of efficacy and/or safety.[12]

ASPIRIN AND OTHER CYCLOOXYGENASE INHIBITORS

Aspirin has been thoroughly evaluated as an antiplatelet drug and has been found to prevent vascular death by approximately 15% and nonfatal vascular events by about 30% in a meta-analysis of > 50 secondary prevention trials in various groups of patients.[13]

Mechanism of Action of Aspirin

The best characterized mechanism of action of the drug is related to its capacity to inactivate permanently the cyclooxygenase (COX) activity of prostaglandin (PG)H synthase-1 and PGH synthase-2 (also referred to as COX-1 and COX-2).[14–18] These isozymes catalyze the first committed step in prostanoid biosynthesis (ie, the conversion of arachidonic acid to PGH_2). PGH_2 is the immediate precursor of PGD_2, PGE_2, $PGF_{2\alpha}$, PGI_2, and thromboxane $(TX)A_2$. COX-1 and COX-2 are homodimers of a approximately 72-kDa monomeric unit. Each dimer has the following three independent folding units: an epidermal growth factor-like domain; a membrane-binding domain; and an enzymatic domain.[18] Within the enzymatic domain, there is the peroxidase catalytic site and a separate, but adjacent, site for COX activity at the apex of a long, hydrophobic channel. There are a number of important differences between COX-1 and COX-2 (Table 1), some of which may contribute to variable inhibitor selectivity.

The molecular mechanism of permanent inactivation of COX activity by aspirin is related to the blockade of the COX channel as a consequence of the acetylation of a strategically located serine residue (ie, Ser529 in the human COX-1 and Ser516 in the human COX-2) that prevents access of the substrate to the catalytic site of the enzyme.[19] Because aspirin has a short half-life (15 to 20 min) in the human circulation and is approximately 50-fold to 100-fold more potent in inhibiting platelet COX-1 than monocyte COX-2,[20] it is ideally suited to act on anucleate platelets, inducing a permanent defect in TXA_2-dependent platelet function. Moreover, since aspirin probably also inactivates COX-1 in relatively mature megakaryocytes, and since only 10% of the platelet pool is replenished each day, once-a-day dosing of aspirin is able to

Table 1—Structure, Distribution, and Regulation of COX-1 and COX-2

Characteristics	COX-1	COX-2
DNA	Chromosome 9; 22 kB	Chromosome 1; 8.3 kB
mRNA	2.8 kB	4.8 kB
Protein	72 kDa: 599 amino acids	72 kDa: 604 amino acids
Homology		Amino acids 90% homologous between species; similar V_{max} and K_m values for arachidonic acid
Differences	Glucocorticoids do not inhibit expression	Glucocorticoids inhibit expression; active site of COX-2 is larger than that for COX-1
Regulation	Predominantly constitutive; increased 2- to 4-fold by inflammatory stimuli	Predominantly inducible (10- to 20-fold) Constitutive in certain organs
Tissue expression	Most tissues, but particularly platelets, stomach, and kidney	Induced by inflammatory and mitogenic stimuli in monocytes/macrophages, synoviocytes, chondrocytes, and fibroblasts; induced by laminar shear stress and platelet microparticles in vascular endothelial cells; induced by hormones in the ovaries and fetal membranes; constitutive in the CNS, kidney, testes, tracheal epithelial cells

maintain virtually complete inhibition of platelet TXA_2 production. In contrast, the inhibition of COX-2-dependent pathophysiologic processes (*eg*, hyperalgesia and inflammation) requires larger doses of aspirin (because of the decreased sensitivity of COX-2 to aspirin) and a much shorter dosing interval (because nucleated cells rapidly resynthesize the enzyme). Thus, there is an approximately 100-fold variation in daily doses of aspirin when it is used as an anti-inflammatory rather than as an antiplatelet agent. Furthermore, the benefit/risk profiles of the drug depend on the dose and indication since its GI toxicity is dose-dependent (see below).

Human platelets and vascular endothelial cells process PGH_2 to produce TXA_2 and prostacyclin (PGI_2), respectively.[17] TXA_2 induces platelet aggregation and vasoconstriction, while PGI_2 inhibits platelet aggregation and induces vasodilation.[17] Aspirin is antithrombotic in a wide range of doses, inhibiting TXA_2 and PGI_2.[20–26] While TXA_2 is largely a COX-1-derived product (mostly from platelets) and, thus, highly sensitive to aspirin inhibition, vascular PGI_2 can derive from both COX-1 (short-term changes in response to agonist stimulation, *eg*, bradykinin[27] sensitive to transient aspirin inhibition) and COX-2[28] (long-term changes in response to laminar shear stress[29] largely insensitive to aspirin inhibition at conventional antiplatelet doses). This may account for the substantial residual COX-2-dependent PGI_2 biosynthesis *in vivo* at daily doses of aspirin in the range of 30 to 100 mg, despite transient suppression of COX-1-dependent PGI_2 release.[27] It has not been established that more profound suppression of PGI_2 formation by higher doses of aspirin is sufficient to initiate or to predispose to thrombosis. However, studies with mice that were deficient in the gene encoding the PGI_2 receptor support the importance of this prostanoid in the prevention of arterial thrombosis.[30] The results of the ACE trial,[2] demonstrating a significantly lower rate of vascular events in patients receiving 80 or 325 mg aspirin than in patients re-

ceiving 650 or 1,300 mg daily, are also consistent with an important role for PGI_2 in preventing thrombosis.

Pharmacokinetics

Aspirin is rapidly absorbed in the stomach and upper intestine. Peak plasma levels occur 30 to 40 min after aspirin ingestion, and inhibition of platelet function is evident by 1 h. In contrast, it can take up to 3 to 4 h to reach peak plasma levels after the administration of enteric-coated aspirin. If only enteric-coated tablets are available, and if a rapid effect is required, the tablets should be chewed. The oral bioavailability of regular aspirin tablets is approximately 40 to 50% over a wide range of doses.[31] A considerably lower bioavailability has been reported for enteric-coated tablets and sustained-release, microencapsulated preparations. Because platelet COX-1 is acetylated in the presystemic circulation,[31] the antiplatelet effect of aspirin is largely independent of systemic bioavailability. Both a controlled-release formulation[27] and a transdermal patch[32] with negligible systemic bioavailability have been developed in an attempt to achieve selective inhibition of platelet TXA_2 production without suppressing systemic PGI_2 synthesis. However, the clinical relevance of preparations that are relatively selective for the presystemic circulation remains to be established (see below).

The plasma concentration of aspirin decays with a half-life of 15 to 20 min. Despite the rapid clearance of aspirin from the circulation, the platelet-inhibitory effect lasts for the life span of the platelet because aspirin irreversibly inactivates platelet COX-1.[14,15] Aspirin also acetylates the enzyme in megakaryocytes before new platelets are released into the circulation.[16,33–35] The mean life span of human platelets is approximately 10 days. Therefore, about 10% of circulating platelets are replaced every 24 h,[36,37] and 5 to 6 days following aspirin ingestion, approximately 50% of the platelets function normally.

Issues Concerning the Antithrombotic Effects of Aspirin

A number of issues related to the clinical efficacy of aspirin continue to be debated. These include the following: (1) the minimum effective dose of aspirin required for antithrombotic efficacy; (2) the relative importance of the effects of aspirin on TXA_2 and PGI_2 synthesis as determinants of clinical efficacy; (3) the suggestion that part of the antithrombotic effect of aspirin is unrelated to inhibition of platelet TXA_2; and (4) the possibility that some patients may develop aspirin *resistance* over time.

The Minimum Effective Dose of Aspirin: Well-designed randomized trials have shown that aspirin is an effective antithrombotic agent when used in doses ranging between 50 and 100 mg/d, and there is a suggestion that it is effective in doses as low as 30 mg/d. Aspirin in a dose of 75 mg/d was shown to be effective in reducing the risk of acute myocardial infarction (MI) or death in patients with unstable angina[38] and chronic stable angina[39] as well as in reducing stroke or death in patients with transient cerebral ischemia[40] and the number of postoperative strokes after carotid endarterectomy.[41] In the European Stroke Prevention Study (ESPS)-2, aspirin, 25 mg bid, was effective in reducing the risks of stroke or death in patients with prior stroke or transient ischemic attack (TIA).[42] Finally, in a study of 3,131 patients after they had experienced a TIA or minor ischemic stroke, aspirin in a dose of 30 mg/d was compared with a dose of 283 mg/d, and no statistically significant difference was found in the incidence of the combined outcome of vascular death, stroke, or MI between the two aspirin regimens.[43] The lowest effective dose of aspirin for these various indications is shown in Table 2.

The clinical effectiveness of different doses of aspirin has been compared directly in a small number of randomized trials.[2,43–47] In the United Kingdom-TIA study,[47] no difference in efficacy was found between regimens of 300 and 1,200 mg/d of aspirin (see below). The Dutch TIA study failed to show a difference between regimens of 30 and 283 mg/d of aspirin.[43] The ACE trial has recently reported that the risk of stroke, MI, or death within 3 months of carotid endarterectomy is significantly lower for patients taking 81 or 325 mg aspirin daily than for those taking 650 or 1,300 mg (6.2% vs 8.4%; p = 0.03).[2] Thus, there is no convincing evidence from randomized studies that have compared different doses of aspirin for a dose dependence of the antithrombotic effect.

The antithrombotic effects of a range of doses of aspirin have also been compared with the effects on an untreated control group in a number of thrombotic vascular disorders. The doses have varied between 50 and 1,500 mg/d. Aspirin has been shown to be effective in patients with the following conditions: unstable angina in which the incidence of acute MI or death was significantly reduced to a similar degree in four separate studies using daily doses of 75 mg,[38] 325 mg,[48] 650 mg,[49] and 1,300 mg[50]; stable angina in which a dose of 75 mg daily reduced the incidence of acute MI or sudden death[39]; aortocoronary bypass surgery in which the incidence of early occlusion was similarly reduced with daily doses of 100 mg,[51] 325 mg,[52] 975 mg,[52] and 1,200 mg[53]; thromboprophylaxis of patients with prosthetic heart valves who also received warfarin in whom the incidence of systemic embolism was reduced with daily doses of 100 mg,[54] 500 mg,[55] and 1,500 mg[56,57]; thromboprophylaxis of patients with arterial venous shunts who were undergoing long-term hemodialysis in whom a dose of 160 mg/d was shown to be effective[58]; acute MI in which a dose of 162.5 mg/d reduced early (35 days) mortality as well as nonfatal reinfarction and stroke[59]; transient cerebral ischemia in which doses between 50 and 1,200 mg/d were effective;[40,42,47,60–62] and acute ischemic stroke in which doses of 160 to 300 mg/d were effective in reducing early mortality and stroke recurrence,[63,64] although the proportional effects of aspirin therapy on vascular events in these patients were small when compared with the effects in other high-risk settings.

Thus, aspirin is an effective antithrombotic agent in doses between 50 and 1,500 mg/d. It is also possible from the results of the Dutch TIA study that a dose of 30 mg/d is effective. There is no evidence that low doses (*ie*, 50 to 100 mg/d) are less effective than high doses (*ie*, 650 to 1,500 mg/d), and, in fact, the opposite may be true. The data from the Antiplatelet Trialists' Collaboration overview are consistent with this conclusion (Table 3).

There is evidence, however, that doses of approximately 300 mg/d produce fewer GI side effects than doses of approximately 1,200 mg/d.[47] There is also some evidence that a dose of 30 mg/d produces fewer side effects than 300 mg/d.[43] In summary, the results of biochemical studies on its mechanism of action, the lack of dose-response

Table 2—*Vascular Disorders for Which Aspirin Has Been Shown To Be Effective and Minimum Effective Dose*

Disorder	Minimum Effective Daily Dose, mg
Men at high cardiovascular risk	75
Hypertension	75
Stable angina	75
Unstable angina*	75
Acute MI	160
TIA and ischemic stroke*	50
Severe carotid artery stenosis*	75
Acute ischemic stroke*	160

*Higher doses have been tested in other trials and not found to confer any greater risk reduction.

Table 3—*Indirect Comparison of Aspirin Doses Reducing Vascular Events in High-Risk Patients**

Aspirin Dose, mg/d	Trials, No.	Patients, No.	Odds Reduction, %
500–1,500	30	18,471	21 ± 4
160–325	12	23,670	28 ± 3
75	4	5,012	29 ± 7

*Data from Antiplatelet Trialists' Collaboration.[13]

relationship in clinical studies evaluating its antithrombotic effects, and the dose dependence of its side effects all support the use of as low a dose of aspirin as has been found to be effective in the treatment of various thromboembolic disorders.[26,65]

The Relative Importance of TXA$_2$ vs PGI$_2$ Synthesis: Although there is some evidence from *in vitro* experiments and *in vivo* studies that aspirin may inhibit platelet function by a mechanism that is unrelated to the inhibition of TXA$_2$ synthesis,[66-69] the results of clinical trials, in which different doses of aspirin ranging from 30 to 1,500 mg/d have been used, are consistent with the hypothesis that the antithrombotic effect of aspirin is caused by the inhibition of platelet TXA$_2$ synthesis. Thus, both the reduction in risk of MI or death in patients with unstable angina, and stroke or death in patients with transient cerebral ischemia, have been reported with daily doses of aspirin as low as 50 to 75 mg, and no further protection was afforded by 20-fold to 30-fold higher doses. There are both theoretical and practical reasons to choose the lowest effective dose of aspirin (Table 2). The GI side effects of aspirin appear to be dose-dependent (see below), and, for many clinical situations, treatment with aspirin is indicated for an indefinite period. There are theoretical reasons to select a dose of aspirin that inhibits TXA$_2$ synthesis without inhibiting PGI$_2$ synthesis. Thus, a low dose might be more antithrombotic because it inhibits PGI$_2$ less than a high dose. Although attempts to identify a dosage (or frequency of usage) of aspirin that blocks TXA$_2$ production without inhibiting PGI$_2$ synthesis have yielded conflicting results,[21-24] use of the lowest effective dose (*ie*, 50 to 100 mg daily for long-term treatment) is probably the most sensible strategy to maximize efficacy and minimize toxicity.

The recently reported Thrombosis Prevention Trial (TPT)[70] employed a novel formulation of controlled-release aspirin (75 mg) designed to minimize the inhibition of vascular PGI$_2$ synthesis by virtue of presystemic acetylation of platelet COX-1.[27] However, because the design of the trial did not include a regular aspirin arm, it is not possible to assess the clinical relevance of absolute vs relative biochemical selectivity in the setting of primary prevention.

Effects of Aspirin Not Related to TXA$_2$: Aspirin has been reported to have effects on hemostasis that are unrelated to its ability to inactivate platelet COX-1. These include dose-dependent inhibition of platelet function,[66-69,71] enhancement of fibrinolysis,[72-74] and suppression of plasma coagulation.[75-78] In contrast to the saturable and well-characterized (*ie*, nanomolar aspirin concentration, rapid time course, physiologic conditions, and single serine modification) inhibition of COX-1 by aspirin,[79,80] the putative mechanisms underpinning the *nonprostaglandin* effects of aspirin on hemostasis are dose-dependent and less clearly defined. For example, the inhibition of shear-induced platelet aggregation depends on the level of aspirin provided, and enhanced fibrinolysis due to N-acetylation of lysyl residues of fibrinogen is seen *in vivo*

with high doses of aspirin (650 mg twice daily)[72] and proceeds more rapidly *in vitro* under nonphysiologic alkaline conditions.[81] Aspirin suppresses plasma coagulation through several mechanisms. The first, initially described by Link and associates in 1943 and confirmed by others,[75,76] is caused by an anti-vitamin K effect of aspirin. It requires very high doses of aspirin and does not contribute to the antithrombotic effect of aspirin when the drug is used in doses up to 1,500 mg/d. The second mechanism is platelet-dependent and is characterized by the inhibition of thrombin generation in a whole-blood system.[77,78] A single dose of 500 mg depresses the rate of thrombin generation, while repeated daily dosing with 300 mg aspirin reduces the total amount of thrombin formed.[82] An interaction with platelet phospholipids, which is blunted in hypercholesterolemia, has been proposed to explain the effects of aspirin on thrombin generation.[82] It is possible (but unproven) that this effect occurs as a consequence of impaired platelet coagulant activity secondary to the inhibition of TXA$_2$-dependent platelet aggregation. It is unknown whether lower doses of aspirin are able to produce this effect. Furthermore, high-dose aspirin therapy can cause abnormal coagulation by direct acetylation of one or more clotting factors. This can be demonstrated in platelet-poor plasma and, thus, is not related to platelet inhibition or vitamin K antagonism.

Additional experimental studies in both animal models and human subjects have detected antithrombotic effects of aspirin that may occur, at least in part, through mechanisms unrelated to inactivation of platelet COX-1. For example, Buchanan et al[68] and Hanson et al,[66] using different animal models, reported that optimal antithrombotic activity of aspirin required doses in excess of those required to inhibit TXA$_2$. Moreover, the results of a subgroup analysis of the North American Symptomatic Carotid Endarterectomy Trial study[83] suggested that aspirin in doses of $\geq$ 650 mg/d might be more effective than $\leq$ 325 mg/d for the prevention of perioperative stroke in patients having carotid artery surgery.[84] Based on these findings, the ACE trial tested the hypothesis that the wide area of collagen exposed by endarterectomy is a sufficiently strong stimulus to platelet aggregation to require a larger dose of aspirin.[2] Thus, approximately 3,000 patients scheduled for carotid endarterectomy were randomly assigned 81, 325, 650, or 1,300 mg aspirin daily, started before surgery, and continued for 3 months. The combined rate of stroke, MI, or death at 3 months was significantly (p = 0.03) lower in the low-dose groups (6.2%) than in the high-dose groups (8.4%) (primary analysis). There were no significant differences between the 81-mg and 325-mg groups, or between the 650-mg and 1,300-mg groups, in any of the secondary analyses of the data.[2] These results underscore the potentially misleading nature of subgroup analyses[85] as well as the weakness of the underlying mechanistic hypothesis being tested, which formed the basis for the design of the ACE trial.

A subgroup analysis of the Physicians' Health Study,[86] based on *post hoc* measurements of baseline plasma C-reactive protein (CRP: the prototypic acute-phase protein, whose serum levels can increase in response to tissue damage, infection, or inflammation) performed in 543

apparently healthy men who subsequently developed MI, stroke, or venous thrombosis, and in 543 study participants who did not report vascular complications, has found that the reduction in the risk of a first MI associated with the use of aspirin (325 mg on alternate days) appears to be directly related to the level of CRP, raising the possibility of anti-inflammatory as well as antiplatelet effects of the drug in cardiovascular prophylaxis.[87] As noted above, the anti-inflammatory effects of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) are largely related to their capacity to inhibit COX-2 activity induced in response to inflammatory cytokines,[18] as these clinical effects can be fully reproduced by highly selective COX-2 inhibitors (coxibs) in patients with rheumatoid arthritis.[88] As shown in Table 4, the dose and time dependence of the effects of aspirin on nucleated inflammatory cells expressing COX-2 vs anucleated platelets expressing COX-1 are markedly different, thus making an anti-inflammatory effect of the drug at a dose of 325 mg every other day pharmacologically implausible given its very short half-life in the human circulation. Finally, aspirin has been reported to modify the way in which neutrophils and platelets[89] or erythrocytes and platelets[90,91] interact, to protect endothelial cells from oxidative stress[92] and to improve endothelial dysfunction in atherosclerotic patients.[93] However, neither the molecular mechanism(s) nor the dose-dependence of these effects has been established clearly.

All of the evidence detailed above suggesting dose-dependent effects for aspirin is indirect and is inconsistent with the failure to show a dose effect in randomized clinical trials and in the overview analysis of the Antithrombotic Trialists.[85] This failure to show a dose effect is the critical point of this discussion, because it correlates with the *saturability* of the aspirin effect on platelet COX-1. For example, in studies with purified enzyme and with isolated platelets, nanomolar concentrations of aspirin will completely block PG synthesis within 20 min after exposure.[65] Higher concentrations and longer exposures will not alter the inhibitory effect of aspirin on PG synthesis because of this *saturable* quality. Exactly the same features (ie, the maximal effect at low doses and the absence of dose effect) are seen in clinical trials with aspirin as an antithrombotic agent. When one raises the dose of aspirin in this situation, no further or additional effect can be appreciated because the critical event has already taken place; namely, the maximal inhibition of platelet TXA_2 synthesis. Thus, the consistency of dose requirements and saturability of the effects of aspirin in acetylating the platelet enzyme,[16] inhibiting TXA_2 production,[25] and preventing atherothrombotic complications[26,65]

constitute the best evidence that aspirin prevents thrombosis through the inhibition of TXA_2 production. It is likely, therefore, that any of the potential effects of aspirin on other determinants of arterial thrombosis are much less important than the inhibition of platelet COX-1 activity.

Aspirin Resistance: Aspirin *resistance* has been used to describe a number of different phenomena, including the inability of aspirin to do the following: (1) to protect individuals from thrombotic complications; (2) to cause a prolongation of the bleeding time; or (3) to produce an anticipated effect on one or more *in vitro* tests of platelet function. A variable proportion of patients (up to one quarter) with cerebrovascular disease only achieve partial inhibition of platelet aggregation at initial testing, and some (up to one third) seem to develop resistance to aspirin over time, even with increasing doses. The results of these long-term studies carried out by Helgason et al[94–96] are at variance with those of a short-term study by Weksler et al[97] showing that 40 mg aspirin daily inhibited platelet aggregation and TXA_2 formation as effectively as higher doses of aspirin in patients who had recently experienced cerebral ischemia. Variable platelet responses to aspirin also have been described in patients with peripheral arterial disease[98] and with ischemic heart disease.[99] In the study of Buchanan and Brister,[99] aspirin *nonresponders* were identified on the basis of bleeding time measurements. Approximately 40% of patients undergoing elective coronary artery bypass grafting showed no prolongation of bleeding time in response to aspirin. This was associated with increased platelet adhesion and 12-hydroxyeicosatetraenoic acid synthesis.[99] In contrast, repeated measurements of platelet aggregation carried out over 24 months of placebo-controlled treatment by Berglund and Wallentin[100] demonstrated that 100 patients with unstable coronary artery disease randomized to receive 75 mg aspirin daily in the RISC (research group on instability in coronary artery disease in southeast Sweden) study[38] had consistently reduced platelet aggregation without attenuation during long-term treatment.

The results of several relatively small studies (n = 39 to n = 180) in stroke patients[101–103] have suggested that aspirin resistance may contribute to lack of response to treatment (ie, recurrent ischemic events while receiving antiplatelet therapy), and that doses of > 500 mg may be more effective than lower doses in limiting this phenomenon. The uncontrolled nature and small sample size of these studies make it difficult to interpret the results. As noted above, a much larger database failed to substantiate

Table 4—Dose-Dependence and Time-Dependence of the Effects of Aspirin on Platelets and Inflammatory Cells

Cellular Target	Enzyme	Single Dose, mg*	Duration of Prostanoid Suppression, h	Cumulative Effects on Repeated Dosing	Daily Dose, mg/d†
Platelets	COX-1	100	24–48	Yes	50–81
Inflammatory cells	COX-2	≥ 650	3–4	No	3,000–5,000

*Dose causing full suppression of prostanoid formation and/or clinically detectable functional effect after single dosing.
†Range of doses shown clinically effective in long-term trials of cardiovascular protection or rheumatoid arthritis.

a dose-dependent effect of aspirin in stroke prevention,[85] an effect that one would theoretically expect if aspirin resistance could be overcome, at least in part, by increasing the daily dose of the drug. The apparent discrepancy between the theoretical predictions originating from studies of aspirin resistance and the actual findings of approximately 50 randomized clinical trials of aspirin prophylaxis in high-risk patients[85] can be reconciled by acknowledging the limitations of platelet function studies. Thus, platelet aggregation, as measured by conventional methods *ex vivo*, has less than ideal intrasubject and intersubject variability and displays limited sensitivity to the effect of aspirin, which often is considered a *weak* antiplatelet agent based on such measurements. Moreover, the relevance of changes in this index of capacity to the actual occurrence of platelet activation and inhibition *in vivo* is largely unknown. Similarly, bleeding time has serious problems of methodologic standardization and is of limited value in predicting hemostatic competence.

In a recent study, Weber et al[104] reported that circulating platelets from healthy subjects express COX-2 protein and messenger RNA, and they suggested that this may represent a factor in aspirin resistance. This finding has been disputed by Patrignani et al.[105] However, incomplete suppression of 11-dehydro-TXB_2 excretion, a noninvasive index of *in vivo* TXA_2 biosynthesis, has been observed episodically in some patients with unstable angina treated with IV low-dose aspirin, despite > 95% suppression of platelet COX-1 activity.[106] COX-2 induction in plaque monocytes/macrophages or activated endothelial cells may contribute to aspirin-insensitive TXA_2 biosynthesis in patients with unstable angina by generating PGH_2 as a substrate for the TX-synthase of the same cell (*constitutive* biosynthesis) or by providing PGH_2 to the TX-synthase of aspirinated platelets (*transcellular* metabolism).[107] The clinical importance of this phenomenon remains to be established.

The coadministration of aspirin and other NSAIDs can lead to pharmacodynamic interactions between the two, which leads to attenuation of the antiplatelet effect of aspirin.[108] Because the ability of aspirin to acetylate a critical serine residue at the apex of the COX channel is dependent on its initial binding to arginine-120,[19] a common docking site for all NSAIDs, the stronger binding affinity of nonaspirin NSAIDs may preclude aspirin from permanently modifying platelet COX-1. Highly selective COX-2 inhibitors (coxibs) are less likely to interfere with the antiplatelet effect of aspirin than conventional NSAIDs because of their limited interaction with platelet COX-1. Finally, it is theoretically possible that polymorphisms and/or mutations in the COX-1 gene affecting Ser529 may represent the structural basis for aspirin resistance in some patients, although this hypothesis remains to be tested.

Thus, in summary, both the mechanism(s) and clinical relevance of aspirin resistance remain to be established. Until its true nature and prevalence are better defined, no test of platelet function is recommended to assess the antiplatelet effect of aspirin in the individual patient.

The Antithrombotic Effect of Aspirin

Prevention of Atherothrombosis in Different Clinical Settings: The efficacy and safety of aspirin are documented from an analysis of > 50 randomized clinical trials that included approximately 100,000 patients at variable risk of thrombotic complications of atherosclerosis. Although a detailed analysis of individual trials is beyond the scope of this article and is more appropriately dealt with in specific clinical sections of this volume, common features of these trials form a basis for general treatment recommendations.

Aspirin has been tested in patients demonstrating the whole spectrum of atherosclerosis, from apparently healthy low-risk individuals to patients presenting with an acute MI or an acute ischemic stroke; similarly, trials have been extended for as short as a few weeks' duration or as long as many years. Although aspirin has been shown consistently to be effective in preventing fatal and/or nonfatal vascular events in these trials, both the size of the proportional effects and the absolute benefits of antiplatelet therapy are somewhat heterogeneous in different clinical settings.

In the Second International Study of Infarct Survival,[59] a dose of a single tablet of aspirin, 162.5 mg, started within 24 h of the onset of symptoms of a suspected MI and continued daily for 5 weeks produced highly significant reductions in the risk of vascular mortality (reduction, 23%), nonfatal reinfarction (reduction, 49%), and nonfatal stroke (reduction, 46%). There was no increase in hemorrhagic stroke or GI bleeding in the aspirin-treated patients and only a small increase in minor bleeding.[59] Thus, aspirin confers conclusive net benefits in the acute phase of evolving MI and should be administered routinely to virtually all patients with suspected acute MI.[109] The treatment of 1,000 such patients with aspirin for 5 weeks will result in approximately 40 patients in whom a vascular event is prevented,[85] with a proportional odds reduction of 30%.

Two separate trials with a similar protocol, the International Stroke Trial[63] and the Chinese Acute Stroke Trial,[64] tested the efficacy and safety of early aspirin use in patients with acute ischemic stroke. Approximately 40,000 patients were randomized within 48 h of the onset of symptoms to 2 to 4 weeks of daily aspirin therapy (300 and 160 mg, respectively) or placebo. As in the Second International Study of Infarct Survival, the fundamental criterion for entry was that the responsible physician was uncertain whether aspirin treatment was indicated for a particular patient. An overview of the results of both trials suggests an absolute benefit of about 10 fewer deaths or nonfatal strokes per 1,000 patients in the first month of aspirin therapy plus an extra 10 patients per 1,000 with a complete recovery. The proportional odds reduction in fatal or nonfatal vascular events is only 10% in this setting. Although the background risk of hemorrhagic stroke was threefold higher in the Chinese Acute Stroke Trial than in the International Stroke Trial, the small absolute increase in this risk associated with early use of aspirin was similar in the two studies (excess of 2 per 1,000 patients).[63,64] The broad clinical implications of these findings are discussed in the article by Albers et al, in this supplement. In terms

of their research implications, these results are consistent with biochemical evidence of episodic platelet activation during the first 48 h after the onset of symptoms of an acute ischemic stroke and with suppression of *in vivo* TXA_2 biosynthesis in patients receiving low-dose aspirin in this setting.[110–112] However, when contrasting the effects of aspirin in acute MI with those in acute stroke, it seems reasonable to assume that TXA_2-driven amplification of the platelet response to acute vascular injury plays a more important role in the coronary than in the cerebrovascular territory.

Long-term aspirin therapy confers a conclusive net benefit on the risk of subsequent MI, stroke, or vascular death among subjects with intermediate-to-high risk of vascular complications. These include patients with chronic stable angina,[39] patients with prior MI,[85] patients with unstable angina,[38,48–50] and patients with TIA or minor stroke,[40,42,47,60–62] as well as other high-risk categories.[85] The proportional effects of aspirin therapy on vascular events in these different clinical settings are rather homogenous, ranging between a 20% and 25% odds reduction based on an overview of all randomized trials.[85] However, individual trial data show substantial heterogeneity, ranging from no statistically significant benefits in patients with peripheral vascular disease[13] to ≥ 50% risk reduction in patients with unstable angina. We interpret these findings as reflecting the variable importance of TXA_2 as a mechanism amplifying the hemostatic response to plaque destabilization in different clinical settings. In terms of absolute benefit, these protective effects of aspirin translate into the avoidance of a major vascular event in 50 patients per 1,000 patients with unstable angina who have been treated for 6 months and in approximately 35 patients per 1,000 patients with prior MI, stroke, or TIA who have been treated for 30 months.[85]

For patients with different manifestations of ischemic heart disease, a widespread consensus exists in defining a rather narrow range of recommended daily doses (*ie*, 75 to 160 mg) for the prevention of MI, stroke, or vascular death.[1,26,65,109] This is supported by separate trial data in > 20,000 patients randomized to treatment with low-dose aspirin or placebo as well as by an overview of all antiplatelet trials showing no obvious dose dependence for the protective effects of aspirin[85] (Table 3).

In contrast, for patients with cerebrovascular disease, a much larger degree of uncertainty still exists about the optimal aspirin dose, with recommendations ranging from 30 to 1,300 mg/d. In particular, a strong case has been put forward by some members of the North-American neurologic community for higher daily doses of aspirin (650 to 1,300 mg) being more effective than lower doses for stroke prevention.[84,113] The evidence supporting this contention is largely based on indirect and selective comparisons of different trial data, mini-meta-analyses, or subgroup analyses of individual trials. In the absence of definitive evidence from direct randomized comparisons of low-dose vs high-dose aspirin therapy in trials of adequate size to detect a moderate difference (one way or the other) between the two, it is reasonable to use the lowest dose of aspirin shown to be effective in patients with transient cerebral ischemia because of safety considerations.[114]

Aspirin has been evaluated in five *primary* prevention trials[39,70,86,115,116] in approximately 53,000 persons at variable cardiovascular risk (Table 5). In the US Physicians' Health Study,[86] among 22,071 healthy physicians, an alternate-day regimen of 325 mg aspirin conferred a statistically significant 44% reduction in risk of first MI. Neither the overall cardiovascular mortality, which was the primary end point of the study, nor the total number of strokes was reduced by long-term aspirin prophylaxis, but there was evidence of a possible increase in the number of hemorrhagic strokes.[86] The British doctors' trial[115] found no statistically significant effects of aspirin, 500 mg daily, but it had a much smaller sample size (5,139) than the US trial. An overview of both trials[117] suggested a highly significant 32% reduction in the risk of first MI, but the data for stroke and cardiovascular mortality were inconclusive. In terms of absolute benefit, the protective effect of aspirin translated into a major vascular event being avoided in 4 healthy physicians per 1,000 treated for 5 years (Fig 1). It should be emphasized that this self-selected group of health-conscious male physicians had an absolute risk of developing a major vascular event, if untreated, of < 1.0% per year, which is much lower than the expected rate in the general population. In the reported TPT,[70] 5,499 men aged between 45 and 69 years were recruited from > 100 general practices in the United Kingdom because they were considered to be at high risk for ischemic heart disease (*ie*, in the top 20 to 25% of the risk score distribution). Low-dose aspirin therapy, 75 mg/d, in the controlled-release formulation discussed above, produced a statistically significant 20% relative reduction in the risk of the primary end point of all ischemic heart disease (*ie*, coronary death and fatal or nonfatal MI), which was almost entirely due to a 32% reduction in nonfatal events. As in the two previous

Table 5—Primary Prevention Trials of Aspirin vs Placebo

Trial	Subjects (No.)	Follow-up, yr	Placebo Event Rate, %/yr	Aspirin RR
UK Doctors[115]	Healthy men (5,139)	5.8	1.4	1.03
US Physicians' Health Study[86]	Healthy men (22,071)	5.0	0.7	0.82
TPT[70]	High-risk men (5,085)	6.3	1.6	0.83
HOT[116]	Hypertensive patients (18,790)	3.8	1.1	0.85
SAPAT[39]	Stable angina patients (2,035)	4.2	3.7	0.71

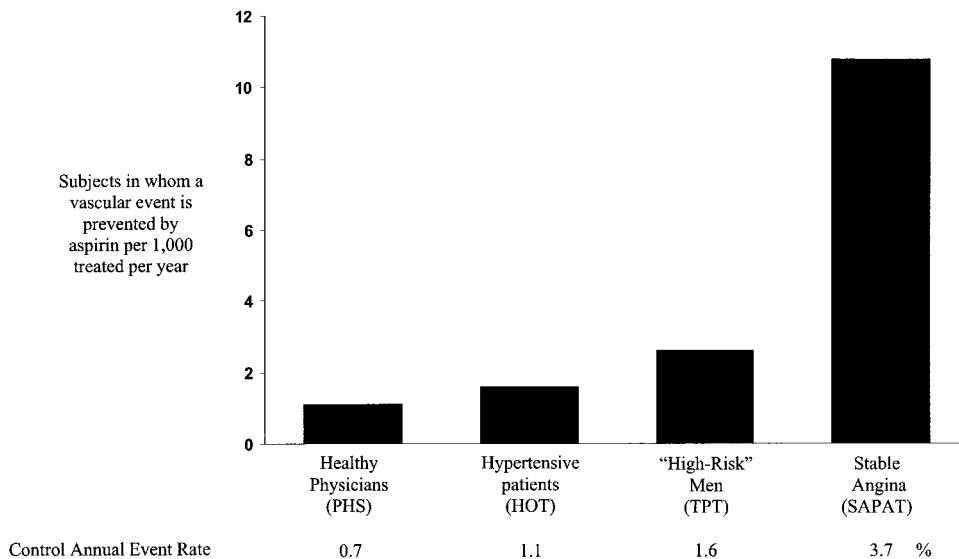RR = relative risk of nonfatal MI, nonfatal stroke or vascular death

FIGURE 1. Absolute benefit of aspirin in primary prevention. Data were calculated from placebo-controlled aspirin trials in different settings characterized by variable cardiovascular risk, as noted on the abscissa. The absolute benefit of aspirin prophylaxis is reported on the ordinate axis as the number of subjects in whom an important vascular event (*ie*, nonfatal MI, nonfatal stroke, or vascular death) is prevented by treating 1,000 subjects with low-dose aspirin for 1 year.

primary prevention trials, neither the total number of strokes nor the overall cardiovascular mortality was modified by aspirin prophylaxis in TPT. Although the men recruited in TPT were selected for being at high risk for ischemic heart disease, their actual risk of developing a major vascular complication, as assessed in the control group, was considerably lower than expected and was only a little more than the rate found in the British doctors' trial (*ie*, approximately 1.5% per year). In this setting, an ischemic cardiac event would be avoided in two high-risk subjects by treating 1,000 such men with aspirin for a year (Fig 1).

Quite similar results were obtained in the Hypertension Optimal Treatment (HOT) study,[116] in which 18,790 male and female patients with intensively treated hypertension were randomly allocated to therapy with aspirin, 75 mg daily, or placebo. Aspirin reduced the incidence of major vascular events by 15% (p = 0.03), and all MI by 36% (p = 0.002), with no effects on stroke or cardiovascular mortality. Because of the low vascular risk of these well-treated hypertensive patients (only about 1.0% per year), a major cardiovascular event would be avoided in one to two patients by treating 1,000 hypertensive men and women with low-dose aspirin therapy for a year (Fig 1). If one compares these absolute benefits of aspirin prophylaxis with those achieved in the primary prevention of MI in patients with stable chronic angina,[39] it becomes apparent that the level of cardiovascular risk in the control population (*ie*, those receiving placebo) represents a major determinant of the absolute benefit of antiplatelet therapy (Fig 1). These results do not support the widespread use of aspirin for primary cardiovascular prophylaxis, because they clearly demonstrate that proper management of modifiable risk factors by current multifactorial strategies

can reduce the actual risk of experiencing a major vascular event to a level at which the additional benefit of aspirin does not clearly outweigh the risk of major bleeding complications (see below).

Additional data assessing the benefit-to-risk ratio of long-term aspirin prophylaxis in apparently healthy persons are currently being collected by the Women's Health Study, an ongoing trial of low-dose aspirin therapy (100 mg every other day) among 40,000 US female health-care professionals.

While the clinical issues related to policy recommendations concerning aspirin in the primary prevention of cardiovascular disease are discussed in detail elsewhere in this supplement, the results of the studies reviewed above do not justify the use of a daily dose of aspirin of > 75 mg when primary prevention with aspirin is considered in the setting of individual clinical judgment by health-care providers.

Atrial Fibrillation: Moderate-dose warfarin alone (international normalized ratio [INR], 2.0 to 3.0) is very effective in reducing the risk of stroke in patients with nonvalvular atrial fibrillation.[118–120] The effectiveness of aspirin in doses between 75 and 325 mg has been compared with that of warfarin and placebo in three randomized trials of patients with nonvalvular atrial fibrillation.[118–122] In one study,[119] aspirin was significantly more effective than placebo, whereas in the other two,[121,122] there was a nonsignificant trend in favor of aspirin. Pooled analysis of the three studies shows a relative risk (RR) reduction in favor of aspirin over placebo of about 25% (range, 14 to 44%). Aspirin was significantly less effective than warfarin in two studies as determined by an intention-to-treat analysis,[121,122] and in the third study by an

efficacy analysis.[119] On pooled analysis, warfarin was significantly more effective than aspirin, with a 47% RR reduction (range, 28 to 61%; p < 0.01).[123] Moreover, adjusted-dose warfarin therapy (INR, 2.0 to 3.0) was more effective than fixed-dose warfarin therapy (INR, 1.2 to 1.5) and aspirin (325 mg/d) in high-risk patients with atrial fibrillation.[124] Thus, aspirin appears to be effective in preventing stroke in patients with atrial fibrillation but is substantially less effective than warfarin. However, aspirin is less expensive, safer, and more convenient than warfarin and may be considered for patients unable to receive anticoagulation therapy or for those with lone atrial fibrillation who have a low risk of stroke.[125,126]

Deep Venous Thrombosis: The PEP trial[3] has now established that aspirin is effective in preventing VTE after surgery for hip fracture. This was a double-blind multicenter study of 13,356 patients undergoing surgery for hip fracture and an additional 4,088 patients undergoing elective hip or knee arthroplasty. Patients were assigned to a regimen of aspirin, 160 mg, or placebo once daily for 5 weeks, with the first dose starting before surgery. Other forms of prophylaxis were allowed, and either heparin or low-molecular-weight heparin (LMWH) was used in about 40% of the patients. Among the 13,356 patients with hip fractures, aspirin produced a 36% reduction in symptomatic deep venous thrombosis (DVT) or pulmonary embolism (PE) (absolute risk reduction, 0.9%; p = 0.0003). A similar RR reduction in patients who were assigned to aspirin therapy was observed in patients who also received heparin. Similar benefits from aspirin were observed at weeks 1, 2, and 3 to 5, with a nonsignificant trend for the risk reduction to be greater after week 1. Surprisingly, PE was a cause of death in < 10% of patients, and aspirin had no effect on total mortality or on other vascular deaths. However, there was an important reduction in fatal PE in patients assigned to aspirin therapy; fatal PE was reduced by 58% (95% CI, 27 to 76%; p = 0.002), from 43 (0.6%) in patients assigned to placebo therapy to 18 (0.3%) in those assigned to aspirin therapy.

Aspirin treatment was associated with a small increase in blood transfusion requirements but not in serious or fatal bleeding. The effect of aspirin on blood transfusion requirements appeared to be greater in those patients also receiving heparin. The beneficial effect of aspirin outweighed its effect on bleeding.

Among the 4,088 elective arthroplasty patients randomly assigned to aspirin or placebo therapy, there was a small and nonsignificant reduction in the incidence of symptomatic DVT or PE observed with aspirin therapy (1.1% vs 1.4%).[3]

This important study, therefore, clearly shows that aspirin reduces the incidence of fatal PE and symptomatic nonfatal DVT or PE in patients with hip fractures. The results of the PEP trial are consistent with the meta-analysis performed by the Antiplatelet Trialists' Collaboration[127] and supersede the findings in most of the previous trials. However, much smaller studies using mandatory venography at or close to hospital discharge indicate that aspirin is not as effective as other forms of prophylaxis. Thus, the overall event rate was high with

aspirin in the studies that used mandatory venography,[128–130] and in indirect comparisons of studies in elective hip surgery, Mohr and associates[128] reported that aspirin use was associated with a pooled incidence of 47% for DVT. Similar conclusions were reached in the analysis by Gallus and associates.[129] The weakness of these two analyses is that they included only a relatively small number of patients treated with aspirin, and the comparisons with other forms of prophylaxis were indirect. However, the indirect comparisons are supported by the results of three randomized studies in patients undergoing major orthopedic surgery comparing aspirin with either warfarin[131] or a LMWH.[132,133] In all three, the incidence of DVT was significantly higher in the aspirin group.

As pointed out by the authors of the PEP trial, the benefit of aspirin is less than with anticoagulants. What then is the role of aspirin in the prevention of VTE?[134] It cannot be recommended for use in combination with LMWH or low-dose heparin until randomized trials show that the possible benefits of such a combination are not outweighed by their risks. It cannot be recommended as a replacement for LMWH or warfarin, because it is less effective than these anticoagulants, although it is likely to be safer and is less expensive. Finally, although the benefits of aspirin continued to be observed after the first week, a definitive recommendation to use aspirin for VTE prophylaxis after hospital discharge cannot be made until such an approach is evaluated in a randomized trial.

Placental Insufficiency: The pathogenesis of preeclampsia and fetal growth retardation is related to reduced placental blood flow, which is believed to be caused by constriction and/or thrombosis of small placental arteries.[135] The initial reports that low-dose aspirin therapy reduces the risk of severe low birth weight among newborns,[136] and the risk of cesarean section in mothers with pregnancy-induced hypertension,[135] led to the widespread use of prophylactic aspirin to prevent preeclampsia. Subsequently, several larger trials reported no beneficial effects of aspirin.[137–142] Although the women in these studies were thought to be at increased risk for preeclampsia, this complication developed in only 2.5 to 7.6% of the women taking placebo. The National Institute of Child Health and Human Development Network of Maternal-Fetal Medicine Units completed a large trial in > 2,500 pregnant women to test the hypothesis that aspirin, 60 mg/d, reduces the incidence of preeclampsia in women at high risk for the disease.[143] Twenty percent of the placebo-treated women did develop preeclampsia during the study. However, aspirin therapy did not reduce the incidence of this maternal complication or improve perinatal outcomes. On a positive note, several months of low-dose aspirin treatment was not associated with adverse consequences for either the mothers or the neonates, there being no increase in abruptio placentae, postpartum hemorrhage, or neonatal intraventricular hemorrhage.[143]

Thus, although aspirin may reduce the risk of preeclampsia by 10 to 15%, this is not reflected in a substantive clinical benefit, such as reduction in perinatal death or intrauterine growth restriction.[144] Whether aspirin given early in the first trimester is more effective in preventing

preeclampsia remains unanswered. Also, the potential involvement of extraplatelet sources of vasoactive eicosanoids expressing COX-2 in response to a local growth-promoting milieu might contribute, at least in part, to the lack of response to low-dose aspirin therapy in this setting.

Adverse Effects of Aspirin

Aspirin does not cause a generalized bleeding abnormality, unless it is given to patients with an underlying hemostatic defect, such as hemophilia, uremia, or that induced by anticoagulant therapy. Aspirin-induced impairment of primary hemostasis cannot be separated from its antithrombotic effect and is similarly independent of the dose.

The balance between preventing vascular occlusion and causing excess bleeding with aspirin depends critically on the absolute thrombotic vs hemorrhagic risk of the patient. Thus, in individuals at very low risk for vascular occlusion (eg, 1% per year), a very small absolute benefit is offset by the exposure of a large number of healthy subjects to undue bleeding complications. In contrast, in patients at high risk for cardiovascular or cerebrovascular complications (eg, > 5% per year), the substantial absolute benefit of aspirin prophylaxis clearly outweighs the risk (Table 6). For example, the absolute excess of major bleeds (ie, those requiring transfusion) in acute MI is approximately 1/100th the absolute number of major vascular events avoided by aspirin therapy.[85]

Hypertension often has been considered a contraindication to aspirin therapy because of the concern that possible benefits in the prevention of cardiovascular events may be counterbalanced by an increased risk of cerebral bleeding. The results of the aspirin component of the HOT study[116] are reassuring in this regard, since

Table 6—Benefit/Risk Ratio of Antiplatelet Prophylaxis With Aspirin in Different Settings

Clinical Setting	Benefit*	Risk†
Men at low to high cardiovascular risk	1–2	1–2
Essential hypertension	1–2	1–2
Chronic stable angina	10	1–2
Prior MI	20	1–2
Unstable angina	50	1–2

*Benefits are calculated from randomized trial data reviewed in this article and depicted in Figures 1 and 2. Values are given as the No. of patients in whom a major vascular event is avoided per 1,000 patients per year.

†Risks of upper GI bleeding are estimated from a background rate of 1 event per 1,000 per year in the general population of nonusers[146] and an RR of 2.0 to 3.0 associated with aspirin prophylaxis.[146, 147] Such an estimate assumes comparability of other risk factors for upper GI bleeding, such as age and concomitant use of NSAIDs, and may actually underestimate the absolute risk in an elderly population exposed to primary prevention. The absolute excess of major bleeding complications in the primary prevention trials reviewed in Table 5 ranged between 0.3 and 1.7 per 1,000 patient-years. Values are given as the No. of patients in whom a major GI bleeding event is caused per 1,000 patients per year.

hypertensive patients whose BP was well-controlled were protected from MI by aspirin without an increase in cerebral bleeding or strokes.

The overall risk of major extracranial and intracranial bleeding associated with antiplatelet drugs is difficult to assess in individual trials because the incidence is low (ie, < 1% per year), making detection of even a 50 to 60% relative increase in risk unrealistic in most trials of a few thousand patients.

Aspirin-induced GI toxicity, as detected in randomized clinical trials, appears to be dose related in the range of 30 to 1,300 mg daily.[145] This is based largely on indirect comparisons of different trials and on a limited number of randomized, direct comparisons of different aspirin doses, as reviewed above. Such a dose-response relationship is thought to reflect at least two COX-1-dependent components, ie, dose-dependent inhibition of COX-1 in the GI mucosa and dose-independent (within the range of examined doses) inhibition of COX-1 in platelets.[26] Thus, it is not surprising that the antithrombotic effect of aspirin can be dissociated, at least in part, from its most common side effect. However, even when administered at low doses, aspirin can cause serious GI bleeding, as reported in studies using 30 to 50 mg daily.[42,43] Because of the underlying prevalence of gastric mucosal erosions related to concurrent use of other NSAIDs and/or *Helicobacter pylori* infection in the general population, it should be expected that any antiplatelet dose of aspirin will cause more bleeding from preexisting lesions than a placebo. Consistent with this mechanistic interpretation, the RR of hospitalization due to upper GI bleeding and/or perforation associated with low-dose aspirin therapy (mostly, 100 to 300 mg daily) (RR, 2.3; 95% confidence interval [CI], 1.7 to 3.2) is comparable to that of other antiplatelet agents (RR, 2.0; 95% CI, 1.4 to 2.7) and anticoagulants (RR, 2.2; 95% CI, 1.4 to 3.4) in a large population-based observational study.[146] A case-control study[147] with hospital and community control subjects has examined the risks of hospitalization for bleeding peptic ulcer associated with three different regimens of aspirin prophylaxis. Odds ratios were raised for all doses of aspirin taken as follows: 75 mg, 2.3 (95% CI, 1.2 to 4.4); 150 mg, 3.2 (95% CI, 1.7 to 6.5); 300 mg, 3.9 (95% CI, 2.5 to 6.3). It has been calculated that approximately 900 of the 10,000 episodes of ulcer bleeding occurring in people > 60 years of age each year in England and Wales could be associated with, and ascribed to, prophylactic aspirin use.[147] A general change to lower doses (75 mg) of aspirin would not eliminate risks but, if these figures are soundly based, would reduce risk by about 40% compared with 300 mg, and by 30% compared with 150-mg doses.[147] Given that the mortality rate among patients who are hospitalized for NSAID-induced upper GI bleeding is about 5 to 10%,[148] such a strategy could save a significant number of lives.

The widely held belief that enteric-coated and buffered varieties of aspirin are less likely to occasion major upper GI bleeding than plain tablets was tested in data from a multicenter case-control study.[149] The RRs of upper GI bleeding for plain, enteric-coated, and buffered aspirin at average daily doses of ≤ 325 mg were 2.6, 2.7, and 3.1, respectively. At doses of > 325 mg, the RR was 5.8 for

plain aspirin and 7.0 for buffered aspirin; there were insufficient data to evaluate enteric-coated aspirin at this dose level.[149] Thus, physicians who recommend aspirin in an enteric-coated or buffered form should not assume that these formulations are less likely to cause GI tract bleeding than plain aspirin.

Suppressing acid secretion is thought to reduce the risk of ulcers associated with regular use of NSAIDs. In patients who required continuous treatment with NSAIDs and who had ulcers or > 10 erosions in either the stomach or duodenum, omeprazole healed and prevented ulcers more effectively than did ranitidine.[150] In these patients, maintenance therapy with omeprazole was associated with a lower rate of relapse and was better tolerated than misoprostol.[151] However, whether suppressing acid secretion might reduce GI toxicity associated with low-dose aspirin remains to be established.

Substantially less information is available concerning the risk of intracranial hemorrhage associated with aspirin use. In the Nurses' Health Study cohort of approximately 79,000 women aged 34 to 59 years, the infrequent use of aspirin (1 to 6 tablets per week) was associated with a reduced risk of ischemic stroke, while high frequency of use (ie, ≥ 15 aspirin per week) was associated with increased risk of subarachnoid hemorrhage, particularly among older or hypertensive women.[152] In the overview of the Antithrombotic Trialists' Collaboration,[85] the absolute excess of intracranial hemorrhage due to aspirin therapy is < 1 per 1,000 patients per year in high-risk trials, with somewhat higher risks in patients with cerebrovascular disease.

Low-dose aspirin therapy has not been reported to affect renal function or BP control,[153] consistent with its lack of effect on renal PG synthesis.[154] Moreover, aspirin therapy, 75 mg daily, did not affect BP or the need for antihypertensive therapy in intensively treated hypertensive patients.[116] The suggestion that the use of aspirin and other antiplatelet agents is associated with reduced benefit from enalapril in patients with left ventricular systolic dysfunction[155] is not supported by the results of a large meta-analysis of MI trials.[156]

Thus, in summary, the inhibition of TXA_2-dependent platelet function by aspirin may lead to the prevention of thrombosis as well as to excess bleeding. Assessing the net effect requires an estimation of the absolute thrombotic vs hemorrhagic risk of the individual patient. In individuals at very low risk for vascular occlusion, a very small absolute benefit may be offset by exposure of very large numbers of healthy subjects to undue bleeding complications. As the risk of experiencing a major vascular event increases, so does the absolute benefit of antiplatelet prophylaxis with aspirin, as shown in Figure 2, for a number of clinical settings in which the efficacy of the drug has been tested in randomized clinical trials. Based on the results of > 50 such trials that are discussed elsewhere in this volume, the antithrombotic effect of aspirin does not appear to be dose-related over a wide range of daily doses (30 to 1,300 mg), an observation that is consistent with the saturability of platelet COX inhibition at very low doses. In contrast, GI toxicity of the drug does appear to be dose-related, which is consistent with dose-dependent and dosing interval-dependent inhibition of COX activity in the nucleated lining cells of the GI mucosa. Thus, aspirin once daily is recommended in all clinical conditions in which antiplatelet prophylaxis has a favorable benefit/risk profile. Because of GI toxicity and its potential impact on compliance,
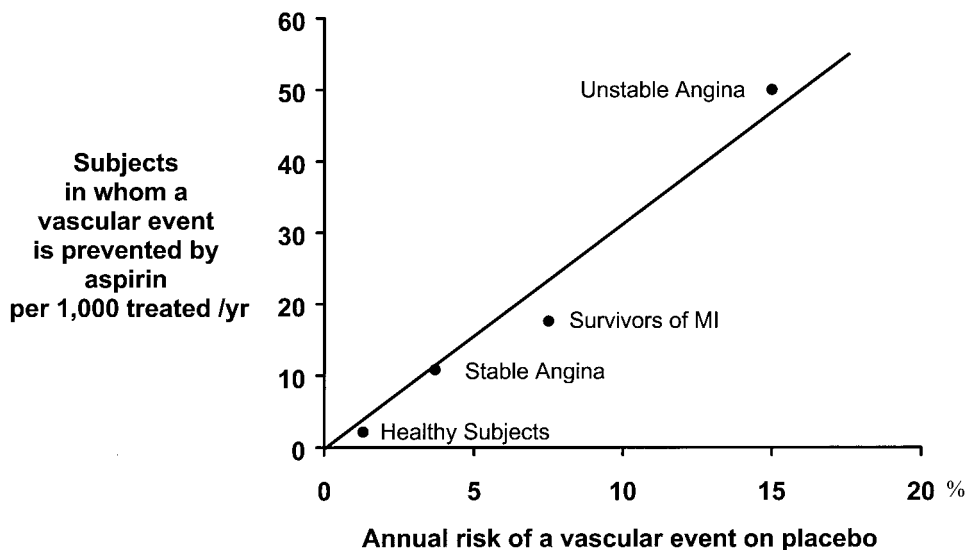

FIGURE 2. The absolute risk of vascular complications is the major determinant of the absolute benefit of antiplatelet prophylaxis. Data are plotted from placebo-controlled aspirin trials in different clinical settings. For each category of patients, the abscissa denotes the absolute risk of experiencing a major vascular event as recorded in the placebo arms of the trials. The absolute benefit of antiplatelet treatment is reported on the ordinate axis as the number of subjects in whom an important vascular event (ie, nonfatal MI, nonfatal stroke, or vascular death) is actually prevented by treating 1,000 subjects with aspirin for 1 year.

physicians are encouraged to use the lowest dose of aspirin that was shown to be effective in each clinical setting.

Reversible COX Inhibitors

A variety of NSAIDs can inhibit TXA_2-dependent platelet function through competitive, reversible inhibition of platelet COX-1.[157] In general, these drugs, when used at conventional analgesic dosages, inhibit reversibly platelet COX activity by 70 to 90%. This level of inhibition may be insufficient to block adequately platelet aggregation *in vivo* because of the very substantial biosynthetic capacity of human platelets to produce TXA_2.[158,159] In fact, in a prospective population-based cohort study of approximately 165,000 postmenopausal women, the long-term use of nonaspirin NSAIDs was not associated with a protective effect against the risk of a first MI (RR, 1.32; 95% CI, 0.97 to 1.81).[160]

The only reversible COX inhibitors that have been tested in randomized clinical trials for their antithrombotic efficacy are sulfinpyrazone, indobufen, flurbiprofen, and triflusal. Sulfinpyrazone is a uricosuric agent structurally related to the anti-inflammatory agent phenylbutazone. When used at the highest approved dosage of 200 mg qid, the drug inhibits platelet COX activity by approximately 60%, after conversion from an inactive sulfoxide to an active sulfide metabolite.[161] The conflicting or negative results obtained in randomized clinical trials of sulfinpyrazone in patients with MI or unstable angina[85] (reviewed in article by Cairns et al, in this volume) are not surprising in light of the drug being a weak COX inhibitor with no other established antiplatelet mechanism of action.

In contrast, indobufen is a very potent inhibitor of platelet COX activity and has comparable biochemical, functional, and clinical effects to those of a standard dose of aspirin. Thus, at therapeutic plasma levels achieved after oral dosing of 200 mg bid, indobufen inhibits serum TXB_2 by > 95% throughout the dosing interval[162] and reduces urinary TXA_2 metabolite excretion to an extent quite comparable to aspirin.[163] The finding that indobufen is as effective as aspirin in preventing coronary graft occlusion in two randomized trials[164,165] is mechanistically consistent with the concept of platelet COX inhibition, which largely accounts for the antithrombotic effect of aspirin, as discussed above. Indobufen also has been investigated in a small placebo-controlled study of patients with heart disease at increased embolic risk[166] and compared with warfarin[167] and ticlopidine[168] in patients with nonrheumatic atrial fibrillation and in patients with recent reversible cerebral ischemia, respectively. However, none of these studies, which comprised > 4,000 patients, clearly established an advantage of indobufen vs standard treatments, although the CIs for these comparisons are wide. Indobufen has been reported to suppress *in vivo* TXA_2 biosynthesis more effectively than low-dose aspirin therapy in patients with unstable angina, an effect possibly related to the inhibition of monocyte COX-2 by therapeutic plasma levels of indobufen.[20] The clinical relevance of these findings remains to be established.

Flurbiprofen has been evaluated in a single placebo-controlled, randomized trial of 461 patients who had experienced acute MIs.[169] The 6-month reinfarction rate was significantly lower in the flurbiprofen group (3%) than in the placebo group (10.5%), with an extremely low mortality rate (1.1%) in both groups. The small sample size of this study limits the interpretation of these potentially interesting findings.

Triflusal, a salicylic acid derivative, reversibly inhibits platelet COX activity after conversion to a long-lived metabolite, 2-hydroxy-4-trifluoromethyl-benzoic acid.[170] While the half-life of the parent compound is only about 30 min, that of the deacetylated metabolite approximates 2 days. Although triflusal is claimed to have negligible effects on vascular PGI_2 production, this is likely to reflect the experimental conditions used for the assessment of PGI_2 production *ex vivo*. Several clinical trials comparing triflusal and aspirin are currently ongoing.

None of these reversible COX inhibitors is approved as an antiplatelet drug in the United States, and it is unclear under what circumstances they are prescribed instead of aspirin in other countries. Because nonaspirin NSAIDs have been investigated inadequately in terms of their potential cardiovascular effects, physicians prescribing these drugs to arthritic patients with prior vascular complications should not discontinue treatment with low-dose aspirin, even though concomitant administration of the two may amplify the risk of upper GI bleeding. The cardiovascular safety of selective COX-2 inhibitors (coxibs) in arthritic patients at low cardiovascular risk is currently being debated, based on the recently reported results of two relatively large GI safety studies (VIGOR, 170a and CLASS, 170b) with short follow-up and inadequate statistical power to detect a realistic difference, one way or the other, in vascular end points between coxibs and conventional NSAIDs.

Dipyridamole

Dipyridamole is a pyrimidopyrimidine derivative with vasodilator and antiplatelet properties. The mechanism of action of dipyridamole as an antiplatelet agent has been a subject of controversy.[171] Both the inhibition of cyclic nucleotide phosphodiesterase (the enzyme that degrades cyclic adenosine monophosphate [AMP] to 5′-AMP, resulting in the intraplatelet accumulation of cyclic AMP, a platelet inhibitor) and the blockade of the uptake of adenosine (which acts at A_2 receptors for adenosine to stimulate platelet adenylyl cyclase and, thus, increases the level of cyclic AMP) have been suggested. Moreover, direct stimulation of PGI_2 synthesis and protection against its degradation have been reported, although the dipyridamole concentrations required to produce these effects far exceed the low-micromolar plasma levels achieved after the oral administration of conventional doses (100 to 400 mg daily).[171]

The absorption of dipyridamole from conventional formulations is quite variable and may result in low systemic bioavailability of the drug. A modified-release formulation of dipyridamole with improved bioavailability has been developed in association with low-dose aspirin.[172] Dipyridamole is eliminated primarily by biliary excretion as a glucuronide conjugate and is subject to enterohepatic

recirculation. A terminal half-life of 10 h has been reported. This is consistent with the twice-daily regimen used in recent clinical studies.

Although the clinical efficacy of dipyridamole, alone or in combination with aspirin, has been questioned on the basis of earlier randomized trials,[1,171] the whole issue has been reopened by the results of ESPS-2.[42] In that study of 6,602 patients who had experienced prior strokes or TIAs, stroke risk in comparison to placebo was reduced by 18% with low-dose aspirin therapy (25 mg bid) (p = 0.013), 16% with dipyridamole alone (200 mg bid) (p = 0.039), and 37% with aspirin plus dipyridamole (p < 0.001). The corresponding RR reductions for the outcome of stroke or death were 13% (p = 0.016), 15% (p = 0.015), and 24% (p < 0.001), respectively.[42] Headache was the most common adverse effect of dipyridamole. Bleeding at any site was almost doubled in the two aspirin arms but was surprisingly indistinguishable from placebo in the dipyridamole-treated patients.[42]

The ESPS-2 study has been criticized for the continued inclusion of a placebo arm after the place of aspirin in the secondary prevention of stroke had been established to the satisfaction of most authorities. Whether the favorable results obtained in ESPS-2 reflect the higher dose (400 vs 225 mg daily) and improved systemic bioavailability of modified-release dipyridamole therapy compared with conventional formulations, or whether they reflect the substantially larger sample size and statistical power of the study compared with previous trials, remains to be established. The combination of modified-release dipyridamole and low-dose aspirin therapy has been recently approved by the US Food and Drug Administration (FDA).

Thienopyridines

Ticlopidine and clopidogrel are structurally related thienopyridines with platelet inhibitory properties. Both drugs selectively inhibit adenosine diphosphate (ADP)-induced platelet aggregation with no direct effects on arachidonic acid metabolism.[173] Although ticlopidine and clopidogrel also can inhibit platelet aggregation induced by collagen and thrombin, these inhibitory effects are abolished by increasing the agonist concentration and, therefore, are likely to reflect blockade of ADP-mediated amplification of the platelet response to other agonists.

Neither ticlopidine nor clopidogrel affects ADP-induced platelet aggregation when added *in vitro* up to 500 μM, thus suggesting that *in vivo* hepatic transformation to an active metabolite is necessary for their antiplatelet effects. Platelets contain two well-characterized receptors for ADP, a ligand gated ion channel (P2X$_1$) and a G-protein-linked receptor that affects platelet shape change and contributes to platelet aggregation (P2Y$_1$). Several lines of evidence suggest that clopidogrel, and most likely also ticlopidine, induce irreversible alterations of a presumed third ADP receptor that is postulated to mediate the inhibition of stimulated adenylyl cyclase activity (P2T$_{AC}$).[173] The inhibition of platelet function by clopidogrel is associated with a selective reduction in the number of ADP binding sites, with no consistent change in the binding affinity. An active metabolite of clopidogrel

has recently been described.[174] The irreversible modification of this ADP receptor site could be explained by the formation of a disulfide bridge between the reactive thiol group of the active metabolite of clopidogrel and that of a cysteine residue of the platelet (P2T$_{AC}$) ADP receptor.[174] The hypothesis of permanent modification of a putative ADP receptor by thienopyridines is consistent with time-dependent cumulative inhibition of ADP-induced platelet aggregation on repeated daily dosing with ticlopidine or clopidogrel and with slow recovery of platelet function after drug withdrawal.[173]

Up to 90% of a single oral dose of ticlopidine is rapidly absorbed in humans.[173] Peak plasma concentrations occur 1 to 3 h after a single oral dose of 250 mg. Plasma levels of ticlopidine increase by approximately threefold on repeated twice-daily dosing over a period of 2 to 3 weeks because of drug accumulation. Greater than 98% of ticlopidine is reversibly bound to plasma proteins, primarily albumin. Ticlopidine is metabolized rapidly and extensively. A total of 13 metabolites have been identified in humans. Of these, only the 2-keto derivative of ticlopidine is more potent than the parent compound in inhibiting ADP-induced platelet aggregation.[173]

The apparent elimination half-life of ticlopidine is 24 to 36 h after a single oral dose and up to 96 h after 14 days of repeated dosing.[173] The recommended regimen of ticlopidine is 250 mg bid, although it is unclear how a twice-daily regimen is related to the pharmacokinetic and pharmacodynamic features noted above. A delayed antithrombotic effect was noted in at least one clinical trial of ticlopidine, in patients with unstable angina, with no apparent protection during the first 2 weeks of drug administration.[175] Therefore, ticlopidine is not useful when a rapid antiplatelet effect is required.

Ticlopidine as a single agent has been evaluated in patients who experienced stroke,[176] transient cerebral ischemia,[177] unstable angina,[175] and intermittent claudication,[178–180] and in patients undergoing aortocoronary bypass surgery.[181] Ticlopidine was significantly more effective than aspirin in reducing the incidence of stroke in patients who have experienced transient cerebral ischemia or minor stroke,[177] although there was no statistically significant difference in the combined outcome of stroke, MI, or death[85]; was more effective than placebo in reducing the risk of the combined outcome of stroke, MI, or vascular death in patients who experienced thromboembolic stroke[176]; was more effective than conventional antianginal therapy in reducing vascular death or MI in patients with unstable angina[175]; was more effective than placebo in reducing acute occlusion of coronary bypass grafts[181]; and was more effective than control in improving walking distance[179] and reducing vascular complications in patients with peripheral vascular disease.[178–180] The association of ticlopidine therapy with hypercholesterolemia and neutropenia (for which the reported rate of occurrence is 2.4% for neutrophil counts < 1.2 × 10^9/L and 0.8% for neutrophil counts < 0.45 × 10^9/L) and its comparative expense has reduced enthusiasm for this therapy as an alternative to aspirin in most situations.[182] Ticlopidine also has been associated with thrombocytopenia,[182] aplastic anemia,[183] and thrombotic thrombocytopenic purpura

(TTP).[184] Ticlopidine has been approved for clinical use in patients with cerebral ischemia when aspirin has failed, cannot be tolerated, or is contraindicated, although this limitation does not apply to all countries where the drug is registered.

The additive effects of ticlopidine and aspirin have been described in rats, in inhibition of ADP-induced platelet aggregation *ex vivo*, tail bleeding time prolongation, and protection from thrombosis in experimental models of platelet-dependent vascular occlusion.[185] Additive anti-platelet effects of aspirin, 40 mg, and ticlopidine, 250 mg, have been reported in healthy volunteers.[186] Several studies have demonstrated the superiority of ticlopidine with aspirin compared to aspirin alone, or aspirin plus warfarin, in preventing thrombotic complications after coronary artery stent placement.[187,188] Ticlopidine has been used routinely in combination with aspirin in patients receiving coronary artery stents, but the better safety profile of clopidogrel has resulted in the substitution of clopidogrel for ticlopidine in many centers (see below). The risk of TTP associated with ticlopidine use has been estimated as 0.02% in patients receiving the drug after stent placement.[189] This compares with an incidence of 0.0004% in the general population. The mortality rate for this rare complication exceeds 20%.[189]

The pharmacokinetics of clopidogrel are somewhat different from those of ticlopidine. Thus, after the administration of single oral doses (up to 200 mg) or repeated doses (up to 100 mg daily), unchanged clopidogrel was not detectable in peripheral venous plasma.[190] Concentrations of 1 to 2 ng/mL were measured in the plasma of patients who received 150 mg/d clopidogrel (twice as much as the dose used in the Clopidogrel vs Aspirin in Patients at Risk of Ischemic Events [CAPRIE] study[191] and approved for clinical use) for 16 days. The main systemic metabolite of clopidogrel is the carboxylic acid derivative, SR 26334. Based on measurements of circulating levels of SR 26334, it has been inferred that clopidogrel is rapidly absorbed and extensively metabolized.[190] The plasma elimination half-life of SR 26334 is approximately 8 h. As noted above, clopidogrel, inactive *in vitro*, is metabolically transformed by the liver into a short-lived active platelet inhibitor, the structure of which recently was elucidated.[174]

Clopidogrel inhibited ADP-induced platelet aggregation in a dose-dependent fashion with an apparent ceiling effect (40% inhibition) at 400 mg after single oral doses in healthy volunteers. The inhibition of platelet aggregation was detectable 2 h after oral dosing of 400 mg and remained relatively stable up to 48 h.[190] On repeated daily dosing of 50 to 100 mg clopidogrel to healthy volunteers, ADP-induced platelet aggregation was inhibited from the second day of treatment (25 to 30% inhibition) and reached a steady-state (ie, 50 to 60% inhibition) after 4 to 7 days. Such a level of maximal inhibition was comparable to that achieved with ticlopidine (500 mg daily), although the latter showed a slower onset of the antiplatelet effect than clopidogrel. No appreciable differences in the maximum inhibitory effects of 50, 75, and 100 mg clopidogrel were noted in this study, suggesting that 50 mg daily may be at or close to the top of the dose-response curve. It is interesting to note that 50 mg is only about 12% of the

dose of clopidogrel necessary to achieve the maximal inhibition of ADP-induced platelet aggregation after single dosing. As would be expected from these pharmacokinetic and pharmacodynamic features, a loading dose (*eg*, 300 mg) of clopidogrel results in a much more rapid onset of platelet inhibition than is achieved with the 75-mg dose.[192] This approach currently is being used in the ongoing CURE trial of clopidogrel plus aspirin vs aspirin alone in patients with unstable coronary syndromes.

The most likely interpretation of the above findings is that the active metabolite of clopidogrel has a pharmacodynamic pattern quite similar to that of aspirin, in causing the cumulative inhibition of platelet function on repeated daily administration of low doses.[157] As in the case of aspirin, platelet function returned 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 a permanent defect in a platelet protein that cannot be repaired during the 24-h dosing interval and can only be replaced as a function of platelet turnover. This also justifies the once-daily regimen of both drugs, despite their short half-life in the human circulation.

Bleeding time measurements performed in the same multiple-dose study described above showed a comparable prolongation (by 1.5-fold to 2.0-fold over control measurements) with clopidogrel, 50 to 100 mg daily, or ticlopidine, 500 mg daily.[190]

Clopidogrel has undergone a quite unusual clinical development, with very limited phase II studies and a single, very large phase III trial (ie, CAPRIE) to test its efficacy and safety at a dosage of 75 mg daily vs aspirin, 325 mg daily.[191] CAPRIE is unique among the studies that have directly compared antiplatelet agents to aspirin in that it incorporated the following three groups of patients, all of whom are recognized to be at an increased risk for recurrent ischemic events: patients who have experienced a recent stroke; patients who have experienced a recent MI; and patients presenting with symptomatic peripheral arterial disease.

Overall, CAPRIE showed a modest difference in effectiveness between aspirin and clopidogrel; the annual event rate calculated for aspirin was 5.83% compared with 5.32% for clopidogrel, a RR reduction of 8.7% (95% CI, 0.3 to 16.5%; p = 0.043).[191] What is particularly interesting, however, are the results obtained when the effects of aspirin and clopidogrel in the three separate groups of patients that were recruited into the study are compared. Each of these comparisons between clopidogrel and aspirin involved approximately 6,400 patients and, therefore, represents the largest head-to-head comparison between aspirin and another antiplatelet agent in that particular clinical setting, although the statistical power of such comparison is inadequate to detect a modest difference between the two. This analysis shows that the majority of the difference in effectiveness occurred in the patients who entered the trial because of symptomatic peripheral arterial disease. A 23.8% reduction in RR (95% CI, 8.9 to 36.2%; p = 0.0028) over aspirin was seen in the clopidogrel-treated patients who had peripheral arterial dis-

ease, while a nonsignificant 7.3% reduction in RR (95% CI, −5.7 to 18.7%; p = 0.26) and a nonsignificant 3.7% increase in risk (95% CI, −22.1 to 12.0%; p = 0.66) were obtained in the clopidogrel-treated groups of stroke and MI patients, respectively. A formal test of heterogeneity of these three treatment effects was statistically significant (p = 0.042), suggesting that the true benefit of clopidogrel may not be identical across the three clinical settings. Although it is possible that such a differential effect may reflect the play of chance, the possibility also exists that TXA_2 and ADP may be equally important in amplifying the platelet response to plaque destabilization when this occurs in the absence of peripheral arterial disease, while ADP may be the key player in platelet activation when it occurs in the presence of peripheral arterial disease. This working hypothesis is consistent with the results of direct and indirect comparisons of aspirin and ticlopidine in similar clinical settings.[85]

Both clopidogrel and medium-dose aspirin therapy were well-tolerated in the CAPRIE study.[191] The frequency of severe rash and severe diarrhea was higher with clopidogrel than with aspirin, while GI discomfort and hemorrhage were more frequent with aspirin than with clopidogrel. No excess neutropenia was found in the clopidogrel group. Based on these findings, clopidogrel has been approved for the reduction of atherosclerotic events in patients who experienced recent strokes or recent MIs, or who have established peripheral arterial disease. TTP can occur after the initiation of clopidogrel therapy. When it occurs, it is often within the first 2 weeks of treatment.[193]

The complementary mechanism of action and safety of clopidogrel and low-dose aspirin therapy suggest that their combination may produce additive antithrombotic effects in a high-risk setting with an acceptable safety margin.[194] Studies are ongoing to test this hypothesis (eg, CURE). Proof of concept is provided by relatively small, short-term studies of aspirin and clopidogrel after coronary stenting.[4–7] A new class of direct $P2T_{AC}$ antagonists (eg, AR-C69931MX) currently is being developed that appears to block this ADP receptor more effectively than clopidogrel.[195]

Integrin αIIbβ3 (GPIIb/IIIa) Receptor Antagonists

Given the redundancy 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 the expression of functionally active integrin αIIbβ3 (GPIIb/IIIa) on the platelet surface is the final common pathway of platelet aggregation, regardless of the initiating stimulus, this glycoprotein has become the target of novel antiplatelet drugs.[196,197] The inhibitors of GPIIb/IIIa include a monoclonal antibody against the receptor, the naturally occurring Arg-Gly-Asp sequence (RGD) containing peptides isolated from snake venoms, synthetic RGD, or Lys-Gly-Asp sequence containing peptides, as well as peptidomimetic and non-peptide RGD mimetics that compete with fibrinogen, von Willebrand factor, and/or perhaps other ligands for occupancy of the platelet receptor. Recent reviews of the subject are available.[198–200]

Blockade of GPIIb/IIIa receptors by a murine monoclonal antibody such as 7E3 essentially induces a functional thrombasthenic phenotype.[201] Approximately 40,000 antibody molecules bind to the surface of platelets, but since they probably bind bivalently, there are probably 80,000 GPIIb/IIIa receptors per platelet.[196] Platelet aggregation is significantly inhibited at antibody doses that decrease the number of available receptors to < 50% of normal. Platelet aggregation is nearly completely abolished at approximately 80% receptor blockade, but the bleeding time is only mildly affected at this level of receptor blockade. It is only with > 90% receptor blockade that the bleeding time becomes extremely prolonged.[196] Because of concerns about immunogenicity of the original 7E3 antibody, a mouse/human chimeric 7E3 Fab (abciximab) was created for clinical development.

Pharmacokinetic data on abciximab indicate that following IV 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 spleen.[202] After bolus injection of abciximab, a dose-dependent inhibition of ADP-induced platelet aggregation was recorded in patients judged to be at moderate to high risk of percutaneous transluminal coronary angioplasty (PTCA)-associated ischemic complications.[202] A bolus dose of 0.25 mg/kg was found to result in the blockade of > 80% of platelet receptors and to reduce platelet aggregation in response to 20 μM ADP to < 20% of baseline. A steep dose-response curve was apparent in this study.[202] Peak effects on receptor blockade, platelet aggregation, and bleeding time were observed at the first sampling time of 2 h after bolus administration of 0.25 mg/kg. Gradual recovery of platelet function then occurred over time, with bleeding times returning to near-normal values by 12 h.[202] Platelet aggregation in response to 20 μM ADP returns to ≥ 50% of baseline within 24 h in most patients and within 48 h in nearly 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.[203]

The receptor blockade, inhibition of platelet aggregation, and prolongation of bleeding time produced by administering a 0.25 mg/kg-bolus dose of abciximab could be maintained for 12 h by administering a 10-μg/min infusion during that time period.[202] This regimen was chosen for a phase III trial (EPIC)[204] that demonstrated the clinical efficacy of abciximab, when added to conventional antithrombotic therapy, in reducing the incidence of ischemic events in patients undergoing PTCA (discussed in the article by Popma et al). Although the 6-month follow-up of patients in the EPIC study suggested a potential effect of abciximab on clinical restenosis,[205] the results of several subsequent studies did not support this finding.[206]

Major bleeding was significantly increased in abciximab-treated patients in EPIC.[204] Subsequently, however, it was found that a reduction in the dosage of concomitant heparin and more rapid sheath removal can greatly reduce the bleeding complications attendant to abciximab administration.[206] Besides hemorrhage, thrombocytopenia represents an important side effect of abciximab treatment. Approximately 1 to 2% of patients treated with abciximab develop platelet counts $< 50,000/\mu L$, of which approximately 0.5 to 1% reflect very rapid decreases (beginning within 2 h of administration) due to abciximab. The abciximab package insert specifies that a platelet count should be obtained 2 to 4 h after initiating therapy, thus permitting the rapid identification of patients who are developing thrombocytopenia. Thus far, all reports indicate that the thrombocytopenia can be treated effectively by stopping the drug therapy, and is reversible, with recovery occurring over several days.[204,206–208] In the EPIC trial, approximately 6% of patients treated with abciximab developed antibodies to the variable region(s) of abciximab (human antichimeric antibody).[204] Few data are currently available to assess the potential risks of reinjecting abciximab,[209] which theoretically include anaphylaxis, neutralization of injected abciximab, and thrombocytopenia.

Although the antiplatelet effect of abciximab in preventing vascular occlusion by suppressing platelet aggregation is likely to be the major mechanism for its beneficial effects, it is quite possible that the potent inhibition of thrombus formation by this antibody may result in decreased thrombin formation.[210] In fact, abciximab produced dose-dependent inhibition of tissue factor-induced thrombin generation, reaching a plateau of 45 to 50% inhibition at concentrations ≥ 15 $\mu g/mL$.[210] Whether the inhibition of thrombin generation by abciximab contributes to its immediate antithrombotic effect remains to be established. Abciximab is unique among the GPIIb/IIIa antagonists in also blocking the $\alpha v \beta_3$ receptor[196] and binding to an activated form of the leukocyte $\alpha M\beta 2$ receptor.[211] It is unclear whether any of the effects of abciximab are due to the inhibition of these receptors.

Tirofiban (MK-383, Aggrastat; Merck;) is a nonpeptide derivative of tyrosine that selectively inhibits the GPIIb/IIIa receptor, with minimal effects on the $\alpha v \beta_3$ vitronectin receptor.[212,213] It inhibits platelet aggregation of gel-filtered platelets induced by 10 μM ADP with an inhibitory concentration (IC_{50}) of 9 nM, but the IC_{50} for the inhibition of human umbilical vein adhesion to vitronectin, which depends on $\alpha v \beta_3$ vitronectin receptors, is 62 μM.[213]

When administered to humans at a concentration of 0.15 $\mu g/kg/min$ for 4 h, tirofiban produced a 2.5-fold increase in bleeding time and a 97% inhibition of ADP-induced platelet aggregation.[214,215] The mean plasma clearance was 329 mL/min, and the half-life in plasma was 1.6 h. After stopping tirofiban therapy, bleeding times returned to normal within 4 h and inhibition of platelet aggregation declined to approximately 20%. When tirofiban was administered with aspirin, the bleeding time increased 4.1 ± 1.5-fold, even though tirofiban plasma levels were unaffected. The plasma concentration of tirofiban needed to inhibit platelet aggregation by 50% decreased, however, from approximately 12 ng/mL to ap-

proximately 9 ng/mL when aspirin was coadministered. Peak plasma concentrations were approximately 40 ng/mL, and the plasma levels decreased to < 3 ng/mL within 6 h after therapy was discontinued.

In a pilot study, 73 patients undergoing PTCA were treated with aspirin, heparin, and bolus doses of tirofiban of 5, 10, or 15 $\mu g/kg$ followed by tirofiban infusions of 0.05, 0.10, and 0.15 $\mu g/kg/min$, respectively.[216] The onset of platelet inhibition was rapid, with platelet aggregation in response to 5 μM ADP inhibited by 93% and 96%, respectively, within 5 min of administering the two higher-dose regimens. Bleeding times for the three regimens at 2 h after starting the infusion were 19.5, > 30, and > 30 min, respectively. At the end of the infusion (16 to 24 h), platelet aggregation was inhibited by 57%, 87%, and 95%, respectively, in response to the escalating tirofiban regimens. Platelet aggregation began to return toward normal within 1.5 h after discontinuing the infusion in all groups; 4 h after discontinuing therapy, platelet aggregation inhibition decreased to $< 50\%$, even in the group receiving the highest dose.

Severe but reversible thrombocytopenia has been reported in a small percentage of patients treated with tirofiban, for which an immunologic mechanism has been proposed, mediated by preformed antibodies to a conformation of the GPIIb/IIIa receptor induced by the binding of tirofiban to the receptor.[217,218] No data are available on the safety of reinfusing tirofiban.

Eptifibatide (Integrilin) is a synthetic disulfide-linked cyclic heptapeptide. It is patterned after the Lys-Gly-Asp sequence found in the snake venom disintegrin obtained from *Sistrurus m barbouri* (barbourin) and has high specificity for the inhibition of GPIIb/IIIa compared with inhibition of the $\alpha v \beta_3$ vitronectin receptor.[219] Preliminary reports suggested that eptifibatide produced less prolongation of the bleeding time than other GPIIb/IIIa inhibitors at doses producing comparable inhibition of platelet aggregation. Later studies found that the citrate anticoagulation used for platelet aggregation studies resulted in an overestimation of the inhibition of platelet aggregation by eptifibatide.[220] Thus, it is unclear whether there is a differential effect of eptifibatide on the bleeding time. The elimination of eptifibatide depends principally on plasma clearance. Patients treated with heparin plus eptifibatide had longer activated clotting times than patients treated with heparin alone,[221] indicating that eptifibatide, like abciximab,[210] can inhibit thrombin generation *in vitro*.

In 21 patients undergoing elective PTCA or directional coronary atherectomy who were treated with aspirin, heparin (10,000-U bolus plus additional doses to maintain an activated clotting time of 300 to 350 s), and a bolus dose of 90 $\mu g/kg$ eptifibatide followed by a 1-$\mu g/kg/min$ infusion for 4 or 12 h, platelet aggregation was measured before the bolus administration, 1 h after the bolus, at the end of the infusion, and 4 h after the end of the infusion.[221] The extent of platelet aggregation in response to 20 μM ADP decreased from approximately 80% before eptifibatide administration to approximately 15% at both 1 h after the bolus dose and at the end of the infusion. There was, however, significant interindividual variation in the inhibitory responses at the two time points tested (95%

CI, 0% to approximately 30% and 0% to approximately 40%, respectively). Four hours after stopping the infusion, the average aggregation response was approximately 55%, but there was marked individual variation (95% CI, approximately 10 to 90%). Median bleeding times were prolonged with eptifibatide therapy, going from approximately 6 min before treatment to approximately 26 min at both 1 h after beginning the infusion and at the end of the infusion. The bleeding times returned toward normal (median, 15 min) within 15 min after stopping eptifibatide therapy, and they declined to approximately 12 min after stopping the drug therapy for 1 h. At each time point, however, there were considerable interindividual differences.

In a later study,[222] the following four eptifibatide regimens were tested in 54 patients undergoing coronary interventions who also were treated with aspirin and heparin: (1) 180 μg/kg bolus plus 1 μg/kg/min infusion for 18 to 24 h (n = 4); (2) 135 μg/kg bolus plus 0.5 μg/kg/min infusion for 18 to 24 h (n = 16); (3) 90 μg/kg bolus plus 0.75 μg/kg/min infusion for 18 to 24 h (n = 6); and (4) 135 μg/kg bolus plus 0.75 μg/kg/min for 18 to 24 h (n = 28). Fifteen minutes after the 180-μg/kg bolus dose, platelet aggregation was inhibited by > 95% in response to 20 μM ADP, with virtually no interindividual variation, whereas the 135-μg/kg bolus dose resulted in 80 to 90% inhibition in 75% of the patients, and the 90-μg/kg bolus produced only slightly less inhibition than the 135-μg/kg dose. The inhibition of platelet aggregation achieved with the 180-μg/kg bolus dose was sustained throughout the infusion by the 1-μg/kg/min dose, but there was a tendency for the platelet aggregation response to return toward normal during infusion in some patients who were given the 0.75-μg/kg/min dose, and the return of the platelet aggregation response toward normal was more marked in those who were given the 0.5-μg/kg/min infusion dose. Two hours after discontinuing the eptifibatide infusion, there was a substantial return of platelet function in all groups and a return of more than half of the baseline aggregation response in all groups after 4 h. Median bleeding times were prolonged in all groups at the time that the infusion was terminated (22, 12, 12, and 17 min, respectively, for the four regimens compared with control values of 7 to 8 min), and they returned toward normal after 1 h (9, 10, 9, and 11 min, respectively). As in the previous study, activated clotting times were longer in patients treated with eptifibatide plus heparin than in those treated with placebo plus heparin. A modest increase in hemorrhagic complications has been reported in patients treated with eptifibatide in the PURSUIT trial.[223] Eptifibatide treatment has not been associated with an increased frequency of cases of overall thrombocytopenia, but it may be associated with a small increase in cases of profound thrombocytopenia.[223] All patients receiving parenteral GPIIb/IIIa blockers should be monitored within 24 h of the initiation of therapy for development of thrombocytopenia. An algorithm for the detection and management of thrombocytopenia after GPIIb/IIIa blockade has been proposed.[218] To our knowledge, no data are available concerning the safety of reinfusing eptifibatide.

The efficacy and safety of GPIIb/IIIa antagonists were evaluated initially in patients undergoing percutaneous coronary intervention (PCI). More than 13,000 patients have been enrolled in six studies of abciximab, eptifibatide, and tirofiban (Table 7). The first of these phase III trials, the EPIC trial,[204] resulted in the approval of abciximab (ReoPro; Eli Lilly; Indianapolis, IN) in many countries in 1994 for PCI patients at high risk of developing ischemic complication. Eptifibatide and tirofiban have been studied in the IMPACT-II[224] and RESTORE[225] trials, respectively. Although neither of these trials achieved their predefined efficacy end points, there was a positive trend in each case (Table 7). Eptifibatide received approval from the US FDA for PCI in 1998 based on data from the IMPACT-II and PURSUIT trials. The CAPTURE trial[226] demonstrated the efficacy of an 18- to 24-h abciximab treatment prior to PCI in patients with unstable angina refractory to conventional antithrombotic and antianginal therapy. The EPILOG trial[206] demonstrated the efficacy of abciximab in a broad patient population undergoing PCI, not just in high-risk patients such as those enrolled in the EPIC and CAPTURE trials. The EPISTENT trial demonstrated that abciximab decreases the frequency of ischemic complications of PCI associated with stent insertion during the first 30 days and that there are fewer ischemic complications during this time period in patients treated with PCI and abciximab alone without stent insertion than in those treated with stents alone.[227] Furthermore, the 1-year mortality rate difference is statistically significant between patients receiving stents alone (2.4%) and those receiving stents plus

Table 7—GPIIb/IIIa Antagonists in PCI*

Trial	N	Compound	Placebo, %	GPIIb/IIIa Antagonist, %	RRR, %
EPIC	2,099	Abciximab	10.3	6.9	30.0
EPILOG	2,792	Abciximab	9.1	3.8†	58.2
CAPTURE	1,265	Abciximab	9.0	4.8	46.7
EPISTENT	1,603	Abciximab	10.2	4.8‡	52.9
IMPACT-II	4,010	Epitfibatide	8.4	6.9	17.9
RESTORE	2,139	Tirofiban	6.4	5.0	21.9

*Rates of death or MI at 30 days are shown for each trial. RRR = RR reduction; EPIC = Evaluation of Platelet IIb/IIIa Inhibition of Prevention of Ischemic Complication.
†Abciximab + low-dose heparin.
‡Abciximab + stenting vs placebo + stenting.

Table 8—GPIIb/IIIa Antagonists in Unstable Angina and Non-Q-Wave MI*

Trial	N	Compound	Placebo, %	GPIIb/IIIa Antagonist, %	RRR, %
PRISM	3,232	Tirofiban	7.1	5.8	18.3
PRISM-Plus	1,570	Tirofiban	11.9	8.7	26.9
PARAGON	2,282	Lamifiban	11.7	10.3	12.0
PURSUIT	10,948	Eptifibatide	15.7	14.2	9.6

*Rates of death or MI at 30 days are shown for each trial. See Table 7 for abbreviations not used in text.

abciximab therapy (1%).[9] Thus, there continues to be support for the hypothesis that periprocedural myocardial damage, as judged by increases in creatine kinase, confers increased long-term mortality risk.

Although it is possible that all GPIIb/IIIa antagonists will be equally effective with optimal dosing, differences in the pharmacokinetics/pharmacodynamics of these agents may explain the apparent differences in effectiveness observed to date in PCI trials.

Four completed trials have examined the efficacy and safety of tirofiban, lamifiban (a nonpeptide GPIIb/IIIa blocker, the development of which has been discontinued), and eptifibatide in approximately 18,000 patients who had had unstable angina or non-Q-wave MI and were randomized to receive a GPIIb/IIIa antagonist or placebo, in addition to conventional antithrombotic therapy (Table 8).[223,228–230] These studies demonstrated a 10 to 27% RR reduction in MI or death at 30 days. Both eptifibatide and tirofiban have received approval from the US FDA for the treatment of acute coronary syndromes, including patients who are to be managed medically and those who are undergoing PCI. Abciximab previously received approval for the treatment of patients with refractory unstable angina who are expected to undergo PCI within 18 to 24 h of beginning abciximab treatment.

The results of phase II trials for abciximab, eptifibatide, and lamifiban, with patients who have had acute MIs, have suggested potential benefits of GPIIb/IIIa blockade as an adjunct to thrombolysis. The Thrombolysis in Myocardial Infarction 14A trial demonstrated that combining abciximab with aspirin and reduced-dose tissue-type plasminogen activator in the treatment of acute MI resulted in improved Thrombolysis in Myocardial Infarction grade 3 flow rates at 60 and 90 min after starting therapy when compared to the best full-dose tissue-type plasminogen activator regimen.[231] The bleeding risk was not substantially increased.[231]

Orally active nonpeptide GPIIb/IIIa inhibitors have been developed, and they have the potential to be given long term.[232,233] However, a number of issues remain unsolved.[198–200,234] These include the following: (1) uncertainty as to the optimal degree of GPIIb/IIIa receptor blockade to maximize safety and efficacy; (2) the difficulty of performing assays to monitor therapy such as ADP-induced platelet aggregation or skin bleeding time; and (3) a steep relationship of dose with a surrogate of safety (ie, bleeding time). Assays to monitor the effects of these drugs have been developed,[235] but the benefits of drug monitoring and dose adjustment remain to be established.

In the largest completed trial of an oral GPIIb/IIIa blocker, SYMPHONY,[11] 9,233 patients whose conditions had stabilized after an acute coronary syndrome were randomized to treatment with aspirin (80 mg bid), low-dose sibrafiban, or high-dose sibrafiban, given within 7 days of and sustained for 90 days after the qualifying event. Sibrafiban doses were based on a model accounting for weight and serum creatinine value and were designed to achieve > 25% (low-dose) and > 50% (high-dose) steady-state inhibition of ADP-induced platelet aggregation. The incidence of the primary end point, a composite of death, nonfatal (re)infarction, or severe recurrent ischemia at 90 days, did not differ significantly between groups (aspirin, 9.82%; low-dose sibrafiban, 10.06%; and high-dose sibrafiban, 10.05%).[11] Similar results were obtained for death or (re)infarction (aspirin, 7.0%; low-dose sibrafiban, 7.4%; and high-dose sibrafiban, 7.9%). Major bleeding was significantly more common with both high-dose (5.7%) and low-dose (5.2%) sibrafiban therapy than with aspirin (3.9%).[11] The same was true for minor bleeding (high-dose sibrafiban, 24.6%; low-dose sibrafiban, 17.7%; and aspirin, 12.6%). There were no significant differences for the occurrence of thrombocytopenia. Two other large trials have tested oral GPIIb/IIIa antagonists in combination with aspirin as a background therapy, OPUS

Table 9—Main Features of Aspirin, Clopidogrel, and Oral GPIIb/IIIa Antagonists for Long-term Therapy

Feature	Aspirin	Clopidogrel	GPIIb/IIIa Antagonists
Targeted platelet protein	COX-1	$P2T_{AC}$	$\alpha IIb\beta3$
Reversibility of the effect	No	No	Yes
Desirability of saturation of the target	Yes	Yes	No
Half-life of the drug or active metabolite	Minutes	Minutes	Hours
Need for monitoring	No	No	Unknown
Need for dose titration	No	No	Unknown

(n = 10,302) and EXCITE (n = 7,232).[12] OPUS was stopped prematurely because of excess mortality in one of the orbofiban groups. An initial review of cause of death suggests that the excess mortality may have been attributable to coronary thrombosis.[12] This finding raises concerns about a paradoxical prothrombotic effect. In fact, induction of fibrinogen binding and platelet aggregation has been described as a potential intrinsic property of various GPIIb/IIIa antagonists.[236] Patients receiving orbofiban in the OPUS trial had a paradoxical increase in platelet reactivity with respect to both fibrinogen binding and α-granule degranulation.[237] Moreover, in each of the three trials of oral GPIIb/IIIa antagonists, the odds ratio for mortality favored aspirin therapy, although it was statistically significant only in OPUS.[12] In all three trials, there were excess bleeding complications in the groups assigned oral GPIIb/IIIa antagonists.

In trying to interpret these largely disappointing results, it should be emphasized that GPIIb/IIIa antagonists have less than ideal features for long-term antiplatelet prophylaxis, particularly when compared to aspirin and clopidogrel (Table 9). The accumulation of evidence that aspirin, clopidogrel, and dipyridamole are all effective at well-tolerated doses in patients with clinical syndromes of platelet-dependent vascular occlusion is likely to antecede evidence supportive of their combination, as heralded by the roughly additive efficacy of aspirin and dipyridamole in ESPS-2. This is welcome news but increases the challenge for novel antithrombotic agents. Particularly in the setting of long-term oral dosing, tolerability may prove to be an even more important consideration than the magnitude of the increment in efficacy. Thus, issues such as predictable pharmacokinetics and shallow dose-response relationships are likely to be fundamental to the success of future oral therapies.

Whether the different binding features of some of the newer oral agents, such as roxifiban, that have much slower rates of dissociation from platelet GPIIb/IIIa than others,[238] or whether new methods of monitoring GPIIb/IIIa antagonist therapy[235] will make a difference for the future development of this interesting class of drugs is currently being debated.[198–200]

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