

Effects of Drugs on Platelet Function

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ABSTRACT

Numerous drugs and chemicals affect the function of human blood platelets. The mechanism of action of some medications is partly understood. Aspirin is the most frequently involved drug. It appears to interfere with the platelet release reaction by acetylation of a platelet membrane protein which may be involved in the synthesis of prostaglandins. Other anti-inflammatory drugs, including indomethacin, phenylbutazone, ibuprophen (Motrin) and clonixin, also interfere with the release reaction but have a shorter acting course than aspirin. Some drugs stimulate adenylyl cyclase (gliclazide) or block phosphodiesterase, (dipyridamole, caffeine) both of which actions lead to an increase in adenosine cyclic 3':5' monophosphate (cAMP) and decrease aggregation by adenosine diphosphate (ADP). These interactions should be known to clinical scientists since patients using these medicaments may manifest abnormal platelet function tests in the laboratory and mild hemorrhagic syndromes in the clinic.

Introduction

Research in platelet function during recent years has shown that platelets adhere to damaged blood vessel walls, particularly to the collagen exposed by the removal of endothelial cells.^{1,4} In subsequent reactions adenosine diphosphate (ADP) is released and induces aggregation of other platelets at the site of injury.^{8,25} The release reaction is accompanied by a change in the platelet's shape from a smooth disk to a sphere with protruding pseudopods.^{2,25} These conformational changes result in greater availability of platelet factor 3 activity which appears to reside in the phospholipid of the platelet membrane.⁴ Because ADP itself is a potent inducer of the release reaction, the process is self-sustain-

ing. Unmasking platelet factor 3 without actual separation from the platelet membrane results in local accumulation of clot accelerating activity at the site of injury with ultimate fibrin formation.

The conformational changes in the platelet surface and the release of ADP, catechol amines, and serotonin require the contraction of actinomyosin like fibrils within the platelets and the utilization of ATP.⁴ *In vitro*, the addition of collagen, ADP in various concentrations, or epinephrine to platelet-rich citrate anticoagulated plasma produces the release reaction and subsequent platelet aggregation.

Platelet aggregation can be measured by platelet aggregometer which records the decrease in optical density as the platelets

aggregate (figure 1). Collagen induces aggregation with a lag phase during which ADP is released from the platelets. ADP ($10^{-5}M$) induces immediate aggregation, but in lower concentrations a short lag phase is seen with moderate primary aggregation and a secondary aggregation phase owing to the release of more endogenous ADP from the platelets. Still lower concentrations of ADP induce slight primary aggregation which is reversible because no release of endogenous ADP occurs. Epinephrine also induces a moderate primary aggregation, release of ADP and subsequent secondary aggregation.

Platelet aggregation can be inhibited in this system by a number of drugs including antiinflammatory agents,^{18, 19, 20} antidepressants,^{12, 23} adrenergic blocking agents⁹ and a number of miscellaneous compounds such as ethanol^{3, 7} clofibrate,¹⁶ dipyridamol,^{16, 28} and others.^{15, 17, 23, 28} The mechanisms by which this inhibition occurs is only partially understood for a few of these compounds (table I).

Some drugs, aspirin for instance, clearly inhibit the release reaction induced by collagen while not interfering with aggregation caused by exogenous added ADP.^{19, 28} Recent evidence suggests that aspirin incorporates into a platelet membrane protein and permanently inactivates the platelets in the circulation at the time of exposure.²¹

Other drugs inhibit the action of ADP induced aggregation. Drugs which increase the levels of cyclic AMP in the platelet interfere with both ADP induced primary aggregation and with ADP induced release reaction.^{16, 22, 23}

At least two direct mechanisms for increasing the cAMP concentration exist in platelets (figure 2), and other indirect mechanisms may also be found.²³ Drugs which stimulate adenylcyclase activity increase the conversion of ATP to cAMP.¹⁰ Similarly, drugs which inhibit cAMP phosphodiesterase also increase cAMP

and inhibit ADP induced aggregation and release.^{12, 16}

Aspirin is the leading drug interfering with platelet function because it is so commonly used and because its effect on the platelet appears to be permanent. The entire cohort in the circulation at the time of aspirin ingestion appears to be involved and new platelets must be produced to

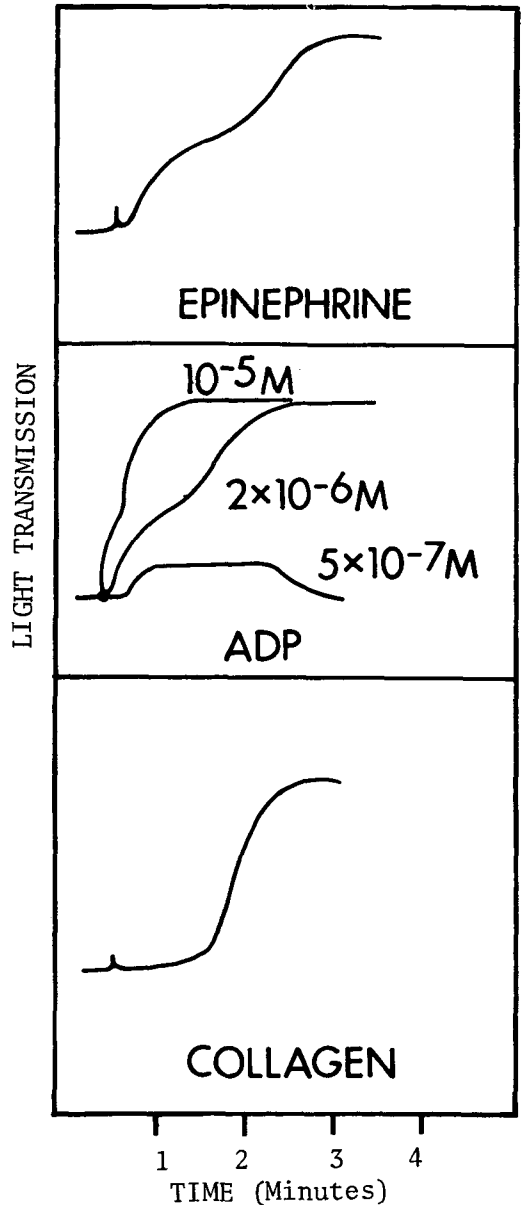


FIGURE 1. Aggregation of platelets.

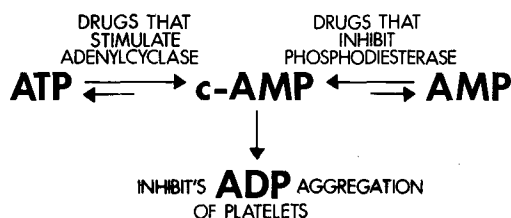


FIGURE 2. Mechanisms for increasing cAMP concentration in platelets.

overcome the defect.^{10,23,28} This, together with the evidence that ¹⁴C labeled acetyl from aspirin labels the platelet membrane while ¹⁴C labeled salicylic acid from aspirin does not, suggests a permanent change occurs in the platelet membrane from the acetylation of a membrane component. Roth and Majerus have isolated a platelet membrane protein (MW 55,000) labeled with ¹⁴C after exposure to ¹⁴C acetyl salicylic acid and speculate that the protein may be the active site of prostaglandin synthetase.^{21,22,23,24} This interaction leads to a prolongation in bleeding time even in normal people.¹⁴

Over 200 drug products on the present pharmaceutical market contain aspirin.¹¹ These drugs should not be prescribed for,

TABLE I

Agents Which Interfere With Platelet Function

Mechanism	Drug
Inhibits release reaction:	Aspirin ^{18,19,20,23,27,29} (antipyretic) Clonixin ²⁵ (analgesic) Dipyridamole ^{16,22,27} Gliclazide ¹⁰ (antidiabetic) Ibuprophen ²⁰ (antiinflammatory) Phenylbutazone ^{23,27} (antiarthritic)
Stimulates adenylylase: (increases cyclic AMP)	Gliclazide ¹⁰ (antidiabetic)
Inhibits phosphodiesterase: (increases cyclic AMP)	Caffeine ^{12,15,23} (stimulant) Dipyridamole ^{16,22,27} Imipramine ^{2,17} (antidepressant) Amitriptyline ^{2,17} (antidepressant)
Blocks collagen receptor:	Phentolamine ^{9,18} (adrenergic blocker) Benadryl ²⁶ (antihistamine)

nor taken by, patients with hemophilia or other plasma coagulation defect, since the platelet is their primary and possibly only hemostatic mechanism.¹³ Furthermore, patients who present with minor bleeding episodes, intermittent bruising, epistaxis and melena who are suspected of having a hereditary platelet defect such as, thrombasthenia, thrombocytopathy, storage pool disease or Portsmouth syndrome, should be carefully interrogated about prescription and proprietary preparations they may be using. Many of the commonly used non-prescription drugs contain aspirin, but give no clue by their names.*

Other antiinflammatory and analgesic agents, such as phenylbutazone,^{6,20,28} ibuprophen²⁰ commonly known as Motrin and used in systemic lupus,⁵ and a new analgesic clonixin,²⁶ also inhibit the release reaction and aggregation induced by collagen but not the aggregation caused directly by addition of ADP. These drugs have a shorter lasting effect on the platelet which is measured in hours rather than days as contrasted with aspirin. Many of these drugs "stabilize" membranes and their mechanism of action is thought to be related to this membrane activity.¹⁵

Still other drugs interfere with platelet function by increasing cAMP directly or indirectly. Gliclazide, a new oral hypoglycemic agent, inhibits the release reaction to a moderate degree. More importantly, however, it stimulates the activity of adenylylase.¹⁰ Thus, either ATP or adenosine is rapidly converted to cAMP and aggregation is inhibited.

Phentolamine is a blocking agent to the adrenergic receptor sites and its presence inhibits epinephrine induced aggregation. Some reduction in ADP induced aggregation occurs with this drug and a partial blockade of the ADP receptor site has been postulated.⁹

* Alka Seltzer, Arthra-Zene Caps, Babylove, Cope, Coricidin, Midol, Percodan Demi, Stanback, Vanguish and Quiet World.

Some drugs increase cAMP by inhibition of phosphodiesterase.^{12,16} Mills and Smith have shown that dipyridamole and other pyrimido-pyrimidine compounds potentiate the effects of prostaglandin E₁ which stimulates adenylcyclase.¹⁶ Dipyridamole and aspirin have such potent effects on platelets they are being tried as antithrombotic agents in patients with a tendency toward thrombosis.²⁸ Unfortunately, the doses of dipyridamole required to produce an effect *in vivo* are so high that vasodilation and hypotension are frequent side effects. Other anti-inflammatory agents, including indomethacin,^{6,20,23,28} are almost as active as aspirin inhibiting aggregation, but their effects are short lived and would be potentially important only in patients taking regular high doses. Phenylbutazone is a relatively weak inhibitor, but it has occasionally been associated with bleeding.

Antiinflammatory agents may inhibit the interaction between actomyosin and ATP,⁶ by increasing cAMP,^{16,22,23} or they may stabilize the platelet membrane by molecular interaction with membrane components,²³ or they may block a receptor⁸ which is necessary for initiation of the release reaction.

Local anesthetics, phenothiazines and tricyclic antidepressants such as imipramine (Tofranil) and amytryptaline (Elavil) in concentrations of 10 to 50 μ M inhibit the release reaction.¹⁷ Even benadryl (50 μ M) inhibits the second wave of ADP induced aggregation, but less so than the phenothiazines and tricyclic antidepressants.²⁷ While benadryl is not usually associated with bleeding episodes, O'Brien has shown that the bleeding time is prolonged when the puncture sites have been previously injected with benadryl.²⁰

Finally, ethanol in susceptible patients has been shown by Cowan et al to prolong the bleeding time, impair primary

and secondary aggregation and reduce platelet factor 3 availability *in vivo*.^{3,7} While no major changes in platelet structure or metabolism were detected in most patients receiving ethanol, at least one patient who developed thrombocytopenia during ethanol infusion showed significantly reduced intracellular ADP.⁷ Platelets from this patient showed giant granules, poor contractile activity and reduced aggregation. The authors suggested an ethanol induced ADP storage pool defect in this patient.

In summary, there are numerous drugs and chemicals which affect platelet function. The mechanism of action of some of these medications is partially understood and should be familiar to the clinical scientist since use of these drugs may cause major or minor hemorrhagic syndromes in susceptible patients.

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