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ASPIRIN AND PLATELETS-A BRIEF REVIEW

by

Rowen K. Zetterman

1969

A THESIS

Presented to the Faculty of
The College of Medicine in the University of Nebraska
In Partial Fulfillment of Requirements
For the Degree of Doctor of Medicine

Under the Supervision of Carol R. Angle, M.D.

Omaha, Nebraska

February 3, 1969

To the members of the Thesis Committee:

This paper is being published in CLINICAL TOXICOLOGY,
Volume I (4), 1968. In the absence of a reprint at this
time, a typewritten copy is submitted for your approval.

Respectfully,

Rowen K. Zetterman

Rowen K. Zetterman

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ASPIRIN AND PLATELETS-A BRIEF REVIEW

Introduction

It has become increasingly apparent that ingestion of aspirin can bring about alteration of hemostatic parameters in normal subjects. Hypoprothrombinemia has been observed in patients 24-36 hr. after taking relatively large and repeated doses of aspirin^{1, 2}, with complete reversal by administration of vitamin K. Gastrointestinal bleeding, unrelated to prothrombin levels, also occurs following aspirin ingestion. This has generally been attributed to a direct gastric mucosal irritant effect³ but is also seen following intravenous administration of aspirin⁴. Bone marrow depression with resultant thrombocytopenia has been reported^{5, 6} and is thought to be secondary to a systemic allergic mechanism. Within the last few years, one of the most intensively studied facets of aspirin is its effect on platelets and platelet aggregation.

Platelet Function

Platelets have two important properties, "adhesiveness" and "aggregation." O'Brien⁷ defines adhesiveness as the property of platelets to adhere to a foreign substance and can be measured by passage of platelet-rich plasma through a glass bead column⁸. Platelets will adhere to glass, collagen, and damaged

cells but a difference in cation dependency suggests different mechanisms for platelet adhesion to glass as compared to organic particles.

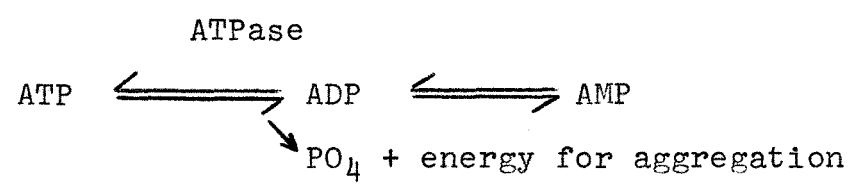
"Aggregation" of platelets is the property of platelets to clump together and is usually measured turbidometrically⁹. Platelet aggregation is calcium-magnesium dependent and is induced by ADP¹⁰ or an initiating factor. Initiating factors may be grouped into a composite of six categories¹¹⁻¹³:

- (1) soluble enzymes such as thrombin and trypsin,
- (2) particulate matter such as collagen,
- (3) amines such as norepinephrine, 5-hydroxytryptamine, and epinephrine,
- (4) saturated fatty acids,
- (5) triethyltin,
- (6) centrifugation.

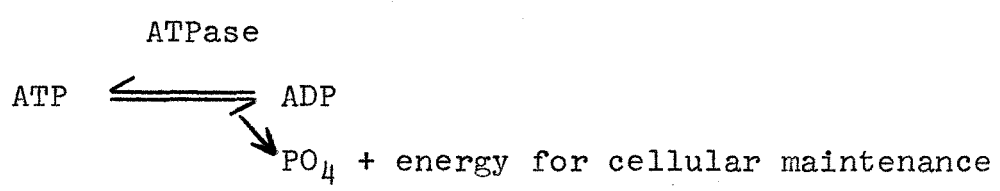
The aggregation produced by initiating factors when added in proper concentrations occurs in two phases: the first produced by the addition of the initiating factor (e.g., extrinsic ADP) and the second by further release of ADP from the platelets¹⁴. An increase in the amount of the initiating factor added to platelet-rich plasma produces monophasic clumping by "abolishing the delay"¹⁴ in the release of ADP from the platelet. Thrombin will produce aggregation after a delay of 5-10 sec. presumably due to conversion of platelet ATP to ADP by thrombin.

One hypothesis for ADP-induced platelet aggrega-

tion is that conversion from nonsticky to sticky platelets is an energy requiring process supplied by breakdown of ATP to ADP¹⁵. Inhibition of aggregation by AMP and adenosine would appear to act by product inhibition.



Salzman et al.¹⁶, however, proposes that platelets remain nonaggregated by an active energy-supplying mechanism which aids the platelet in preservation of its size and shape. The energy is supplied by breakdown of platelet ATP to ADP.



When ADP is added to platelet suspension, the ATP is blocked by product inhibition, preventing energy production. The low energy state allows the platelet to swell, exposing receptor sites, and permits secondary bridging by fibrinogen (or another plasma protein) and calcium. Chambers et al¹⁷ has described an "ecto-ATPase"¹⁸ present on platelets which is inhibited by thrombasthenia

antisera and is calcium-magnesium dependent. This may be the needed ATPase to fit the theory of energy production.

The Effect of Aspirin

In 1966, Quick¹⁹ introduced the "aspirin tolerance test" whereby the ingestion of 1.3 gm. of aspirin resulted in a small "but significant" increase in the bleeding time of more than one-half of his normal subjects. This effect was not seen with 1.15 gm. of sodium salicylate. Quick attributed the increased bleeding times with aspirin to its acetyl linkage, suggesting that it was perhaps a competitive inhibitor of an enzyme in the platelet.

Aspirin and Platelets

Using a glass-bead method for determination of platelet stickiness, Morris²⁰ noted a depression of platelet stickiness greater than 10% with 0.5 and 1.0 gm. in a dose-response fashion. The bleeding time was also prolonged. Using Born's method²¹, Morris also showed that in vitro addition of salicylic acid produced an inhibition of ADP-induced aggregation.

When platelet-rich plasma is drawn from subjects who have ingested aspirin, Weiss and Aledort²² demonstrated significantly decreased platelet aggregation in the presence

of added connective tissue. This decreased aggregation was associated with an impaired ability to release intrinsic platelet ADP. Primary aggregation of platelet-rich plasma by added ADP was not impaired. Zucker and Peterson²³ have also demonstrated that the secondary aggregation phase (presumably secondary to release of endogenous ADP activity) produced by added ADP is abolished 1-2 hr. following ingestion of 1.3 gm. of aspirin, but the primary aggregation phase is unaffected.

O'Brien^{24, 25} has been able to demonstrate that ingestion of as little as 0.15 gm. of aspirin will inhibit the secondary aggregation phase of adrenalin on platelet-rich plasma from these volunteers. This abnormality was found to persist for 2-5 days, in the absence of residual serum aspirin. However, incubation of "normal" platelet-rich plasma with "aspirin" platelet-rich plasma on subsequent days after aspirin ingestion resulted in a normal biphasic response to adrenalin, presumably by release of intrinsic ADP from the "normal" platelets. Ingestion of sodium salicylate had no effect on secondary aggregation by adrenalin of the patient's platelet-rich plasma. This finding would suggest that the short-lived aspirin molecule may permanently damage a single enzyme system of the platelet¹². MacMillan²⁶ also feels that the action of aspirin is "inside" the platelet because primary phase aggregation by the addition

of extrinsic ADP is not affected.

Because aspirin inhibits the second wave of aggregation induced by added ADP, adrenalin, collagen, triethyltin, and centrifugation, O'Brien has inferred that the basic mechanism is inhibition of release of intrinsic ADP. Because aspirin blocks the self-stimulating cyclic release of ADP, both O'Brien and MacMillan have suggested aspirin might be ideal as an antithrombotic agent.

O'Brien²⁷ has also shown that other anti-inflammatory agents can inhibit secondary aggregation of platelet-rich plasma by adrenalin. He proposed two alternate theories for these findings: (1) that these drugs inhibit inflammatory changes and the platelet response by the same mechanism; or (2) that their anti-inflammatory activity is mediated (at least in part) by the effect on platelets.

The effect of aspirin on platelet aggregation has many implications. Quick¹⁹ has shown that aspirin ingestion can be used as a diagnostic aid in evaluation of patients with clinical Minot-Von Willebrand syndrome. In addition, he suggests that his "aspirin tolerance test" may be used to detect the syndrome in patients with a characteristic history of bleeding and a typical hereditary pattern, but with a normal bleeding time. Such prolongations of the bleeding time suggests that a platelet

function is impaired.

Thrombasthenin is postulated as a surface enzyme leading to contractility that is absent in one type of thrombasthenia and considered by Chambers¹⁷ to be identical with "ecto-ATPase." After preparing antibody to thrombasthenin he demonstrated inhibition of ecto-ATPase, but no investigation has been made of enhanced inhibition by aspirin.

The production of hemorrhagic diatheses by aspirin in normal patients should also be considered. The Portsmouth syndrome as defined by O'Brien²⁸ consists of (1) a history of bleeding, (2) a moderately prolonged bleeding time, (3) increased adhesiveness of platelets during passage through a glass bead column, (4) normal plasma AHG levels, and (5) decreased aggregation of platelets in the presence of collagen. Evans²⁹ suggested that this syndrome could be due to the administration of aspirin, yet the majority of patients had no history of recent aspirin ingestion and, in addition, aspirin is not known to affect platelet survival during passage through a glass bead column³⁰.

Nicolaides³¹ also reported spontaneous bruising in patients with a history of aspirin ingestion, the phenomenon subsiding after cessation of aspirin. Our own studies³² suggest an individual susceptibility to

prolongation of the bleeding time by aspirin, as only 38% of the "normal" volunteers showed an increase in bleeding time two hours' post-aspirin ingestion. It is suggested that this difference in response represents a common genetic variance in enzymatic activity.

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Aspirin and Platelets—A Brief Review

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INTRODUCTION

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Platelets have two important properties, "adhesiveness" and "aggregation." O'Brien [7] defines adhesiveness as the property of platelets to adhere to a foreign substance and can be measured by passage of platelet-rich plasma through a glass bead column [8]. Platelets will adhere to glass, collagen, and damaged cells but a difference in cation dependency suggests different mechanisms for platelet adhesion to glass as compared to organic particles.

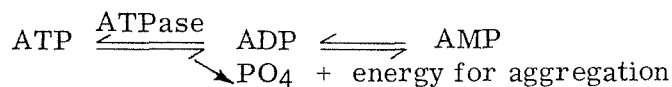
"Aggregation" of platelets is the property of platelets to clump together and is usually measured turbidometrically [9]. Platelet aggregation is calcium-magnesium dependent and is induced by ADP [10] or an initiating factor. Initiating factors may be grouped into a composite of six categories [11-13]:

*Supported by Poison Control Fellowship, Division of Maternal and Child Health, Department of Health, State of Nebraska at Childrens Memorial Hospital, Omaha, Nebraska.

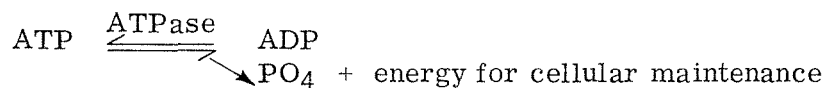
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