EDITORIAL

Why Aspirin?

RECENT INTEREST in the use of aspirin as an agent for prevention of myocardial infarction has focused attention on the pharmacology of the drug.¹ The rationale for the use of aspirin depends on the hypothesis that platelet aggregation and release have a role in the pathogenesis of myocardial infarction and/or atherosclerosis. The evidence for the hypothesis will not be presented here but it is certainly far from conclusive. Rather, I will present recent data which elucidates the role of prostaglandin synthesis in platelet physiology and the mechanism by which aspirin interferes with prostaglandin synthesis.

Role of Platelets in Hemostasis

The details of the mechanisms by which platelets function in hemostasis are essentially unknown. While a number of experimental observations suggest that collagen, cyclic prostaglandin endoperoxides, and adenosine diphosphate (ADP) play central roles in the processes of platelet aggregation and secretion, the precise role of these agents in vivo hemostasis and their relationship to each other are not clear.

The first step in hemostasis that can be defined morphologically is the adhesion of platelets to collagen or other substances found in the tissues beneath the vascular endothelium. This interaction does not explain the major growth of the platelet plug, however, since most platelets do not come into contact with the vessel wall. When platelets aggregate they secrete certain granular constituents in a process analogous to secretion reactions in other cells. The secreted materials include coagulation factors, vasoconstrictors, and other substances which promote further aggregation, thrombus formation, and hemostasis (for recent review, see reference 2). This secretion reaction is termed the release reaction.

Most investigators postulate that aggregation and release are mediated by adenosine diphosphate (ADP), or by prostaglandin G₂ or thromboxane A₂ (see below⁶,⁷) released by the platelets initially interacting with the vessel wall. According to this hypothesis, platelet aggregation and secretion or release occur prior to and independent of the coagulation reactions leading to fibrin formation. An alternative hypothesis suggests that minute amounts of thrombin which are formed prior to generation of a fibrin clot serve as the initial stimulus to platelet function in hemostasis. Thus, a number of potentially important hemostatic reactions are catalyzed by concentrations of thrombin well below that required to clot fibrinogen. These include the activation of factors VIII and V as well as the initiation of platelet aggregation and the release reaction. This hypothesis has not been subject to test since assays which could detect minute amounts of thrombin which may be generated early in hemostasis are not available.

Inhibition of Platelet Function by Aspirin

Early experiments indicated that aspirin blocks the platelet release reaction induced by ADP and collagen in vitro.⁴,⁷ Furthermore, aspirin minimally prolongs the bleeding time of normal subjects, but markedly prolongs the bleeding times of patients with coagulation factor defects,⁷ indicating an in vivo effect of aspirin. Further studies indicated that the observed defects occur at low aspirin concentrations, <50 μM, which are easily achieved in vivo by an aspirin dosage of no more than 300–600 mg, and that the defects persist for the life-span of the aspirin-treated platelet. Vane⁶ originally showed that aspirin is a potent inhibitor of prostaglandin synthesis in several tissues. Smith and Willis demonstrated that aspirin inhibits platelet prostaglandin synthesis and postulated that the inhibition of prostaglandin synthesis by aspirin might account for the antiplatelet action of the drug.¹¹ It was difficult to support the hypothesis since classical prostaglandins do not cause platelet release and some (e.g., PGE₂) even inhibit the platelet release reaction.¹² The recent work of Samuelsson and co-workers has greatly clarified the role of prostaglandin synthesis in platelet reactions.

Role of Prostaglandin Synthesis in Platelet Reactions

Arachidonic acid exists in platelets and other cells esterified to the 2 position of glycerol of phospholipids. Addition of thrombin or collagen to washed platelets is thought to stimulate a specific lipase (phospholipase A–2) resulting in arachidonic acid hydrolysis. Arachidonic acid serves as substrate for an enzyme cyclooxygenase (fig. 1) which catalyzes the formation of labile cyclic endoperoxide forms of prostaglandins designated prostaglandin G₂ and prostaglandin H₂ (PGG₂, PGH₂).³ This reaction appears to be regulated by the availability of substrate and thus stimulation of platelets presumably provides arachidonic acid substrate for the enzyme. Unlike the classical prostaglandins, the cyclic endoperoxides are potent inducers of platelet

From the Divisions of Oncology and Hematology, Departments of Internal Medicine and Biochemistry, Washington University School of Medicine, St. Louis, Missouri.

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Address for reprints: Dr. Philip W. Majerus, Divisions of Hematology and Oncology, Washington University School of Medicine, 660 South Euclid, Box 8125, St. Louis, Missouri 63110.

357
aggregation and release and thus can explain the stimulation of the platelet release reaction by the prostaglandin pathway. The further metabolism of these cyclic endoperoxides is also of interest since it appears that over 95% of these compounds are converted to non-prostanoid metabolites and only a small fraction are converted to classical prostaglandins such as PGE₂ and PGF₂α.¹² One of the non-prostaglandin metabolites recently has been found to be even more potent as a vasoconstrictor and platelet aggregating agent than PGG₂. This compound has been designated thromboxane A₂ by Hamberg et al.⁴ and is the most potent vasoconstrictor ever described (100-fold more potent on a molar basis than angiotensin II).¹⁴ These compounds are extremely labile in aqueous media (PGG₂ T½ = 5 min, thromboxane A₂ T½ = 30 sec) and these properties suggest a possible physiological role in primary hemostasis. The most direct evidence that these new compounds are of physiological importance comes from the recent description of a patient with a mild bleeding disorder who apparently lacks the cyclo-oxygenase enzyme and is thus unable to form cyclic endoperoxides or thromboxanes.¹⁴

Inhibitory Mechanism of Aspirin on Prostaglandin Synthesis

Hamberg and co-workers⁴ as well as Smith and Willis⁵ have demonstrated that aspirin blocks the conversion of arachidonic acid to the pharmacologically active intermediates. We have recently defined the mechanism of this inhibition. By using radioactive acetylsalicylic acid (fig. 2) labeled in the acetyl moiety, we have demonstrated that aspirin acetylates a single protein of 85,000 molecular weight from the particulate fraction of human platelets.¹⁶ We have postulated that the acetylated protein is cyclo-oxygenase.¹⁷ Thus, study of cyclo-oxygenase preparations purified from sheep seminal vesicles shows that the acetylated protein copurifies with cyclo-oxygenase.¹⁷ Further, arachidonic acid, the cyclo-oxygenase substrate, inhibits the acetylation reaction competitively. Other cyclo-oxygenase inhibitors, including fatty acid analogues and indomethacin, inhibit enzyme activity and acetylation in parallel.¹⁸ Thus we conclude that aspirin acetylates some residue in the active site of cyclo-oxygenase, thereby inactivating the enzyme.

The evidence that the acetylation explains the inhibitory action of aspirin on platelets includes the following: 1) The time course and aspirin concentration dependence of the acetylation reaction parallels the same parameters of aspirin’s effect on platelet function. 2) Acetylation is permanent and persists for the life-span of the aspirin-treated platelet. The latter point was demonstrated by treating normal subjects with aspirin and then measuring the degree of protein acetylation achieved in vitro using platelets isolated from blood drawn from 1 to 13 days later.¹⁹ We assumed that any cyclo-oxygenase present in circulating platelets at the time of aspirin ingestion would be permanently acetylated. Since platelets are not capable of significant protein synthesis, any protein acetylation observed in vitro at later times after aspirin ingestion would reflect new platelet production. Immediately after and 1 day after aspirin ingestion there was no detectable protein acetylated in vitro by [acetyl-³H]-aspirin. We subsequently observed increasing acetylation over the next 13 days, finally reaching pre-aspirin levels by day 13. The time course of reappearance of acetylation agrees with the known platelet survival time of 9–12 days. It is clear from measurement of cyclo-oxygenase activity, as well, that the inhibition by aspirin is permanent and this result has been used recently to measure platelet survival.¹⁸

The hypothesis that platelet aggregation and secretion are mediated through the prostaglandin metabolites is attractive in that it provides a possible rationale for the efficacy of polyunsaturated fatty acids in the diet. Thus, as linoleic acid or other polyunsaturated fats are increased in proportion to arachidonic acid in the 2 position of glycerol in platelet lipids the synthesis of prostaglandins would be decreased upon stimulation by thrombin or other agents. This decrease might alter the sensitivity or threshold of platelets responding to any hemostatic stimulus. From in vitro experiments using human platelets it is known that linoleic acid and other polyunsaturated fatty acids can inhibit prostaglandin synthesis by competing with arachidonic acid for the cyclo-oxygenase enzyme. This results in diminished platelet reactivity.¹⁹ An in vivo effect on platelet sensitivity has also been demonstrated in dietary experiments using rats fed dihomo-γ-linoleic acid. Platelets from these animals showed decreased aggregation responses to added collagen or epinephrine.²⁰ Whether dietary manipulations actually alter the proportion of arachidonic acid to other polyunsaturated fatty acids in the platelets of man remains to be demonstrated.

While the above hypothesis regarding prostaglandins is interesting, it seems clear that prostaglandins or their intermediates are not primary or obligate intermediates in platelet function in hemostasis. This fact is most clearly illustrated by the finding that thrombin can induce full platelet aggregation and release in either aspirin-treated platelets²¹ or in platelets from the patient with cyclo-oxygenase deficiency.¹⁴ Similarly, while collagen-induced aggregation is inhibited by aspirin, the inhibition can be overcome by higher concentrations of collagen. Thus, it seems more likely that prostaglandins may serve as positive modulators of
platelet reactions which potentiate the effects of other aggregating agents. This might also explain why aspirin induces only a minimal hemostatic defect in normal subjects, where thrombin generation can overcome the platelet defect. According to this hypothesis, hemophiliaacs and other patients with coagulation factor defects with impaired thrombin generating capacity would depend greatly on the potentiating effects of prostaglandins to maintain normal platelet function. This could explain why these patients may have a severe hemostatic defect after aspirin therapy.

Therefore, I would suggest that aspirin induces a mild defect in platelet function which may serve only to increase the threshold for platelet aggregation and release in normal subjects. Whether this mild alteration in function is sufficient to prevent or diminish platelet aggregation which could lead to thrombosis can only be answered by clinical trials. However, the very mild nature of the defect induced makes aspirin particularly attractive as an agent to be used as a preventive medicine (i.e., low toxicity). Even if the incidence of myocardial infarction or atherosclerosis is reduced only 10–20%, the therapy of many “normal” individuals could be justified. Since aspirin results in permanent inhibition of prostaglandin synthesis, therapy does not require continuous blood levels of the drug, thus making it the most desirable of the drugs which inhibit platelet function by interfering with prostaglandin synthesis. The fact that other prostaglandin synthesis inhibitors can block acetylation of cyclo-oxygenase by aspirin suggests that combinations of agents such as sulfinpyrazone or indomethacin with aspirin would not be expected to be more effective than aspirin alone and in fact might actually decrease the effectiveness of aspirin by blocking acetylation of cyclo-oxygenase.

I have not discussed the feasibility of clinical trials of aspirin in prevention of either initial or subsequent myocardial infarctions but numerous authors have pointed out the obvious difficulties relating to the ready availability of the drug without prescription and the large intake of aspirin by potential control subjects for a randomized trial. It seems likely that high risk subjects who become aware of the theoretical benefits of aspirin therapy are likely to take the drug without waiting for the results of trials designed to determine its efficacy.

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Editorial: Why aspirin?
P W Majerus

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