Whole Blood Platelet Function in Acute Ischemic Stroke

Importance of Dense Body Secretion and Effects of Antithrombotic Agents

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We studied platelet function in whole blood, a situation that better reflects the in vivo state, from 85 patients with acute ischemic stroke and from 19 healthy controls. Patients receiving no antithrombotic drugs demonstrated increased platelet dense body secretion without an associated increase in platelet aggregation, thus raising the possibility that dense body secretion may be of separate importance in cerebral infarction. Our results also suggest that dense body secretion may occur independently of aggregation. Heparin and heparin plus warfarin were ineffective in reducing the high level of dense body secretion seen in acute cerebral infarction, whereas treatment with aspirin plus dipyridamole inhibited both dense body secretion and platelet aggregation. It seems worthwhile to investigate the usefulness of antiplatelet drugs in the treatment of acute ischemic stroke wherein clinical outcome is correlated with the extent of suppression of platelet dense body secretion. (Stroke 1989;20:38–44)

Normal platelet function includes dense body secretion and aggregation.1 While it is generally considered that platelets may contribute to the pathogenesis and/or propagation of cerebral infarction by forming occlusive aggregates,2-5 the role of vasoactive platelet dense body release products has been less frequently studied. Therefore, we simultaneously investigated platelet dense body secretion and aggregation in whole blood, which better reflects the natural milieu, from patients suffering acute ischemic stroke and from healthy controls.

Even though platelet and coagulation functions are frequently regarded as separate processes in hemostasis, they are in fact closely linked6 so that simultaneous suppression of platelet activity may be necessary to fully inhibit thrombosis. The limited value of heparin and warfarin, potent inhibitors of thrombin generation, in the treatment of acute cerebral ischemia therefore may be in part due to inadequate suppression of platelet activity. To date there is little information from stroke patients concerning the effects of antithrombotic agents on platelet activity, particularly dense body secretion, that would support this contention. Accordingly, we have also studied the effects of heparin, heparin plus warfarin, and aspirin plus dipyridamole on platelet dense body secretion and aggregation in whole blood from patients with acute ischemic stroke.

Subjects and Methods

We studied 85 consecutive patients with acute ischemic stroke admitted to the stroke unit of the Department of Neurology, Henry Ford Hospital, Detroit, Michigan (stroke group) and 19 healthy controls (control group) (age 40±20 years, 37% male, hematocrit 40±6%). All values are expressed as mean±SD. All 104 subjects were without a known primary platelet or coagulation disorder. Ischemic stroke was diagnosed based on a history of the sudden onset of a persistent focal neurologic deficit lasting for >24 hours. Computed tomography (CT) helped exclude hemorrhage. The stroke group was divided into four subgroups based on infarct size and treatment (see below), and the control group was divided into two subgroups based on age (age <40 years, n=12; age >41 years, n=7).

Based on the absence of clinical signs of cortical involvement (language impairment, neglect, cortical sensory loss), 56 of the 85 patients were diagnosed as having suffered small subcortical infarcts attributed to small-vessel disease; this was supported by CT in 38 patients (68%), and the remaining 18 (32%) had negative CT scans. These 56
patients were randomly assigned to treatment with 400±140 mg aspirin/day plus 186±51 mg dipyridam- 
ole/day (AD subgroup, n=29, age 65±15 years, 62% 
mal e, hematocrit 42±6%, 69% positive scans) or no 
drugs (ND subgroup, n=27, age 66±13 years, 59% 
mal e, hematocrit 38±2%, 67% positive scans) before 
the study. Patients in the AD subgroup were treated 
for 1–10 days before the study; 6.4±5.6 days had 
elapsed from the onset of neurologic dysfunction. 
Twenty AD patients (69%) were hypertensive, seven 
(24%) had diabetes mellitus, and 10 (34%) smoked. 
Patients in the ND subgroup took no antiplatelet 
medication before the study; 4.2±3.7 days had 
elapsed from the onset of neurologic dysfunction. 
Twenty ND patients (74%) were hypertensive, seven 
(26%) had diabetes mellitus, and seven (26%) 
smoked. Of the 27 ND patients, nine were younger 
than 60, seven were aged 61–70, and 11 were older 
than 71 years; 48 hours had elapsed from the onset 
of neurologic dysfunction in eight, 60–120 hours in 
eight, and >6 days in 11. 

Based on the presence of clinical signs of cortical 
involvement (see above), 29 of the 85 patients were 
diagnosed as having suffered cortical infarcts attrib- 
uted to large-vessel occlusion; this was supported 
by CT in 17 patients (59%), and the remaining 12 
(41%) had negative CT scans even though the 
clinical signs were consistent with a cortical infarc-
tion. These 29 patients were randomly assigned to 
treatment with intravenous infusion of 1007±341 
units heparin/hr (H subgroup, n=18, age 66±9 
years, 67% male, hematocrit 44±6%, 56% positive 
scans) or intravenous infusion of 913±352 units 
heparin/hr plus 8.6±2.2 mg warfarin/day (WH sub-
group, n=11, age 65±13 years, 36% male, hemato-
crit 40±4%, 64% positive scans). Patients in the H 
subgroup were treated for 1–10 days before the 
study; 4.8±1.9 days had elapsed from the onset of 
neurologic dysfunction. Fourteen H patients (78%) 
were hypertensive, three (17%) had diabetes mellit-
us, and 10 (56%) smoked. Patients in the WH 
subgroup were treated for 1–10 days before the 
study; 6±2.7 days had elapsed from the onset of 
neurologic dysfunction. Six WH patients (55%) were 
hypertensive, two (18%) had diabetes mellitus, and 
three (27%) smoked.

Antihypertensive and antidiabetic drug therapies 
were matched between all the subgroups. Platelet 
function in whole blood was studied within 10 days 
after the onset of neurologic dysfunction. 

Peripheral venous blood (10 ml from the antecu-
bital vein) from each subject was collected into 
one-tenth its volume of 3.8% sodium citrate. Whole 
blood was diluted with an equal volume of 0.9% 
isotonic saline, and the hematocrit was estimated. 
No adjustments were made to achieve a fixed hemat-
ocrit. Platelet dense body secretion (release of adenosine triphosphate, ATP release) and aggregation 
were measured simultaneously in diluted whole blood 
using the Model 500 Chrono-Log Lumiaggregometer 
(Havertown, Pennsylvania).

ATP release was measured using the luminescence 
principle.8 Firefly luminous organ extracts that have 
become dark react in a dose-dependent fashion to 
added ATP by producing light.8 ATP released from 
platelets is reliably estimated by this system.10 Ali-
quots of 900 μl diluted whole blood were placed in 
cuvettes, warmed for 2–3 minutes at 37°C in the
aggregometer heater block, and stirred at 1200 rpm using a Teflon-coated magnetic stirrer bar; 100 μl (4 mg) luciferase-luciferin reagent (Chrono-Log Corp. #395) was added for estimating ATP release. The maximum height of the ATP release curve was derived from internal ATP standard concentrations in picomoles per 900 μl diluted whole blood.

The principle of whole-blood aggregometry involves measuring the electrical resistance (impedance) to the passage of a small electric current across two platinum electrodes immersed in a sample of blood. Following initial immersion, the electrodes become coated with a layer of platelets. The addition of an inducing agent results in further accumulation of platelets on the electrodes, simultaneously increasing the impedance across them. This change in impedance is recorded on a strip chart recorder. Collagen is a potent platelet-stimulating agent acting directly on platelets. It may be the principal thrombogenic stimulus in ulcerated atherosclerotic plaques. Accordingly, native collagen derived from equine tendon (Chrono-Log Corp. #380) used as the inducing agent was applied in final concentrations of 1, 2, and 4 μg/ml. For measuring aggregation, the gain of the aggregometer was calibrated at 12 mm = 5 Ω.

As indicators of platelet dense body secretion (ATP release), the following measurements were made on each set of tracings based on modifications of our previous studies: secretion lag time (SLT), time in seconds from the addition of collagen to the start of ATP release; 0–30-second secretion slope (SS 0–30) (x/y in Figure 1), slope of the ATP release curve between the onset of ATP release (time 0) and a point 30 seconds later; 30–60-second secretion slope (SS 30–60) (x/y in Figure 1), slope of the ATP release curve between 30 and 60 seconds after time 0; time in seconds to achieve half-maximal ATP release after addition of collagen (SED/50), twice SED/50 was considered to be the time to reach the peak height of the ATP release curve; secretion peak height (SPH), the maximum height of the ATP release curve relative to a curve derived from ATP standard concentrations in picomoles per 900 μl diluted whole blood. As indicators of platelet aggregation, the following measurements were made on each set of tracings based on modifications of our previous studies: aggregation lag time (ALT), time in seconds from the addition of collagen to the start of aggregation; 0–60-second aggregation slope (AS 0–60) (x/y in Figure 1), slope of the aggregation curve between the onset of aggregation (time 0) and a point 60 seconds later; 60–120-second aggregation slope (AS 60–120) (x/y in Figure 1), slope of the aggregation curve between 60 and 120 seconds after time 0; 120–180-second aggregation slope (AS 120–180) (x/2y in Figure 1), slope of the aggregation curve between 120 and 180 seconds after time 0; time in seconds to achieve half-maximal aggregation after addition of collagen (AED/50), twice AED/50 was considered to be the time to reach the peak height of the aggregation curve; aggregation peak height (APH), the maximum height of the aggregation curve in units of electrical resistance (ohms). As an indicator of the relation between platelet dense body secretion and aggregation, the time in seconds between the onset of ATP release and the onset of aggregation (secretion-lag-aggregation, SLA) was measured on each set of tracings; secretion always preceded aggregation. These 12 platelet function variables allowed reconstruction of secretion and aggregation graphs for each subgroup based on their mean±SEM, permitting comparison of the "entire" platelet dense body secretion and aggreg-

![Figure 2](http://stroke.ahajournals.org/)
We used profile analysis to test for differences between subgroups across the three collagen concentrations. If an overall difference was significant by profile analysis, then pairwise comparisons using two-sample t-tests were done adjusting for multiple testing (0.05/3 = 0.016). Comparisons were made to determine the effect of age and time after stroke onset in controls and ND patients. The control group was compared with the ND subgroup, and the ND subgroup was compared with the AD, H, and WH subgroups separately.

**Results**

Within the control group, the overall difference between subgroups was not significant for any platelet function variable, and no sex-related effects on platelet function were observed. The effect of collagen on platelet dense body secretion and aggregation was concentration-dependent.

There were no significant differences for any platelet function variable among the ND patients when the subgroup was further stratified by age. Even though we observed no age-related effects on platelet function in the controls or the ND patients, we used analysis of covariance before comparing platelet function in the control group with that in the ND subgroup. Platelet dense body secretion was increased in the ND subgroup compared with the control group at all three collagen concentrations (Figure 2), but platelet aggregation was similar (Figure 3, Table 1).

**TABLE 1. Platelet Function Variables in Whole Blood From Stroke Patients**

<table>
<thead>
<tr>
<th>Platelet function variable</th>
<th>Time course</th>
<th>ND (n=27)</th>
<th>Profile analysis (p value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secretion (ATP release)</td>
<td>Control (n=19)</td>
<td>&lt;48 hr (n=8)</td>
<td>60–120 hr (n=8)</td>
</tr>
<tr>
<td>Lag time (sec)</td>
<td>24±8</td>
<td>22±6</td>
<td>29±11</td>
</tr>
<tr>
<td>Slope 0–60 (pM/sec)</td>
<td>7.3±6.2</td>
<td>10.6±7.7</td>
<td>8.9±5.8</td>
</tr>
<tr>
<td>Slope 30–60 (pM/sec)</td>
<td>10.7±7.3</td>
<td>13.9±7.3</td>
<td>11.2±7.5</td>
</tr>
<tr>
<td>ED/50 (sec)</td>
<td>61±17</td>
<td>65±16</td>
<td>67±20</td>
</tr>
<tr>
<td>Peak height (pM)</td>
<td>621±360</td>
<td>923±329</td>
<td>782±331</td>
</tr>
</tbody>
</table>

| Aggregation                | Control (n=19) | <48 hr (n=8) | 60–120 hr (n=8) | >6 days (n=11) | H (n=18) | WH (n=11) | AD (n=29) |
| Lag time (sec)             | 45±17       | 51±19    | 56±23        | 47±12        | NS       | NS       | <0.03      |
| Slope 0–60 (fL/sec)        | 0.20±0.05   | 0.22±0.08 | 0.16±0.07    | 0.20±0.07    | <0.05    | NS       | <0.0001   |
| Slope 60–120 (fL/sec)      | 0.14±0.04   | 0.16±0.03 | 0.11±0.04    | 0.13±0.04    | <0.03    | NS       | <0.0001   |
| Slope 120–180 (fL/sec)     | 0.09±0.02   | 0.10±0.02 | 0.07±0.03    | 0.09±0.03    | <0.03    | NS       | <0.03      |
| ED/50 (sec)                | 141±31      | 149±38  | 158±31       | 152±30       | <0.05    | NS       | 0.06      |
| Peak height (fL)           | 34±6        | 37±7    | 29±11        | 35±9         | <0.04    | NS       | <0.02      |

| Relation                   | Lag (sec) (n=21±12) | 30±19 | 26±18 | 22±9 | NS | <0.0009 |

All values per 900µl of 50% diluted blood. ND, no drug; H, 1007±341 units heparin/hr for 1–10 days; WH, 8.6±2.2 mg warfarin/day plus 913±352 units heparin/hr for 1–10 days; AD, 400±140 mg aspirin/day plus 186±51 mg dipyridamole/day for 1–10 days; ED/50, time to achieve half-maximal value; Lag, time between onset of secretion measured by ATP release and onset of aggregation; NS, not significant. Time after onset of neurologic dysfunction. Profile analysis across three collagen concentrations (1, 2, and 4 µg/ml) was used to compare differences between control and ND patients, within the ND group based on time after onset of stroke, and between the ND group and treated subgroups (H, WH, and AD). Compared with control, platelet secretion was increased in ND patients (Figure 2) without alteration of aggregation (Figure 3). Compared with ND patients, H patients showed only decreased platelet aggregation, WH patients showed no inhibition of platelet activity, and only AD patients showed decreased platelet secretion and aggregation.
There were no significant differences for any platelet function variable among the ND patients when the subgroup was further stratified by time elapsed from the onset of neurologic dysfunction. The overall differences within the ND subgroup among collagen concentrations was significant for all platelet function variables except SS 30–60. The means of all variables reflected increased platelet dense body secretion at <48 hours that decreased at 60–120 hours and then began increasing at >6 days after the onset of neurologic dysfunction; the results were significant for AS 0–60 and AS 120–180 (Table 1).

Compared with the ND subgroup, the H subgroup showed only decreased platelet aggregation at all three collagen concentrations (Figure 4, Table 1). Treatment with heparin did not reduce the increased platelet dense body secretion seen after acute cerebral ischemia. No difference was observed between the ND and WH subgroups for secretion or aggregation at any collagen concentration (Figure 4, Table 1). Compared with the ND subgroup, platelet secretion and aggregation were both decreased in the AD subgroup at all three collagen concentrations (Figure 4, Table 1); SLA was also prolonged in the AD subgroup at all three collagen concentrations (Figure 5, Table 1). Of the antithrombotic agents studied, only treatment with aspirin plus dipyridamole reduced both secretion and aggregation.

Discussion

Studying platelet function in whole blood, we have shown an increase in platelet dense body secretion without a simultaneous increase in platelet aggregation in patients suffering cerebral infarction. Previous studies have shown increased platelet aggregation in acute ischemic stroke by techniques requiring the use of platelet-rich plasma, 2 an approach that appears to poorly reflect the in vivo state. Study of platelet function in whole blood reflects the in vivo state better since it allows assessment of platelet activity while still in its natural milieu.14–16 It is now known that other cellular elements in blood (erythrocytes17–19 and leukocytes14,17,20 in particular) may significantly affect platelet function. Furthermore, epinephrine, a potent inducer of platelet aggregation in platelet-rich plasma, does not function as such in whole blood.21–23 Several studies have shown the impedance technique8 to be reliable in assessing platelet dense body secretion and aggregation in whole blood.14–16,24 Therefore, our results may better reflect in vivo platelet function in acute ischemic stroke patients.

A common sequence of events, the basic platelet reaction, is believed to follow platelet activation.1 Platelet dense body and α-granule release are believed to precede irreversible aggregation, that is,
Thus, our article adds to the body of literature indicating that platelets are activated in stroke patients, based on the release of platelet dense body secretion and onset of platelet aggregation in whole blood diluted 50% after acute cerebral ischemia. ND, no drugs, n=27; H, 1007±341 units heparin/hr for 1–10 days, n=18; W, 8.6±2.2 mg warfarin/day plus 913±352 units heparin/hr for 1–10 days, n=11; AD, 400±140 mg aspirin/day plus 186±51 mg dipyridamole/day, n=29. Profile analysis was used to compare across collagen concentrations. Only AD subgroup showed increased lag time compared with ND subgroup (see Table 1).

The threshold for the initiation of dense body secretion is lower than that for aggregation. These results also suggest that platelet dense body secretion may occur as an independent function, without necessarily leading to platelet aggregation. Our results support previous observations of the presence of activated platelets in acute ischemic stroke, particularly during the first 48 hours after onset.

ATP release measured by the luminescence principle reliably indicates platelet dense body secretion in whole blood.24,25 Platelet dense bodies contain large amounts of vasoactive substances including ADP, ATP, calcium, and serotonin. The release of these substances, particularly serotonin, can result in vascular and neuronal injury.26,27 For example, ADP and arachidonic acid, potent platelet activating agents, injected directly into the carotid artery of rats and rabbits cause functionally significant structural damage in the ipsilateral middle cerebral artery territory, probably mediated by platelet release products and unassociated with occlusive platelet aggregates.28–31 Platelet secretion from the α-granule has been documented previously to be increased in stroke patients based on the release of β-thromboglobulin and platelet factor 4, but there is no known functional significance for these proteins.32,33 Thus, our article adds to the body of literature indicating that platelets are activated in patients with acute cerebral infarction but raises the possibility that dense body secretion may be another mechanism by which platelets play a role in this condition. Although we have shown an increase in platelet dense body secretion in patients shortly after the onset of cerebral infarction, the significance of this finding vis-à-vis the pathogenesis and/or propagation of infarction is uncertain. We studied no patient before the onset of stroke; therefore, we do not know if the changes in platelet function preceded the clinical event. One speculation is that dense body release products may contribute to stroke worsening by having access to cerebral tissue across a disrupted blood–brain barrier.

We next investigated the effect of currently used antithrombotic agents on platelet function, particularly dense body secretion, in whole blood. Aspirin plus dipyridamole inhibited whereas heparin and heparin plus warfarin had no effect on platelet dense body secretion. Aspirin plus dipyridamole also inhibited platelet aggregation, as did heparin. The effects of heparin and heparin plus warfarin we noted were observed in patients judged on a clinical basis to have suffered large cortical infarcts, unlike the unmedicated stroke patients, who had small subcortical infarcts. However, it is sometimes difficult to clearly distinguish cortical from subcortical infarcts, and we believe that pathophysiologic mechanisms for stroke in at least some of these patients were mixed. Moreover, in a recent study involving measurement of cytosolic ionized calcium in unmedicated subjects with "cortical" and "subcortical" stroke, no differences were observed in platelet function.34

Of the antithrombotic agents we investigated, only aspirin plus dipyridamole reduced both platelet dense body secretion and aggregation in whole blood. This inhibition extended to nearly all platelet function variables studied and was significantly more marked than that of heparin or heparin plus warfarin. Even though recent experience suggests that aspirin treatment does not alter long-term prognosis in stroke patients,35 the clinical benefit of adding antiplatelet drugs to the treatment regimen of acute stroke has not been assessed previously,36 nor is it addressed in our study. However, based on the platelet function data that we obtained, it seems worthwhile to investigate the usefulness of aspirin alone or with dipyridamole in the treatment of acute ischemic stroke, wherein clinical outcome is correlated with the extent of suppression of platelet dense body secretion.

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References


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