

Increased Platelet Aggregates in Patients With Transient Ischemic Attacks

BY KENNETH K. WU, M.D., AND JOHN C. HOAK, M.D.

Abstract:
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■ In order to evaluate the pathogenetic importance of platelet aggregates in cerebrovascular disease, a platelet count ratio method was used to study 66 patients with transient ischemic attacks (TIAs). Thirty normal subjects and 22 patients without thromboembolic disorders were also included as controls. The mean platelet aggregate ratio of the TIA group was 0.75 ± 0.03 SEM which was significantly lower than that of normal subjects (0.90 ± 0.02) or patient controls (0.88 ± 0.01) ($P < 0.01$). Seventeen patients with TIA were then treated with aspirin (1,200 mg) and dipyridamole (200 mg) daily. The platelet aggregate ratios were normalized in 13 patients. Of four patients who did not respond to this regimen, one did respond to sulfipyrazone. When sulfipyrazone was discontinued, recurrence of symptoms was preceded by an increase in platelet aggregates. These findings suggest that platelet aggregates may play an important role in the pathogenesis of cerebrovascular insufficiency. The determination of platelet aggregates appears useful in selecting patients for antiplatelet therapy.

Additional Key Words
dipyridamole

platelet count ratio method
sulfipyrazone

aspirin

Introduction

□ Based upon detailed autopsy studies, it has been shown recently that platelet aggregates may play a key role in the pathogenesis of arterial thromboembolism and could result in cerebral, myocardial or pulmonary infarction.¹⁻³ In experimental animal studies, the infusion of adenosine diphosphate or norepinephrine induced intravascular platelet aggregates which in turn occluded small branches of coronary or pulmonary arteries and caused corresponding organ infarction.⁴⁻⁶ Despite these important observations and their potential clinical implications, the part that platelet aggregates play in the cause of human transient ischemic attacks (TIAs) is less understood. With the availability of a platelet count ratio method for the quantitative detection of platelet aggregates developed in our laboratory,⁷ it was the purpose of this study to investigate the importance of platelet aggregates in cerebrovascular ischemia and to study the effects of antiplatelet agents on platelet aggregates.

Patients and Methods

QUANTITATION OF PLATELET AGGREGATES

A platelet count ratio method reported previously was used for the quantitative detection of platelet aggregates.⁷ In

brief, venous blood (0.5 ml) was drawn directly into two separate polypropylene syringes, one containing 2 ml of buffered formalin-EDTA solution and the other containing 2 ml of buffered EDTA solution. After incubation at room temperature for 15 minutes, both samples were centrifuged to prepare platelet rich plasma (PRP). Platelet counts were then performed on the PRP samples using a Model B Coulter Counter. The result was expressed as follows:

$$\text{platelet aggregate ratio} = \frac{\text{platelet count (formalin-EDTA PRP)}}{\text{platelet count (EDTA PRP)}}$$

This method was based on the idea that platelet aggregates, when present, would be fixed by formalin and centrifuged out. Therefore, the platelet count in the formalin-EDTA PRP would be decreased, resulting in a lower ratio. The validity of this method was confirmed by *in vivo* studies in which thrombin was infused into rabbits and by *in vitro* studies in which ADP was added to blood obtained from normal subjects.

PATIENTS STUDIED

Sixty-six patients with TIAs formed the main study group. Their age ranged from 21 to 79 years with an average age of 58 years. Fifty-one were men. All of the patients had sudden onset of well-defined episodes of transient focal neurological deficits, lasting from several minutes to 24 hours and clearing without significant deficits. All of them had recurrent attacks. Patients with progressing stroke, completed stroke, familial hyperlipoproteinemia and cardiac arrhythmia were excluded. None of the patients studied had polycythemia, thrombocytosis, hypergammaglobulinemia or uncontrolled diabetes mellitus. Cerebral angiography was performed on most patients with clinical manifestations consistent with carotid artery involvement. Patients with extracranial artery occlusion were excluded from this study. Blood samples for the platelet aggregate study were drawn at the quiescent period and within 48 hours of the last episode of TIA.

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Thirty healthy subjects and 22 patients without thrombosis were also included to serve as normal and patient controls. None of the patients or normal subjects took aspirin or agents known to inhibit platelet aggregation at least seven days prior to the study.

To study the effects of antiplatelet agents on platelet aggregates, 17 patients were given a combination of aspirin (1.2 gm) and dipyridamole (200 mg) daily. Sulfipyrazone, 200 mg three times a day, was given to a patient after the aggregates had failed to respond to aspirin and dipyridamole. Three to seven days later, repeat platelet aggregate determination was obtained. To serve as controls, platelet aggregates were followed for one to two months in five patients who did not take antiplatelet agents.

Results

PLATELET AGGREGATES IN PATIENTS WITH TIA

The mean platelet aggregate ratio in 66 patients with TIA was 0.75 ± 0.03 SEM, which was significantly lower than those of normal subjects (0.90 ± 0.02) and patient controls (0.88 ± 0.01) with a P value less than 0.01 (fig. 1). These results suggested that patients with TIA had increased platelet aggregates.

The range of platelet aggregate ratios of 30 normal subjects was 0.80 to 1.0 (0.90 ± 0.10 SD). Forty-four of these 66 patients had a ratio below 0.80. There was no difference between the low ratio and the normal ratio groups in regard to age, sex, blood sugar,

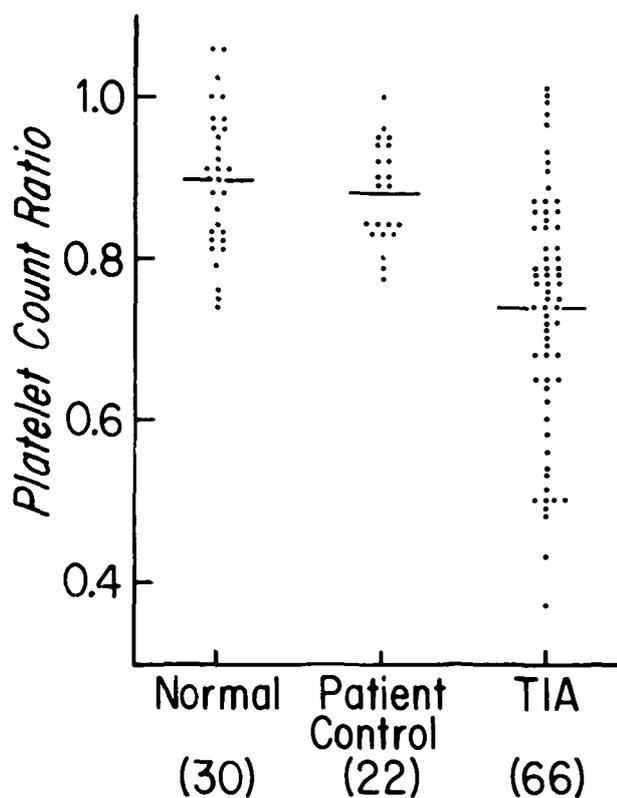


FIGURE 1

Platelet aggregate ratios in patients with TIAs, normal subjects and patients without thromboembolic disorders.

cholesterol, hematocrit, platelet counts, blood pressure or systemic atherosclerosis.

Five patients who were under the age of 45 years and were healthy previously had sudden onset of TIAs. All of them had increased platelet aggregates (table 1). A thorough search for lipid abnormalities, diabetes mellitus, premature atherosclerotic changes, abnormal proteins, cardiac diseases, polycythemia and thrombocytosis was negative.

EFFECTS OF ANTIPLATELET AGENTS ON PLATELET AGGREGATES

Taken as a group, the post-treatment platelet aggregate ratio was significantly higher than the pre-treatment ratio, indicative of a favorable response of platelet aggregates to aspirin and dipyridamole (fig. 2). However, the effects of antiplatelet agents were not uniform. The platelet aggregates normalized in 13 patients, whereas the abnormal platelet aggregate ratios remained unchanged in four patients even four weeks after treatment with aspirin and dipyridamole. One of these patients subsequently responded to sulfipyrazone. This patient, a 21-year-old woman, presented with sudden onset of right homonymous hemianopia preceded by dizziness. The platelet aggregate ratio performed 24 hours after the attack was 0.65. Despite treatment with aspirin and dipyridamole for five days, the ratio remained abnormal; she was then placed on sulfipyrazone. Three weeks later the ratio went up to 0.90. Sulfipyrazone was then discontinued to discover the drop of the ratio to 0.64 four days later. Two weeks later, she started to

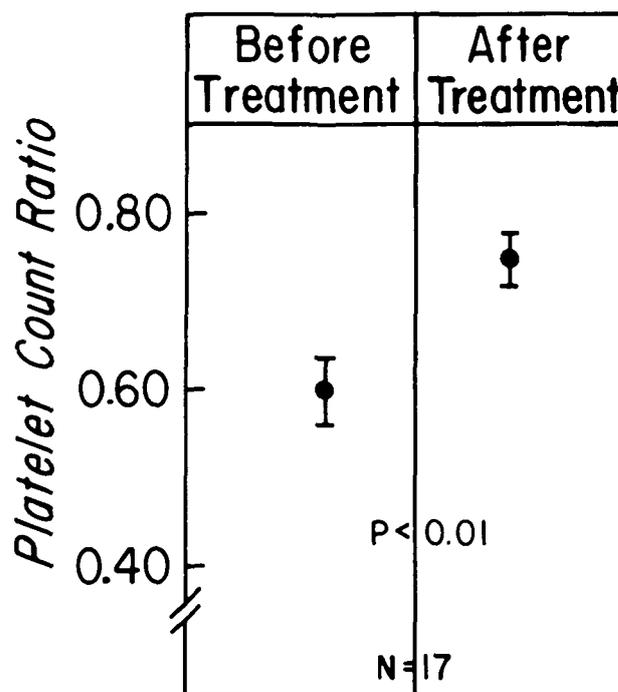


FIGURE 2

The effect of antiplatelet agents on platelet aggregates in vivo.

INCREASED PLATELET AGGREGATES IN PATIENTS WITH TIAs

TABLE 1

Clinical Data and Platelet Aggregate Studies in Five Young TIA Patients Who Had no Clinically Demonstrable Atherosclerotic Diseases

Case no.	Age, sex	Clinical manifestations	Associated diseases	Platelet aggregate ratio
1	21 F	Homonymous hemianopia	Chronic active hepatitis	0.65
2	32 M	Amaurosis fugax	None	0.57
3	35 M	Amaurosis fugax	None	0.58
4	40 F	Hemiparesis	None	0.68
5	44 M	Hemiparesis	None	0.50

complain of recurrent dizziness similar to her initial attack. Sulfinpyrazone was then reinstated and she has been free from any dizziness since then with the normalization of her platelet aggregate ratios. The platelet aggregate ratios remained unchanged in five untreated patients who were followed for one month.

Discussion

Several methods have been used to elucidate the relationship between abnormal platelet function and cerebrovascular disease. Platelet adhesiveness⁸ and platelet aggregation⁹ have been shown to be increased in patients with TIAs. In this study, we demonstrated increased platelet aggregates in patients with TIAs. These results seem to serve as a bridge connecting the clinical demonstration of increased platelet function in patients with TIA on one hand and the autopsy findings of occlusion of cerebral arteries by platelet aggregates on the other. We postulate that the platelets from these patients are hyperactive and are prone to form aggregates in the circulation. These aggregates may then occlude small branches of cerebral arteries resulting in ischemic attacks. Since patients included in this study were randomly selected, it is not surprising to find increased aggregates in only two-thirds of the patients selected. The diverse distribution of platelet aggregate values, therefore, is suggestive of a heterogeneous patient population.

Though it is not fully established that increased platelet function and platelet aggregates demonstrated in patients with TIA are the causes rather than the results of the ischemic attacks, results derived from this study and other reports tend to favor the idea that platelet aggregates^{8, 9} are of pathogenetic importance. Platelet aggregates persisted for four weeks in five patients with recurrent TIAs. This is consistent with the observations that platelet aggregation and adhesiveness remained abnormal six weeks after the acute stroke. In addition, sequential studies of a patient with recurrent dizziness and homonymous hemianopia documented a cause-and-effect relationship between the increased aggregates and ischemic

attacks. It is difficult to pinpoint the factors that induced increased platelet aggregates in this patient, but immune complexes might be involved. Other factors such as viral infections, increased serum catecholamines and free fatty acid concentration may also induce platelet aggregation¹⁰ and may have clinical importance in causing TIAs. Platelet aggregates may be increased in older patients with systemic atherosclerosis. However, the most intriguing finding is the demonstration of increased aggregates in four young patients who were not associated with any precipitating factors or diseases despite a thorough search. Therefore, the platelet aggregates may either play a primary role in causing acute ischemic episodes in patients who are young and lack obvious arterial lesions or in precipitating an occlusion of the cerebral arteries in patients in whom the vascular endothelial surface has already been disrupted.

Our demonstration of a favorable response of platelet aggregates to antiplatelet agents further substantiates the belief that antiplatelet agents may be useful in the prophylaxis and treatment of patients with cerebrovascular disease. This concurs with the results obtained from several clinical trials.¹¹

Of the antiplatelet agents used in those clinical trials, aspirin or dipyridamole as single agents seemed to be of little beneficial effect, whereas sulfinpyrazone seemed to decrease the frequency of transient attacks.¹²⁻¹⁴ In this study we used combination therapy based on the observation that the antiplatelet effect of dipyridamole could be potentiated by the concomitant use of aspirin.¹⁵ Even with this regimen, the platelet aggregate ratios did not normalize in four patients. One of them eventually responded to sulfinpyrazone. When this drug was discontinued, her symptoms recurred preceded by the increase in platelet aggregates. Both the symptoms and platelet aggregates again responded to the reinstatement of sulfinpyrazone. From this sequential study, it appears promising that the determination of blood platelet aggregates may be useful in the selection of patients with cerebrovascular disease for antiplatelet therapy.

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