Pharmacology of Platelet Inhibitors

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Although many drugs have inhibitory effects on platelet function, none of them inhibits all of the mechanisms that may be involved in the various forms of thrombosis. Choice of suitable drugs is hampered by lack of full knowledge concerning the reactions that make the major contributions to the formation of arterial thrombi at sites of repeated vessel wall injury or on atherosclerotic lesions. Drugs such as aspirin that inhibit the arachidonate pathway in platelets can only be expected to be effective against thromboembolic events in which the generation of thromboxane A₂ plays a major part. If thrombin and fibrin formation are dominant, oral anticoagulant agents or heparin should be beneficial; thus, experimental evidence indicates that with repeated vessel wall injury, the formation of platelet fibrin thrombi on the vessel wall is probably influenced more by inhibitors of thrombin generation than by the subendothelial constituents such as collagen.

Pharmacologic modification of platelet function reduces the thromboembolic complications in a number of arterial vascular disorders and in patients with prosthetic cardiovascular devices (1–3). However, the indications for the clinical use of antiplatelet agents are not entirely clear despite extensive basic experimental animal and clinical studies. This uncertainty is explained, at least in part, by our lack of knowledge of the precise pathogenesis of many clinical vascular syndromes, by the lack of suitable pharmacologic agents for definitive clinical testing and by the inconclusive results obtained in some of the clinical studies. In this study, the current pharmacologic status of antiplatelet drugs used in clinical practice is reviewed.

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Agents like prostacyclin that raise platelet cyclic adenosine monophosphate (AMP) levels in platelets by stimulating adenylate cyclase are potent inhibitors of the reaction of platelets to all aggregating and release-inducing stimuli, but these agents are not suitable for long-term administration. The effect of dipyridamole on platelet cyclic AMP levels is weak, and this drug may act through other effects on platelets or on other cells. Indeed, several of the drugs that have been tested in clinical trials may exert their effects through unrecognized mechanisms. Many combinations of drugs have been used to affect platelets or platelets and coagulation. This practice has been based on the theory that because several mechanisms may be involved in thrombus formation, combinations of drugs that inhibit different mechanisms may be beneficial.

(J Am Coll Cardiol 1986;8:21B–32B)

Ideally, a clinically useful, platelet-modifying drug should be nontoxic, orally effective, have sustained action and have good antithrombotic potency without excessive risk of abnormal bleeding. None of the currently available clinical agents satisfies all these requirements. Aspirin, sulfinpyrazone, dipyridamole, sulocitidil, ticlopidine and prostacyclin have been the agents evaluated in clinical trials to date. Many agents with more defined pharmacologic actions have been synthesized for evaluation and are presently at various stages of development.

The disorders for which antiplatelet therapy has been studied may have quite different pathogenetic mechanisms. Because the effects of a given drug may be different for each pathogenetic process, it is probably inappropriate to extrapolate the results for a given drug from one clinical setting to another. In general, however, kinetic studies (4) in patients using radioactively labeled platelets and fibrinogen indicate that arterial thrombosis is characterized by selective platelet consumption that can be interrupted by some inhibitors of platelet function but not by heparin, whereas venous thrombosis produces combined and equivalent consumption of both platelets and fibrinogen that is blocked by anticoagulant therapy but not by drugs that modify platelet function.

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Pathways of Platelet Activation and Their Modulation

Process of platelet adhesion and aggregation (Fig. 1). Vascular injury with endothelial denudation exposes subendothelial structures to circulating blood and induces platelets to adhere to subendothelial collagen (5). The process of platelet adhesion involves interaction of the platelet membrane glycoprotein receptor GPIb, subendothelial collagen and plasma von Willebrand factor and fibronectin (6). Platelet interaction with collagen leads to the release of adenosine diphosphate (ADP) and activation of the prostaglandin arachidonate pathway (7,8) to release thromboxane A2. Both ADP and thromboxane A2 recruit circulating platelets (9,10) to change shape and aggregate with the layer of already adherent platelets on the subendothelial surface (Fig. 1). A third pathway of platelet recruitment involves thrombin generation. Thrombin formation is produced through both extrinsic and intrinsic coagulation processes at the injury site and around the aggregating platelet mass. Although thrombin induces the release of both ADP and thromboxane A2, more importantly, thrombin induces platelet aggregation and release through a direct mechanism that is independent of ADP release or the formation of thromboxane A2 (11–13). In addition, the formation of polymerizing fibrin in close proximity to the platelet aggregate by the action of thrombin on fibrinogen stabilizes the aggregate through the adherence of polymerizing fibrin to the platelet surface (14). Platelet aggregates that are not stabilized by fibrin deaggregate readily and are broken up under the force of blood flow.

All platelet recruitment requires the rapid expression of the platelet-membrane complex GPIIb/GPIIIa as the fibrinogen receptor (6,9,15). That is, platelet-platelet aggregates are formed through calcium-dependent fibrinogen binding with the expressed receptor. This reaction may also be modulated by platelet alpha-granule proteins, fibronectin, von Willebrand factor or thrombospondin (16).

Limitation of thrombus formation. Intact endothelium actively limits thrombus formation and its extension by multiple mechanisms including 1) inactivation of released ADP (17), 2) active clearance of vasoactive amines (18), 3) thrombin-mediated release of prostacyclin (19), 4) direct inactivation of circulating thrombin by plasma protease inhibitors (20), 5) inactivation of thrombin by enhancement of the endothelial surface thrombin-antithrombin III complex formation (21), 6) thrombin complexing with thrombomodulin on the endothelial cell surface to activate protein C (which reduces thrombin formation by destroying factors Va and VIIIa both in plasma and bound to the platelet surface) (22), and 7) thrombin-mediated release of tissue plasminogen activator from intact vascular wall (23) (Fig. 2). Thus, thrombin initiates important negative feedback mechanisms to control its own generation and extent of effect.

Calcium-dependent platelet activation. There is increasing evidence that some platelet responses occur through calcium-regulated contractile processes (24), which are in turn modulated by cyclic adenosine monophosphate (AMP) (25) and prostaglandin or arachidonic acid products (26). In general, contractile and secretory processes are thought

Figure 1. Pathways of platelet recruitment. Endothelial disruption initiates the attachment of platelets to the subendothelium through the interaction of a platelet receptor (GPIb), subendothelial collagen and a plasma cofactor VIII/von Willebrand factor (vWF). Fibronectin and thrombospondin derived from plasma, platelets and endothelium may also play roles in this process. An unstable platelet mass forms through several interactive but independent recruitment mechanisms, including the generation of thromboxane (TX) A2 release of dense granule, adenosine diphosphate (ADP) and thrombin-mediated platelet activation. Aggregation requires the rapid expression of platelet membrane fibrinogen receptor complex (GPIIb and GPIIIa) and calcium-dependent interplatelet bridging by fibrinogen. Thrombin is generated locally on the platelet surface through both extrinsic and intrinsic pathways. Thrombin-modified factor V (factor VmA) binds to platelets. Platelet-bound factor VmA serves as a high affinity platelet receptor for factor Xa in the final conversion of prothrombin to thrombin. Thus, the generation of thrombin initiates potent positive feedback mechanisms on the platelet surface for explosive activation of the coagulation cascade and fibrin formation. βTG = β-thromboglobulin; FDP = fibrin degradation products; PDGF = platelet-derived growth factor; PF4 = platelet factor 4.
Figure 2. Mechanisms limiting thrombus extension. Intact endothelium actively resists thrombus formation. Platelet aggregate formation is prevented by the endothelium despite the presence of ADP, epinephrine or thrombin through the inactivation of ADP, active clearance of vasoactive amines, facilitated complexing of thrombin with antithrombin III and the explosive thrombin-mediated synthesis and release of inhibitory PIB. These mechanisms markedly decrease the possibility of thrombus forming in the presence of intact endothelium. The potent effects of thrombin are also actively limited to the site of vascular injury by plasma protease inhibitors, enhancement of the endothelial surface of thrombin-antithrombin III complex formation, binding of circulating thrombin to thrombomodulin (a receptor of luminal surface of the endothelial cells) thereby activating protein C to destroy factors V and VIII and induced release of tissue plasminogen activator from vascular endothelium. Thus, thrombin initiates negative feedback mechanisms controlling its own generation. Abbreviations as in Figure I.

to be mediated by an increase in the concentration of cytoplasmic calcium derived from intracellular storage sites or from the extracellular environment. Calcium-dependent platelet activation occurs through actomyosin-mediated contractile mechanisms including pseudopod formation, aggregation, release and clot retraction. It appears that the release reaction is triggered by release of calcium from an intracellular storage site, possibly the dense tubular system, into the cytoplasm. Calcium regulation of contractile proteins in platelets is similar to the processes of contraction in muscle. Calcium and the calcium-binding protein calmodulin are involved in activation of the specific enzyme myosin light chain kinase and, hence, promotion of the interaction between platelet actin and myosin (27).

Platelet cyclic AMP. This compound inhibits both platelet secretion and aggregation (28). The amount of cyclic AMP in the platelet is determined both by the activity of membrane adenylyl cyclase and by the specific phosphodiesterase that hydrolyzes cyclic AMP to adenosine triphosphate (ATP); that is, platelet cyclic AMP is increased by agents that either increase the activity of adenylyl cyclase or decrease the activity of phosphodiesterase. The basal level of cyclic AMP appears to modulate release and aggregation by controlling the storage and release of calcium from platelet intracellular storage sites.

Role of prostaglandins. As discussed in more detail in the first section of the symposium by Vermylen et al. (29) (also see Fig. 1 and 2 in reference 29), prostaglandins (PG) are important in the regulation of platelet activation for two reasons (26,30). First, as mentioned, stimulated platelets produce biologically active prostaglandins and derived substances that regulate platelet function. Second, other prostaglandins produced by other cells (such as PGI2 by vascular cells) may either inhibit or activate platelets. Platelet stimulation results in the hydrolysis of arachidonic acid from membrane phospholipid, possibly through a calmodulin-mediated process. Both phosphatidylinositol (through hydrolysis by phospholipase C) and phosphatidylycholine (through hydrolysis by phospholipase A2) may serve as the source of arachidonic acid. The arachidonic acid thus liberated becomes converted by cyclooxygenase to labile prostaglandin endoperoxide intermediates (PGG2 and PGH2), which are potent platelet-aggregating agents, or through the lipoxygenase pathway to other prostaglandin derivates. By means of thromboxane synthetase, the most important conversion of PGH2 is to the highly labile nonprostaglandin substance thromboxane A2, which is the most potent platelet-aggregating agent derived from arachidonic acid yet discovered. In addition, thromboxane A2 is a potent vasoconstrictor. PGH2 is also converted to several other (stable) prostaglandins, namely, PGE2 and PGF2α, which do not directly affect platelets but may potentiate aggregation by other substances, and PGD2, which inhibits aggregation and could play the role of a negative feedback mechanism in regulating aggregation.

On the other hand, vascular tissue, cultured endothelial
cells and postinjury neointima synthesize PGI₂ from the arachidonic acid pathways (26,31,32). Moreover, endothelial cells can utilize PGG₂(H₂) derived from platelets to synthesize PGI₂ (33). PGI₂ is about 30 to 40 times more potent than PGE₁ as an inhibitor of platelet aggregation induced by ADP, epinephrine, collagen, thrombin and arachidonic acid. Its inhibitory effects are mediated through elevation of platelet cyclic AMP through activation of adenylcyclase (34).

Finally, prostaglandins, cyclic AMP and Ca²⁺ interact in a number of complex ways to enhance platelet function (7,8,35). For example, both phospholipase A₂ and phospholipase C are activated by Ca²⁺, and the inhibitory effect of cyclic AMP on platelet aggregation may act by maintaining calcium in a bound form, thereby preventing activation of phospholipases. Thromboxane A₂ also has properties resembling those of a calcium ionophore, and some of the platelet-stimulatory effects of thromboxane A₂ may be related to its ability to mobilize intracellular calcium from storage sites.

Drug Testing

The first assessment of a new compound to test for platelet-inhibitory activity usually involves adding it to citrated platelet-rich plasma in vitro and examining the effect on aggregation and the release of granule contents in response to such substances as ADP, epinephrine, collagen and arachidonic acid. Suspensions of washed or gel-filtered platelets are more suitable for tests with thrombin and also permit experiments in the presence of physiologic concentrations of ionized calcium and magnesium. Unfortunately, these results have little predictability regarding antiplatelet efficacy in vivo, and it is for this reason that the effects of these agents on in vitro testing will not be addressed in detail in this review. Thus, although many agents that strongly inhibit aggregation and release in vitro have been identified, few have proved useful in human patients, particularly for long-term administration. Most of the drugs that have been used in clinical trials were in use long before their effects on platelets were recognized, and their value as antithrombotic agents would not have been recognized by the type of routine screening systems now being used. There have been no reports of major trials of new drugs that have been designed specifically as inhibitors of platelet function. Other in vitro tests of platelet function of uncertain interpretation include studies of platelet adherence to glass beads, artificial surfaces, collagen or subendothelium and examination of the contribution of platelets to the acceleration of coagulation. In vivo testing in experimental animals has a number of advantages: 1) the drug is tested at concentrations that can be achieved clinically, 2) active metabolites of the drug are also assessed, 3) the time during which effective concentrations of the drug or its metabolites remain in the circula-

Pharmacology of Drugs That Modify Platelet Function

The pharmacology of the drugs that inhibit platelet function and thrombus formation will be considered in four broad categories: 1) drugs that inhibit the arachidonate pathway, 2) drugs that affect platelet cyclic AMP levels, 3) drugs that inhibit thrombin formation and action, and 4) drugs that act through less well defined mechanisms.

Drugs that inhibit the arachidonate pathway. Drugs that affect this prostaglandin pathway include agents that inhibit platelet cyclooxygenase, drugs that block the action of phospholipases and drugs that inhibit thromboxane synthetase. Because these drugs affect only thrombus formation that is dependent on thromboxane A₂, thrombus formation that is predominately mediated by the other pathways and stimuli (that is, thrombin) will be largely unaffected by this class of drug.

Drugs that inhibit cyclooxygenase include the nonsteroid anti-inflammatory drugs such as aspirin, indomethacin, ibuprofen and many others (1–3,42). (Although the uricosuric agent sulfipyrazole has effects on cyclooxygenase, its antithrombotic mechanism is unclear and is, therefore, discussed later.)

Aspirin and platelet function. Aspirin inhibits the second but not the first wave of ADP-induced platelet aggregation. Aspirin affects, in part, collagen-induced platelet aggregation caused by its inhibitory effect on platelet secretion. In addition to inhibiting the release of ADP, aspirin in vitro and in vivo inhibits the collagen-induced release of ATP, serotonin and platelet antiheparin activity (platelet factor 4). Similar results have been obtained with epinephrine-induced aggregation. The effects of aspirin on thrombin-induced
aggregation and secretion are dose-related. Inhibition occurs at low but not high concentrations of thrombin (43). Finally, the inhibitory effects of aspirin on platelet aggregation to different agents are detected for 4 to 7 days after a single oral dose when salicylate is no longer detectable in the blood. This period of inhibition reflects an irreversible effect of aspirin on the platelet.

Aspirin irreversibly acetylates the cyclooxygenase in platelets (44). Because platelets are unable to synthesize new enzyme to replace that which has been inactivated, it is not until new platelets enter the circulation that platelet function is restored. However, aspirin also acts on megakaryocytes. This is shown by the finding that human platelets harvested at times up to 48 hours after administration of aspirin cannot be acetylated in vitro with radiolabeled aspirin (45), and also by direct experiments with megakaryocytes that indicate that aspirin administration abolishes their ability to synthesize prostaglandins (46). The long-term administration of 1 mg/kg of aspirin once a day effectively inhibits platelet cyclooxygenase and thromboxane A₂ formation in humans.

In contrast, aspirin inhibition of the cyclooxygenase of the cells of the vessel wall is not irreversible because these cells synthesize new cyclooxygenase. Regeneration of the ability of cultured human endothelial cells from umbilical veins to synthesize PGI₂ requires many hours (47). Indeed, long-term administration of 1 mg/kg of aspirin once a day will also effectively inhibit vascular cyclooxygenase and prostaglandin production in humans (48). Thus, the notion that low dose, infrequently administered aspirin exerts its antithrombotic effects by preferentially sparing vascular prostacyclin production may not be correct. Furthermore, high doses of aspirin over prolonged periods to patients with rheumatoid arthritis may decrease thrombosis despite inhibition of PGI₂ production (49). Earlier data concerning the low incidence of myocardial infarction as a cause of death of patients with rheumatoid arthritis who consumed large amounts of aspirin also support this view (50).

Under physiologic conditions of flow and protein concentration and hematocrit, aspirin does not inhibit adherence of the initial layer of platelets to the subendothelium (51,52). Aspirin and indomethacin also do not inhibit the release of amine storage granule contents from platelets that adhere directly to collagen or the subendothelium (53), and released ADP and serotonin from these platelets contribute to thrombus formation in the presence of the cyclooxygenase inhibitors. Because aspirin does not inhibit release of platelet-derived growth factor from the alpha granules of the adherent platelets, it is not surprising that these drugs do not prevent smooth muscle cell proliferation in response to vessel damage (54). However, these drugs may inhibit platelet aggregate formation on the layer of adherent platelets (52), presumably by blocking thromboxane A₂ release.

In some experimental animal studies, aspirin has partially inhibited thrombus formation. In other studies no significant effects have been observed and, in a few, aspirin has been reported to potentiate thrombus formation (55). The last effect has been attributed to aspirin inhibiting PGI₂ production by the vessel wall.

Aspirin in unstable angina and ischemia. In the myocardial and cerebral circulations, platelet aggregate formation and, perhaps, associated vasospasm (related to the release of thromboxane A₂ or platelet-derived growth factor [56,57]) may lead to the acute coronary syndromes (that is, unstable angina, myocardial infarction, arrhythmia-related sudden death) or transient ischemic attacks, respectively. In dogs with experimentally stenosed coronary arteries, aspirin inhibits the formation of platelet plugs and the occurrence of fatal arrhythmias (58). Studies (59,60) with ischemic dog and cat myocardium have shown that aspirin can prevent ventricular fibrillation caused by ischemia. The beneficial effects of aspirin in patients with unstable angina or transient ischemic attacks and in the experimental preparations just referred to, may be related not only to its ability to inhibit platelet aggregation, but also to the inhibition of release of thromboxane A₂ and platelet-derived growth factor, preventing vasoconstriction so that thromboemboli do not readily lodge and persist in the microcirculation.

Aspirin and thromboembolism. Aspirin has been shown to be clinically effective in reducing thromboembolic complications of artificial heart valves in one study (61), thrombotic occlusion of arteriovenous cannulas in uremic patients (62), stroke and death in patients with transient ischemic attack (63), myocardial infarction in men with unstable angina (64,65), and, possibly, the occlusion of saphenous vein coronary artery bypass grafts (66). It may be important in this context to note that an additional hemostatic abnormality was associated with the beneficial effect of aspirin in the studies of artificial heart valves (oral anticoagulation) and arteriovenous cannulas (platelet dysfunction in uremic patients treated with long-term hemodialysis). The reported antithrombotic effect of aspirin in patients undergoing saphenous vein coronary artery bypass surgery is provisional in that the study involved a relatively small number of patients with an unusually high frequency of graft occlusion in the control group. Thus, aspirin may exert limited antithrombotic effects unless it is used in association with another antithrombotic drug or perturbation of the hemostatic mechanism.

Regarding aspirin dose, the beneficial effects of low doses of aspirin (100 to 150 mg/day) reported to prevent thrombosis in saphenous vein aortocoronary bypass grafts and arteriovenous shunts must be considered to be provisional because, as already mentioned, the former study requires confirmation because of questions regarding the control group, and the conclusions of the latter study may not be extrapolated to other clinical settings because of the associated platelet dysfunction in those patients undergoing long-term
hemodialysis. On the other hand, there are clinical reports of high dose-dependent antithrombotic effects of aspirin. In this context, recent studies (67) in experimental thromboembolism indicate that aspirin has antithrombotic effects that are independent of its effect on cyclooxygenase and that these effects occur at high doses.

Nonsteroid anti-inflammatory drugs. Other nonsteroid anti-inflammatory drugs also inhibit platelet activation through inhibition of platelet cyclooxygenase (67). However, these agents differ in potency and duration of action because they inhibit competitively. In addition, some of these drugs may have other effects on platelets besides inhibiting cyclooxygenase. For example, indomethacin may inhibit phospholipase activation. At equimolar concentrations, meclofenamic acid, aspirin and indomethacin and ibuprofen were the most active, whereas phenylbutazone was a relatively weak inhibitor (68).

Other agents. Drugs that affect the generation of arachidonate from the cell membrane have been considered to be inhibitors of phospholipase A₂; they include the antimalarial drug quinacrine hydrochloride (mepracrine hydrochloride) and some of the steroid anti-inflammatory drugs. None of these agents has been shown to have clinically useful antithrombotic effects in humans (42).

Inhibitors that block thromboxane synthetase include imidazole and its derivatives. Although a number of these compounds have been synthesized and have undergone testing in animal models and human subjects (69), none of these agents has been shown to prevent thrombosis in humans.

Drugs that increase platelet cyclic AMP levels. When the cyclic AMP level in platelets is elevated, many platelet functions are inhibited (42). Platelet cyclic AMP is increased by agents that stimulate adenylate cyclase and, to a lesser extent, by drugs that inhibit platelet phosphodiesterase (79) and, thus, prevent the breakdown of cyclic AMP in platelets (42). Adenylate cyclase is stimulated by PG₁₂, PGF₂ and PGE₁. The combination of PG₁₂ or PGE₁ with a phosphodiesterase inhibitor such as dipyridamole or theophylline strongly inhibits platelet functions (71,72).

PG₁₂ and PG₁₁₂. Agents that increase cyclic AMP levels diminish rabbit platelet adherence to damaged vessel walls (72), inhibit platelet aggregation induced by all stimuli and inhibit the release of platelet granule contents induced by all stimuli (26,42,74). When cyclic AMP levels are raised, the change in platelet shape in response to aggregating and release-inducing agents is also inhibited. This prevents platelet membrane sites involved in the intrinsic coagulation pathway from becoming available to accelerate the generation of thrombin on the platelet surface. Agents such as PGE₁ or PG₁₂, when given in high doses, have been shown to be strong inhibitors of platelet aggregation, thrombus formation and their consequences under a variety of situations in humans and experimental animals (75–80). PG₁₂ is the most potent inhibitor of platelet aggregation thus far described, and it is 30 to 40 times more potent than PGE₁ in inhibiting platelet aggregation by ADP. The duration of these effects in vivo is very short; they disappear within 30 minutes of dosing. PG₁₁₂ is a strong dilator in several vascular beds such as those of the heart, kidney, mesenteric and skeletal muscle (81,82). Intravenous infusion of relatively small amounts (5 ng/kg per min) of PG₁₁₂ in human patients produces vaso-dilation of peripheral blood vessels in the head, neck and palms, a decrease in diastolic but not systolic blood pressure and an increase in heart rate (83). Platelet aggregation in response to ADP and the number of circulating aggregates decrease during PG₁₁₂ infusion, while the bleeding time increases. One hour after termination of the infusion, the responsiveness of the platelets to ADP returns to normal.

Dipyridamole. Dipyridamole is another platelet-inhibitor drug. It has been proposed that it acts to elevate platelet cyclic AMP by both blocking platelet phosphodiesterase-dependent breakdown and increasing cyclic formation by PG₁₂-mediated stimulation of platelet adenylate cyclase (72). It seems more likely that an increase in blood adenosine levels may be involved because dipyridamole is a potent inhibitor of vascular and erythrocyte adenosine uptake (84,85) and produces an elevation in plasma adenosine levels (86). The higher plasma concentrations of adenosine could mediate the inhibition of platelet reactivity by stimulating platelet adenylate cyclase activity (87). For example, 10 µM of dipyridamole in vivo markedly inhibits the uptake of adenosine by endothelial cells and produces potent antiaggregating activity that is not blocked by aspirin (that is, in the absence of prostacyclin). The vasodilating effects of dipyridamole also appear to be related to the elevation of plasma adenosine levels. Indeed, vasodilation may account for its actions in some experimental situations in which it has been shown to be beneficial (88).

Dipyridamole, when given experimentally at a very high dose, does inhibit platelet adherence to collagen (89) and the subendothelium (90), but most importantly, when given at a dose more comparable with that used in humans, dipyridamole inhibits platelet activation over artificial surfaces (67,91). Indeed, the capacity of dipyridamole to decrease experimental thromboembolism in a dose-dependent fashion correlates well with its ability to normalize platelet survival in patients with artificial heart valves (92,93) and arteriovenous cannulas (94) and its efficacy in preventing thromboembolism in patients with prosthetic heart valves (95). Moreover, the dose (10 mg/kg per day) and resulting blood levels that produce complete interruption of experimental thromboembolism at prosthetic surfaces are in reasonable accord with the dose (5 to 7 mg/kg per day) reported to normalize platelet survival (92,94,96) and prevent thromboembolism in patients with artificial heart valves.
Aspirin potentiates the efficacy of dipyridamole in experimental thromboembolism on prosthetic surfaces (67). Moreover, the full potentiation depends on administering a relatively high dose of aspirin simultaneously with every dose of dipyridamole (20 mg/kg per day). These results in baboons are in accord with the capacity of the combination of aspirin and dipyridamole to prolong platelet survival in patients with Dacron vascular grafts (96). On the other hand, the data regarding the beneficial effects on platelet survival and thromboembolism of the combination of aspirin and dipyridamole in biologic surfaces such as in coronary artery atherosclerosis (97–99), renal microvascular disease (100) and saphenous vein bypass grafting (101,102) has raised the possibility of aspirin being the main effective component. That is, in four other different trials (103–106), the combination of aspirin and dipyridamole was as effective as aspirin alone; in only one trial (107) addressing the rate of progression of peripheral vascular disease did this combination appear to be more effective than aspirin alone, but this remains to be confirmed.

The mechanism whereby aspirin potentiates the antithrombotic activity of dipyridamole, particularly in experimental prosthetic surfaces, is not: 1) mediated through aspirin’s conversion to salicylic acid; 2) due to altered pharmacokinetic results; or 3) dependent on inhibition of the cyclooxygenase-thromboxane A₂ pathway since the specific blockade of thromboxane A₂ formation with dazoxiben does not by itself produce detectable antithrombotic activity or potentiation of dipyridamole’s antithrombotic activity (67).

Drugs that inhibit thrombin. The effects of thrombin on platelets and fibrin formation can be prevented by inhibiting its action with heparin. Heparin, through its effect on antithrombin III, also inhibits other serine proteases produced during activation of the coagulation cascade, such as factors IXa, Xa and Xla (108).

Heparin. Reports of the effects of heparin on platelet reactions are complex and contradictory (109,110) perhaps, in part, because of the variability among different commercially available heparin preparations. In vitro, heparin prevents the effects of thrombin on platelets, but may potentiate or inhibit aggregation and release induced by other aggregating agents (109,110). It may itself cause platelet aggregation.

It is now recognized that heparin is heterogeneous with respect to its molecular size and its affinity for antithrombin III (111,112). More than 60% of some commercial forms of heparin are practically inactive as an anticoagulant. In one study (109), heparin induced platelet aggregation in citrated platelet-rich plasma. Fractions of high molecular weight (approximately 20,000 daltons) were more reactive with platelets than were fractions of low molecular weight (approximately 7,000 daltons). These findings raise the possibility that it may be feasible to select fractions of low molecular weight and high antithrombin III activity for antithrombotic therapy without the undesirable effects of unfractionated heparin on platelets.

Heparin combined with prostaglandins. Two observations should be considered in the relation between the effects of heparin and PGI₂ on platelet function. In the presence of PGI₂, the inhibitory effect of heparin on coagulation should be enhanced (113). On the other hand, heparin has been shown to inhibit the activation of adenylate cyclase by PGI₂ (114). Thus, a combination of heparin with one of these prostaglandins might prove beneficial in preventing the formation of thrombi to which the coagulation pathway makes a major contribution, but might be less useful if platelet aggregation in response to collagen, ADP and thromboxane A₂ has a dominant role and PGI₂ acts in a regulatory role. Overall, when well defined anticoagulant fractions of heparin become available for more extensive testing, it will be important to reassess the antplatelet effects of heparin in a variety of experimental and clinical situations.

An alternative strategy being developed to block thrombin-mediated platelet thrombus formation involves the synthesis of peptides that show specificity for the inhibition of thrombin (115–117). A number of these agents have been studied in vitro and in experimental animals. No clinical experience is available.

Agents that inhibit platelet function by other mechanisms. Many other agents have been shown to inhibit platelet reactions and thrombus formation, although their mechanisms of action remain to be defined (1–3,42).

Sulfinpyrazone. Sulfinpyrazone inhibits platelet aggregation and secretion in vitro, but at concentrations higher than that achieved after standard clinical doses (42). Inhibition of platelet aggregation and secretion after oral administration of high doses of sulfinpyrazone has also been reported (118). However, the concentration of collagen used in performing in vitro tests is critical in demonstrating an inhibitory effect of sulfinpyrazone after oral administration of the drug in humans (119). Although it has been suggested that sulfinpyrazone, in contrast to aspirin, is a competitive inhibitor of platelet cyclooxygenase, present data suggest that other mechanisms are more relevant in vivo (42,67). The inhibitory effects of sulfinpyrazone on collagen-induced aggregation in rabbits has persisted for many hours after the drug was cleared from the blood. This prolonged inhibitory effect has been attributed to mediated drug metabolites in plasma (120). Sulfinpyrazone has also been found to inhibit the formation of thrombi on subendothelium (121) and to protect endothelium from chemical injury in vitro and possibly in vivo (122). No mechanism has been identified for this postulated endothelial protective effect. In addition, a dose-dependent inhibition by sulfinpyrazone of experimental thromboembolism has been reported (67) that correlates with its capacity to normalize platelet survival in patients.
with artificial heart valves (123) and with its ability to reduce thrombotic events in patients with arteriovenous cannulas (124). Experimentally on prosthetic surfaces, aspirin also potentiates this antithrombotic effect of sulfinpyrazone, with a maximal effect at 20 mg/kg per day, the same dose found to optimally potentiate dipryidamole (67). These results with sulfinpyrazone on prosthetic surfaces have been more consistent than with biologic surfaces. Thus, despite a beneficial trend in decreasing vascular events after myocardial infarction (125) and saphenous vein coronary artery bypass (126), the results were negative in the Canadian stroke study (63) and in the Canadian unstable angina study (65).

Ticlopidine. Ticlopidine is unusual in two respects: 1) its effects are only manifest 24 to 48 hours after administration, but they last for several days after the drug is stopped; and 2) it is an inhibitor of the primary phase of ADP-induced aggregation (127,128). It also inhibits aggregation and release induced by collagen, epinephrine, arachidonic acid and thrombin, prolongs the bleeding time, prolongs shortened platelet survival (129) and inhibits intimal proliferation (130) in the rabbit aorta from which the endothelium has been removed. As well as being an inhibitor of platelet reactions in its own right, ticlopidine has been shown to enhance the inhibitory effect of PGI2 on platelets (131). Thus, of the antiplatelet drugs currently available for clinical investigation, ticlopidine is one of the most potent and has several important theoretical advantages over existing drugs. Ticlopidine is chemically unrelated to other antiplatelet drugs and appears to have a unique, albeit unknown, mechanism of action. It is neither a prostaglandin synthesis inhibitor nor a cyclic AMP-phosphodiesterase inhibitor. There are data that indicate that ticlopidine may act on the platelet membrane to alter its reactivity to activating stimuli (128). Clinical evaluation of this drug is underway in a number of European and American trials.

Antibiotics. In high doses, carbenicillin and penicillin produce a bleeding tendency in patients (132). Studies with human volunteers have shown that these antibiotics also prolong the bleeding time and inhibit platelet aggregation induced by a variety of agents. The drugs also inhibit platelet activation in vitro at concentrations greater than those obtained in vivo (133). Similar findings have been described for ticarcillin. In vitro studies suggest that these drugs interfere with the binding of substances such as ADP, epinephrine and factor VIII/von Willebrand factor with their specific receptors on the platelet membrane, perhaps as a result of properties that permit them to bind to membrane proteins or, as shown with artificial phospholipid bilayers, to platelet membrane lipids (134). The inhibitory effects in vitro are observed immediately, but the prolongation of the bleeding time occurs after 12 to 24 hours of parenteral administration (132,133). Therefore, either a metabolite or prolonged contact of platelets with lower concentrations of the antibiotics may account for the inhibition of platelet activation observed in subjects who received the drugs (134). Although these antibiotics appear to have platelet-inhibiting effects, no studies have been performed demonstrating that they prevent clinical thrombosis.

Dextran infusions. Dextran of a molecular weight of 65,000 to 80,000 daltons produces a prolonged bleeding time after patients receive one or more liters (135–137). The inhibitory effects of dextran infusions on platelet function are dose-related and are more pronounced with dextran of high molecular weight. The finding that platelets absorb dextran, with resultant changes in electrophoretic mobility, suggests that the inhibitory effect of dextran on platelet function might be due to some alteration of platelet membrane function. Other findings suggest that dextran may temporarily impair platelet function by interfering with the factor VIII/von Willebrand factor complex (138). Anti-thrombotic effects of dextran have been shown by experimental and clinical studies (3,42).

Sulocitidil. This compound, originally introduced to prevent vasospasm at high concentrations, has been reported to have a weak effect on platelet aggregation and secretion (139). Although it has been reported to be antithrombotic in some thrombotic models and to prolong shortened platelet survival time in patients with artificial heart valves (140,141), sulocitidil is ineffective in the secondary prevention of stroke (142) and in experimental arterial thromboembolism (143).

Vitamin E. This agent inhibits collagen-induced aggregation and the second phase of aggregation induced by ADP or epinephrine in citrated human platelet-rich plasma (144). It has been suggested that vitamin E may prevent the internal calcium flux that mediates internal platelet contraction and secretion induced by aggregating and release-inducing agents (145). However, the clinical antithrombotic usefulness of vitamin E remains unproved.

Other drugs. A variety of other drugs have been reported to have in vitro antiplatelet effects (1–3,42): calcium channel blockers, clofibrate and halofenate, furosemide and other diuretic drugs, serotonin antagonists, anesthetics, phenothiazines, tricyclic antidepressants, antihistamines, propranolol and other beta-adrenergic blocking agents, glyceryl guaiacolate, furadantin, monoamine oxidase inhibitors, pyridyal carbamate, hydroxychloroquine, alcohol, pyrazidine derivatives, pyridoxal phosphate and chlorotetracycline. Clinically useful antithrombotic effects have not been demonstrated using any of these agents.

Summary of Drug Effects

The principal antithrombotic effects of those drugs adequately evaluated are summarized as follows:

Aspirin. This nonsteroid anti-inflammatory agent potently and irreversibly inactivates platelet cyclooxygenase by acetylation. Although all of aspirin’s antithrombotic effects have been attributed to this blockade of thromboxane
A₂ formation by platelets, there is evidence for antithrombolytic effects independent of its inactivation of cyclooxygenase. At present, there is intense interest in aspirin therapy for vascular disease.

Aspirin has been shown in well designed clinical trials to be beneficial in at least five and possibly six clinical settings: 1) reduction of thromboembolic complications associated with artificial heart valves; 2) prevention of stroke and death in patients with transient ischemic attacks; 3) decrease in thrombotic occlusion of arteriovenous Silastic cannulas in uremic patients undergoing hemodialysis; 4) reduction in myocardial infarction and cardiac death in patients with unstable angina; 5) probable effect in the secondary prevention of myocardial infarction; and 6) possible increase in the patency of saphenous vein coronary bypass grafts. These reported benefits of aspirin require some comment. First, the antithrombotic effects of aspirin in patients with prosthetic mitral valves are evident in association with oral anticoagulant therapy, and this combination is associated with an unacceptably high frequency of gastrointestinal bleeding. Second, the capacity of aspirin in low doses to reduce the thrombotic complications of arteriovenous cannulas was shown in patients undergoing long-term hemodialysis and, thus, associated with significant platelet dysfunction. It cannot be assumed that a similar dose would be antithrombotic in the absence of associated platelet dysfunction.

Sulfipyrazone. This urocosuric agent has weak anti-inflammatory properties. The mechanism of antithrombotic action remains to be defined. Sulfipyrazone reduces arteriovenous cannula occlusion and early coronary artery saphenous vein graft occlusion. Since the claimed benefit in reducing mortality in the secondary prevention of myocardial infarction remains controversial, no recommendations can be made regarding that possible indication. The reports that it may also decrease thromboembolism in patients with artificial heart valves require confirmation.

Dipyridamole. This agent, a coronary vasodilator with weak inhibitory effects on phosphodiesterase activity, appears to increase inhibitory cyclic AMP levels in platelets by elevating blood adenosine levels through the blockade of adenosine uptake by red blood cells and vascular wall cells. Experimentally, on artificial surfaces, aspirin potentiates the antithrombotic effects of dipyridamole by mechanisms independent of platelet inactivation of platelet cyclooxygenase. Dipyridamole decreases thromboembolism in patients with artificial heart valves (in combination with anticoagulants). Dipyridamole in association with aspirin may be more effective than aspirin alone in reducing the progression of peripheral vascular disease, but this remains to be confirmed. When used in combination with aspirin, it has been shown to decrease coronary mortality and non-fatal myocardial infarction in patients who have previously sustained a myocardial infarction, to reduce both early and late coronary artery saphenous vein graft occlusion and to preserve renal function in patients with membranoproliferative glomerulonephritis. However, the benefit of adding dipyridamole to aspirin in these latter settings of biologic surfaces remains to be established.

Ticlopidine. This agent exhibits global antiplatelet activity of prolonged duration through some apparently novel mechanism of action. The fact that ticlopidine does not inhibit prostacyclin synthesis in the arterial wall, but can still inhibit aggregation induced by thromboxane A₂ and prostaglandin endoperoxide, gives it a theoretical advantage over aspirin. A number of important well designed multicenter trials are in progress. These trials should determine the ultimate clinical role of ticlopidine.

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