BRIEF REPORT

Aspirin Ingestion Compared with Bleeding Disorders—Search for a Useful Platelet Antiaggregant

By Harvey J. Weiss

PLATELETS PARTICIPATE in both hemostasis and thrombosis by forming aggregates on an injured intimal surface. Although the mechanisms ultimately responsible for both processes are unknown, recent studies suggest that the release of intrinsic platelet adenosine diphosphate (ADP) plays a major role in the formation of a platelet aggregate. These studies have demonstrated that when platelets are stirred with connective tissue or purified collagen, ADP is released, which results in their aggregation. Packham and coworkers demonstrated that when phenylbutazone, an agent which inhibits ADP release, is administered to dogs, the arrest of bleeding in transected mesenteric vessels is markedly retarded. Since the intimal attachment of a thrombus, particularly of an arterial thrombus, consists primarily of platelets, the question naturally arises as to whether agents which inhibit platelet aggregation may, in addition to producing a defect in primary hemostasis, also protect against the formation of arterial thrombi.

Recent studies indicate that collagen-induced aggregation of human platelets is significantly decreased after aspirin ingestion and that this defect is associated with, and probably due to, a decreased release of platelet ADP. Aspirin also inhibits the response of platelets to epinephrine. Whereas the addition of epinephrine to platelet-rich plasma results in two waves of aggregation, the second of which is due to the release of platelet ADP, only the first wave is seen after aspirin ingestion or after its addition in vitro. The effects of a single oral dose may be detected for four–seven days. Unlike aspirin, ingestion of sodium salicylate neither prolongs the bleeding time nor significantly inhibits platelet ADP release.

The effects produced by aspirin are similar, in many ways, to those observed in some patients with idiopathic bleeding disorders. In 1967, Hardisty and Hutton, and Weiss independently described a group of patients with bleeding disorders in whom one of the major findings was an impairment of collag-
induced platelet aggregation associated with, and presumably due to, inhibition of platelet ADP release. This type of defect may be familial in some cases.\textsuperscript{13} Similar abnormalities in collagen-induced platelet aggregation were also described by Hirsch, Castelan and Loder,\textsuperscript{14} O'Brien,\textsuperscript{15} and, more recently, by Caen, Sultan and Larrieu,\textsuperscript{16} and Sahud and Aggeler,\textsuperscript{17} although in the cases described by these investigators an associated abnormality in collagen-induced ADP release was either not found\textsuperscript{14} or not reported.\textsuperscript{15-17} We have tentatively called the condition thrombopathia because, in most cases, a defect in platelet-factor 3 (PF-3) release was also present and was, similarly, associated with a decreased release of platelet ADP.\textsuperscript{12} The studies cited strongly suggest that the hemostatic defect (prolongation of the bleeding time) induced by aspirin is a result of its inhibitory effect on platelet aggregation and further suggest the possibility that aspirin, or drugs with similar properties, may be useful antithrombotic agents in man. It would seem reasonable to assume that the antithrombotic properties of a drug might be proportional to the magnitude of both its antithrombotic activity and to the accompanying defect in hemostasis. We wish to report here on the relative magnitude of the aspirin-induced defect by comparing the effects produced by aspirin with the findings in patients with thrombopathia. For comparison, and to further emphasize the nature of the aspirin induced defect, the findings in patients with other disorders of primary hemostasis are also reported.

**Subjects**

Patients with bleeding disorders are defined as follows:

(a). Glanzmann's thrombasthenia (TS)—absent clot retraction and failure of platelets to aggregate with ADP. (b). von Willebrand's disease (VW)—prolonged bleeding time and decreased values of antihemophilic globulin (AHG) or, occasionally, less-rigid criteria where indicated by other studies.\textsuperscript{18} (c). thrombopathia (TP)—impaired release of PF-3 activity and decreased aggregation by collagen associated, in both cases, with a diminished release of platelet ADP.

Studies on some of these patients have been reported previously.\textsuperscript{12,18,19} The data reported here include some of the results of more recent studies on these and recently discovered patients. More extensive details will be presented elsewhere.

The effects of aspirin ingestion were studied in 10 normal subjects who received 3 Gm. per day for 2½ days [high dose, first aspirin study\textsuperscript{6}], six different subjects who received a single 1.5 Gm. dose [low dose, second aspirin study\textsuperscript{8}] and four more-recently studied normal subjects (third aspirin study) treated the same as in the second study. Tests were performed two hours after the last aspirin dose in the first (high dose) study and two hours after ingestion in the second and third single dose studies.

**Methods**

In vitro bleeding time was performed on a 3-mm. deep forearm puncture and platelet adhesiveness to glass beads determined by a modification of Salzmann's method.\textsuperscript{12} Kaolin-induced PF-3 activity and collagen (connective tissue)-induced platelet aggregation were
determined using citrated platelet-rich plasma (PRP) and the amount of ADP released during these tests determined as previously described.\(^6,8,12\) The suspension of connective tissue used in the original study of patients with bleeding disorders\(^12\) and in the first aspirin study\(^9\) was different from that in the second, and later, aspirin study.\(^8\) The values shown in this report, some of which were obtained by restudying the original patients, were all obtained with the later suspension, which was prepared from human subcutaneous tissue as previously described\(^8\) and kept frozen in aliquots until the day used. Platelet aggregation by epinephrine was studied at 37\(^\circ\)C.\(^8\)

In the aspirin studies, reactivity to kaolin (10 subjects) and platelet adhesiveness (four subjects) were determined in the first study only. Bleeding times were not performed during the third study (four subjects).

Control values for collagen-induced aggregation and ADP release were obtained by studying 48 normal male and female subjects, ages 22-50, all of whom were requested to abstain from all medications for one week prior to testing, although this request was not specifically made for all of the control subjects used in the other studies. A similar request was made of the patients.

To determine the means, S.E. and S.D. for the kaolin-induced reactions (PF-3 and ADP release), the values were first converted to logarithms, since it was found that the distribution of these transformed values more closely approximated a normal curve than did the distribution of the original values.\(^20\)

**RESULTS**

The analysis of the results obtained on 16 subjects in the previously reported aspirin studies\(^6,8\) was designed to determine the change in values in each subject after aspirin ingestion. So that the results obtained after aspirin ingestion may be compared with those in a large normal population and in patients with bleeding disorders, only the postingestion values are shown here. The results obtained on all tests are summarized in Table 1.

The hemostatic defect produced by aspirin, as reflected by the increase in bleeding time, is generally less severe than in patients with idiopathic bleeding disorders (Fig. 1). This may also be reflected by the results obtained on tests of platelet adhesiveness to glass beads (Fig. 1). In patients with bleeding disorders, platelet adhesiveness was almost invariably decreased, although the mechanism responsible for this abnormality may not be the same for each disorder. Following aspirin ingestion, platelet adhesiveness values did not differ from those obtained in 58 normal subjects.

| Table 1.—Degrees of Abnormality in Bleeding Disorders and after Aspirin Ingestion * |
|---------------------------------------------|-------------------|-----------------|--------------------------|-------------------|
| von Willebrand's | Glanzmann's Thrombasthenia | Thrombopathia | Aspirin |
| **Number Studied** | 15 | 3 | 7 | 20 |
| **Bleeding Time** | ++ + | + + + + | + + | + |
| **Platelet Adhesiveness** | ++ | + + + + | + | 0 |
| **Reactivity to Collagen** | Platelet Aggregation | 0 | + + + + | + | + |
| ADP Release | 0 | + + + 0 | + + | + |
| **Aggregation by Epinephrine †** | Primary | 0 | + + + | 0 to + + | 0 |
| Secondary | 0 | + + + | + + + | + + + + |

* Average degree of abnormality indicated from 0 (none) to + + + + (marked), adapted from data shown in Figs. 1–3.

† Final Concentration 5μM.
As shown in Fig. 2, collagen-induced platelet ADP release is diminished after aspirin ingestion. The abnormality is similar to the defect in patients with thrombopathia, but is present to a lesser degree. Consistent with this difference, collagen-induced aggregation, which is ultimately caused by released ADP, is inhibited less after aspirin ingestion than in the thrombopathic patients. In Glanzmann’s disease, where there is no impairment in ADP release, the platelets nevertheless do not aggregate with collagen. In this disorder, the platelets are totally unreactive to ADP, and hence do not aggregate with collagen even though ADP is normally released. In von Willebrand’s disease, both ADP release and aggregation are normal.

In thrombopathia, the defect in ADP release is also strikingly demonstrated by the abnormal response of their platelets to kaolin (Fig. 3). Kaolin-induced platelet ADP release is markedly diminished and, since ADP is required for the activation of PF-3, the latter is significantly abnormal. The results obtained in normal subjects after aspirin ingestion again demonstrates the lesser magnitude of the aspirin-induced defect. As reported previously, the amount of ADP released from platelets by kaolin is significantly decreased, compared with the subjects pretreatment values after aspirin ingestion. However, when only the posttreatment values themselves are considered, and compared with those
Fig. 2.—Degree of platelet aggregation (left) and ADP release (right) produced by stirring platelet-rich plasma with standard suspension of connective tissue (collagen). Results obtained in 42 and 30 normal subjects (N), and in patients with von Willebrand's disease (VW), Glanzmann's thrombasthenia (TS) and thrombopathia (TP) compared with values obtained in normal subjects who ingested a low dose of aspirin (ASA<sub>L</sub>) (see subjects). * means that amount of ADP released was less than that indicated by point, the lowest amount detectable on day assay performed. Ninety-five per cent confidence limits of normal values (-----) and their means (- - -) indicated. Some data adapted from Ref. 8.

obtained in 70 normal subjects (Fig. 3), it is seen that the defect in ADP release is minimal and, in most cases, the values obtained are still within the 95 percent confidence limits of the normal values. Perhaps as a consequence, the values obtained for PF-3 activity after aspirin ingestion were completely normal. In von Willebrand's disease, ADP is released normally, similar to the results obtained with connective tissue, and there is no defect in PF-3 activation. In Glanzmann's disease, PF-3 activity is abnormal and, in contrast to the results obtained with connective tissue, ADP release is decreased.

Aspirin completely inhibits the second wave of epinephrine-induced platelet aggregation, which requires ADP release, but has no effect on primary aggregation. In thrombopathic patients, the first wave is normal in most patients, but decreased in some. The second wave is almost invariably absent. Both phases are normal in patients with von Willebrand's disease.

DISCUSSION

The inhibitory effect of aspirin on platelet aggregation is now well established and appears to provide the best explanation, at present, for the mild
defect in hemostasis which occurs following its ingestion. The results of these studies indicate that this type of platelet defect produced in normal subjects after aspirin ingestion is similar, in some respects, to the abnormality observed in some patients with primary bleeding disorders. In both cases, collagen-induced platelet aggregation is impaired and is associated with decreased release of platelet ADP, although the mechanism producing this defect may not necessarily be the same. The magnitude of both the platelet defects and the impairment of hemostasis (bleeding time prolongation) is, in general, less after aspirin ingestion than in the patients with thrombopathia. The inhibitory effect of aspirin on platelet aggregation suggests its possible use as an antithrombotic agent and recent studies indicate that aspirin ingestion prevents some types of experimentally induced arterial thrombosis in dogs. Because of its generally low toxicity, aspirin would appear to be the logical agent to use in initial clinical trials designed to evaluate the potential antithrombotic properties of agents which inhibit platelet aggregation. While it is tempting to speculate that the antithrombotic properties of a drug may be related to the
magnitudes of its antiaggregant activity, in the final analysis this can only be determined by clinical trials and it is to be anticipated that such trials will be forthcoming. Nevertheless, aspirin appears to be a weak antiaggregant and this suggests that other, more potent agents, may prove to be more effective in the treatment of thrombosis.

**Summary**

The effects on human platelets produced by ingesting aspirin have been compared with the types of platelet abnormalities observed in patients with bleeding disorders. The inhibitory effect of aspirin on platelet function appears to be relatively weak and suggests a continued search for antiaggregant agents which may prove to be useful in preventing thrombosis.

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**References**

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