IDIOPATHIC RECURRENT DEEP VEIN THROMBOSIS/Wu et al.

The documentation that platelet survival time may be partially or completely corrected with available drugs, sulfipyrazone and clofibrate,1 affords the opportunity to evaluate the use of these drugs in patients who undergo coronary bypass surgery.

Acknowledgment

The authors appreciate the expert technical assistance of Mrs. Gloria Smith, Jan Lacher, Ann Burns and of Miss Jean Baughman and the secretarial assistance of Mrs. Peggy Corbin.

References


Platelet Hyperaggregability in Idiopathic Recurrent Deep Vein Thrombosis

KENNETH K. WU, M.D., ROBERT W. BARNES, M.D., AND JOHN C. HOAK, M.D.

SUMMARY Studies of platelet function were performed in 30 patients with idiopathic recurrent venous thrombosis. Evidence of platelet hyperactivity was found in 14 patients who exhibited spontaneous platelet aggregation and in 13 patients who had evidence of circulating platelet aggregates. No other differences in clinical characteristics or coagulation parameters could be elucidated between these two subgroups. In nine patients who had 45Cr-labeled platelet survival studies, there was a good correlation between the platelet hyperactivity and shortened platelet survival. Spontaneous platelet aggregation was inhibited both in vivo and in vitro by aspirin.

ALTHOUGH IT HAD BEEN THOUGHT earlier that platelets did not play a significant role in the pathogenesis of deep vein thrombosis (DVT), several recent studies have reported shortened platelet survival in patients with recurrent venous thrombosis.1, 2 These observations have revived interest in the possible pathogenetic relationship between platelets and venous thromboembolic disorders. In order to help elucidate this problem, the present study was performed to assess the platelet aggregability in 30 patients with idiopathic recurrent deep vein thrombosis and to evaluate the effects of antiplatelet agents.

Patients and Methods

All patients were evaluated at the University of Iowa Medical Center. Only patients with recurrent idiopathic deep vein thrombosis were included. Criteria for the selection of patients in this prospective study included 1) objec-
tive evidence of deep vein thrombosis demonstrated by the Doppler ultrasound technique, venography, and the 125-I fibrinogen test; 2) recurrent deep vein thrombosis and pulmonary embolism, arbitrarily defined as having at least three episodes of well documented thromboembolic disorders in the previous years; 3) absence of known predisposing factors or underlying diseases to account for the recurrence. A detailed history and physical examination were performed on each potential patient. Patients whose recurrence was associated with surgery, trauma, oral contraceptive agents, pregnancy, delivery, or long-term immobilization were excluded. In addition, a complete blood count, battery of blood chemistry tests, urinalysis and chest X-ray were obtained. Patients with obvious neoplastic diseases, hematological disorders including paroxysmal nocturnal hemoglobinuria and myeloproliferative disorders, and renal failure were excluded. From September 1973, to June 1975, 30 patients were selected. Their ages ranged from 19 to 64 years (mean, 39 years). Nineteen were men. At the time platelet function studies were performed, none had experienced acute thromboembolic episodes within the previous month. Fifteen normal subjects, matched for sex and age, were included to serve as controls (table 1). None of the subjects or patients took aspirin for at least seven days prior to the platelet studies. None of the patients received oral anticoagulants or heparin during the study.

Tests for spontaneous platelet aggregation (SPA) were performed using a platelet aggregometer (Payton Associates, Inc., Buffalo, N.Y.) following the principle of Born. Nine volumes of free flowing venous blood were drawn into a siliconized glass tube containing one volume of 3.8% sodium citrate. After thorough mixing, the sample was immediately centrifuged at 220 × g at 22° C for 8 min to obtain platelet-rich plasma (PRP). The remaining sample was centrifuged at 1800 × g for 15 min to obtain platelet poor plasma (PPP). The platelet concentration of the PRP sample was determined with a Coulter counter. Since the platelet counts of all patients were normal and the platelet concentrations of the PRP were within the range of 300,000 to 500,000 per cu mm, adjustment of the platelet concentrations was unnecessary. For the determination of SPA, 0.4 ml of PRP from a patient or a normal subject was placed in a cuvette containing a siliconized stirring bar and was stirred at 37° C at 1000 RPM for 10 min. The interval from time of venipuncture to the performance of platelet aggregation studies was less than 1 hour. The plasma samples were kept at room temperature during the interval. The aggregometer was so standardized that the platelet poor plasma blank would indicate 100% light transmission and the platelet rich plasma 0% light transmission. At the end of 10 min, the extent of the aggregation of the test PRP samples as measured by the height of the curve on the chart was determined and the result was expressed as follows:

\[
\text{% of increase in light transmission} = \frac{\text{extent of SPA (cm)}}{\text{PPP blank (cm)}} \times 100.0.
\]

The result was confirmed further by examinations for the presence of gross and microscopic platelet aggregates.

Circulating platelet aggregates (CPA) were measured by a method described previously by Wu and Hoak. Free-flowing venous blood (0.5 ml) was drawn directly into two separate syringes through a 19 gauge siliconized needle. One syringe contained 2 ml of buffered formalin-EDTA solution and the other buffered EDTA solution. After thorough mixing, they were transferred into siliconized glass tubes and kept at room temperature for 15 min. They were again mixed and centrifuged at 22° C at 220 × g for 8 min. Platelet counts were then determined on the supernatant platelet rich plasma (PRP) samples. The result was expressed as the ratio of the platelet count in the formalin-EDTA PRP to that of the EDTA PRP. This method was based upon the concept that formalin would fix existing platelet aggregates which would then be centrifuged out, causing the platelet count to be decreased in the formalin-EDTA sample. The concept also involves the ability of EDTA to deaggregate the non-fixed platelet aggregates in the EDTA sample. Most platelet aggregation is calcium dependent, but irreversible platelet aggregates would not be detected by this method. The greater number of platelet aggregates in a given sample, the lower the ratio. This method was reproducible. When five sets of CPA were performed on three normal subjects, the variation of the results was minimal: i.e., 1.09, 1.09, 1.09, 1.00 in subject A; 0.94, 0.93, 0.87, 0.96, 0.85 in subject B; and 1.00, 1.00, 1.00, 1.04, and 0.88 in subject C. Day-to-day variability was determined on two normal subjects for four consecutive days. The daily CPA ratios were 0.90, 0.84, 0.81, and 0.89 in one subject and 0.95, 0.85, 0.84, and 0.88 in the other. Spontaneous platelet aggregation was repeatedly negative in both subjects throughout the study.

Platelet survival studies were performed with 51Cr-labeled autologous platelets following the principle of Aster. Platelet half-life (T½) was determined with a computer-assisted program using the least-squares technique.

To study the in vitro effect of aspirin on spontaneous platelet aggregation, 10 μl of aspirin solution (Merck Chemical Division, Rahway, N.J.) with final concentrations ranging from 10 μg/ml to 100 μg/ml was added to the PRP which was incubated at 37° C for 5 min before the aggregation study. To study the in vivo effect of aspirin, a patient was given aspirin 1200 mg in divided doses and the spontaneous aggregation study was repeated in 24 hours. In addition, a combination of aspirin (1200 mg daily) and dipyridamole (200 mg daily) was given to eight patients and the platelet aggregate ratio was repeated 3–7 days later.

Statistical evaluation was carried out by the Student's t-test. Mean values were expressed together with the standard error of the mean (SEM) unless otherwise specified.

### Table 1. Platelet Function Studies in 30 Patients with Recurrent Venous Thrombosis and 15 Healthy Subjects

<table>
<thead>
<tr>
<th></th>
<th>DVT</th>
<th>Normal</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>30</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>39 (19–64)*</td>
<td>37 (25–53)*</td>
<td></td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>19/11</td>
<td>9/6</td>
<td></td>
</tr>
<tr>
<td>Platelet count</td>
<td>(×10⁹/mm³)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.6 ± 20.4</td>
<td>35.7 ± 16.6</td>
<td></td>
</tr>
<tr>
<td>CPA (μl)</td>
<td>0.73 ± 0.02</td>
<td>0.91 ± 0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>SPA (%)</td>
<td>17.0 ± 5.5</td>
<td>0.7 ± 0.4</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

*The number in the parentheses indicates the range of the age. Abbreviations: DVT = deep vein thrombosis; CPA = circulating platelet aggregates; SPA = spontaneous platelet aggregation.
## Results

### Spontaneous Platelet Aggregation in Recurrent DVT

The mean SPA value in 30 patients with idiopathic recurrent DVT was 16.5% ± 5.5, which was significantly increased from the controls (P < 0.01) (fig. 1). The normal range (mean ± 3 sd) of the SPA value as determined in our laboratory from 150 normal subjects was 0 to 6.76%. A positive SPA result was defined as an increase in light transmission of more than 7% with the presence of gross platelet clumps. By this definition, 14 of the 30 patients had increased SPA. Their values ranged from 8% to 100%.

### Platelet Aggregates in Recurrent DVT

The mean platelet aggregate ratio in 30 patients with recurrent DVT was 0.73 ± 0.02 which was significantly lower than that of normals (P < 0.01), indicative of increased aggregates in these patients (fig. 2). The normal range of CPA (mean ± 2 sd) obtained from 15 normal subjects was 0.77 to 1.00. Thirteen (43%) patients had increased platelet aggregates. The comparison of SPA and CPA values in these patients was shown in table 2. Both tests were found to be positive in ten patients and both were negative in 13.

Simultaneous determinations of SPA, CPA, and platelet survival time were performed on nine patients (table 3). These patients were randomly selected mainly because of their cooperation to participate in the study. In six patients in whom both SPA and CPA were increased, the platelet survival was all decreased. On the other hand, platelet half-life was normal in two patients whose CPA and SPA values were also normal. The platelet half-life was shortened in one patient whose CPA was abnormal but SPA was normal.

### Effects of Aspirin on Spontaneous Aggregation

The in-vitro inhibition of spontaneous platelet aggregation was dose-related. The SPA was not inhibited at the concentration of 10 µg/ml, partially inhibited at 25 µg/ml and, completely inhibited at 50 µg/ml. In this same patient, the spontaneous aggregation was completely inhibited 24 hours after aspirin, 1200 mg, was given. On the other hand, dipyridamole (200 mg daily) alone did not normalize the platelet aggregation.

### Persistence and Response of Increased CPA to Antiplatelet Agents

The platelet aggregate ratio was repeated from eight to 164 days later in nine patients who were found to have increased aggregates initially. The condition of these patients was stable during the study. The platelet aggregate ratios remained low in all patients and the SPA remained little changed (table 4). However, CPA returned to normal in three to seven days after combination of aspirin and dipyridamole was given in all patients except W.T. who had an episode of pulmonary embolism at the end of the platelet survival study. He was therefore removed from the study and treated with intravenous heparin. Spontaneous platelet aggregation returned to normal in six of seven patients. This combination antiplatelet therapy failed to reduce the SPA value in one patient (E.M.).

Four patients were followed adequately and the clinical response could be assessed. Three of the four patients (C.L., R.W., and R.S.) had no recurrence within one year after the start of antiplatelet therapy. These patients had two to four episodes of deep vein thrombosis per year previously. The fourth patient (T.S.) had a recurrence of deep vein thrombosis and pulmonary embolism when he was treated with aspirin and dipyridamole. Despite an initial normalization of the CPA and SPA values one week following the an-

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**Figure 1.** Spontaneous platelet aggregation in 30 patients with recurrent deep vein thrombosis and 15 healthy subjects.

**Figure 2.** Platelet aggregates in patients with recurrent venous thrombosis. The area above the dotted line represents the normal range.

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**Table 2.** Comparison of Circulating Platelet Aggregates (CPA) and Spontaneous Platelet Aggregation (SPA) Results in Patients with Recurrent Venous Thrombosis

<table>
<thead>
<tr>
<th>SPA</th>
<th>CPA</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>10</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>13</td>
</tr>
<tr>
<td>+</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>-</td>
<td>+</td>
<td>3</td>
</tr>
</tbody>
</table>

* + = positive, indicating increased values as compared with normal mean data.
  - = negative, indicating values commensurate with normal mean data.
  N = number of patients.
Table 3. Correlation of Platelet Function and Survival Studies in Nine Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>CPA</th>
<th>SPA (%)</th>
<th>Platelet T50 (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R.S.</td>
<td>0.39</td>
<td>10</td>
<td>1.2</td>
</tr>
<tr>
<td>C.F.</td>
<td>0.68</td>
<td>12.5</td>
<td>3.2</td>
</tr>
<tr>
<td>T.S.</td>
<td>0.63</td>
<td>8</td>
<td>3.0</td>
</tr>
<tr>
<td>R.W.</td>
<td>0.57</td>
<td>100</td>
<td>2.9</td>
</tr>
<tr>
<td>C.L.</td>
<td>0.67</td>
<td>100</td>
<td>3.8</td>
</tr>
<tr>
<td>W.T.</td>
<td>0.61</td>
<td>43</td>
<td>3.0</td>
</tr>
<tr>
<td>B.C.</td>
<td>0.90</td>
<td>0</td>
<td>4.0</td>
</tr>
<tr>
<td>M.H.</td>
<td>0.88</td>
<td>5</td>
<td>4.1</td>
</tr>
<tr>
<td>A.P.</td>
<td>0.65</td>
<td>3</td>
<td>4.0</td>
</tr>
<tr>
<td>Normal range</td>
<td>0.77 – 1.0</td>
<td>&lt;7</td>
<td>3.3 – 4.9</td>
</tr>
</tbody>
</table>

Antiplatelet therapy in this patient, both SPA and CPA had become abnormal when the venous thrombosis recurred. He was subsequently treated with intravenous heparin.

Discussion

In this study of patients with idiopathic recurrent deep vein thrombosis, both spontaneous platelet aggregation and circulating platelet aggregates were enhanced in almost 50% of the patients. The platelet function studies were performed while they were free from active thrombosis and while they were not taking any anticoagulant or antiplatelet agents. The abnormal platelet function persisted from weeks to months and became normal when diprydamole and/or aspirin were given. These findings suggest that platelet hyperaggregability may play an important role in the recurrence of venous thrombosis in these patients.

From this study, it also appears that idiopathic recurrent deep vein thrombosis represents a heterogeneous group of patients which can be divided into two subgroups: group I in which a platelet abnormality could not be detected and group II in which platelet hyperaggregability was detected. The clinical manifestations, as well as the platelet count, fibrinogen concentration, partial thromboplastin and prothrombin times were similar in both groups of patients. The absence of hyperaggregability in group I patients suggests that the pathogenetic mechanisms for the initiation and recurrence of venous thrombosis may involve abnormalities in the coagulation or fibrinolytic systems. Although recent studies have found decreased fibrinolytic activity and anti-thrombin III in patients with deep vein thrombosis, their importance with respect to the pathogenesis remains to be further studied. On the other hand, the pathogenesis of recurrent deep vein thrombosis in group II patients is more readily understood on the basis of enhanced SPA, increased CPA, and shortened platelet survival. However, questions concerning the etiology of platelet hyperaggregability remain to be answered. Does the increase in platelet function merely reflect the fact that the platelet population in these patients may be abnormally young and metabolically hyperactive? What are the roles of extrinsic factors such as immune complexes, bacterial and viral injuries, etc. in triggering platelet hyperaggregability? Could the platelet abnormality be related to intrinsic platelet defects, particularly the altered platelet surface? Pertinent to the last question are recent observations that removal of surface sialic acid with neuraminidase enhances platelet aggregability to aggregating agents and shortens platelet survival while addition of sialic acid decreases platelet aggregability. It has also been shown that platelet sialotransferase may play an important role in the platelet aggregation and platelet adhesiveness. Alteration of the enzyme activity should also be considered as a possible mechanism for platelet hyperaggregability.

That the SPA was inhibited with aspirin in vivo and in vitro was consistent with the observation of Vreeken and VanAken. Additional studies performed in our laboratory revealed the SPA could also be inhibited with 2-chloro-adenosine, prostaglandin E1, or apyrase in a dose-related manner. These indirect evidences and the demonstration of increased ADP release by direct measurement indicate that the phenomenon involved the release of endogenous ADP from the stirred platelets.

The rationale for using the aspirin-diprydamole therapy in this study was based on the observation by Harker and Slichter that aspirin may potentiate the effect of diprydamole on the prevention of platelet consumption in patients with arterial thromboembolic disorders. On the other hand, aspirin alone has little modifying effect on platelet consumption. After treatment with this combination regimen, CPA returned to normal in all eight patients and SPA returned to normal in six of the seven patients. Three of the four patients who were followed for at least a year did not have recurrence. This is quite an improvement considering that they usually had two episodes of deep vein thrombosis and/or pulmonary embolism per year. These preliminary data suggest that CPA and SPA tests may be useful in the selection of patients who may benefit from therapy with agents that inhibit platelet aggregation.

Acknowledgments

The authors wish to thank Mrs. Trudi Krapf, Mrs. Donna Haycraft, and Mr. Don Harmon for their excellent technical assistance.

References

6. Aster RH: Effect of anticoagulant and ABO incompatibility on recovery
Heparin Kinetics in Venous Thrombosis and Pulmonary Embolism

JACK HIRSH, M.D., WILLEM G. VAN AKEN, M.D., PH.D., ALEXANDER S. GALLUS, M.B.B.S., COLIN T. DOLLERY, M.B.CH.B., JOHN F. CADE, M.D., PH.D., AND WILLIAM L. YUNG, B.SC.

SUMMARY The response to a standard dose of heparin was studied in 20 patients with venous thromboembolism. The heparin regimen consisted of intravenous injection of 70 units per kg, followed after 90 minutes by a maintenance dose of 400 units per kg per 24 hours given by continuous infusion. Plasma heparin activity and the activated partial thromboplastin time (APTT) were measured at intervals to determine clearance of the initial injection and the response to maintenance dose. Large inter-individual variations were found in the anticoagulant effect and these were due in part to differences in heparin clearance and in part to differences in the APTT response to given amounts of heparin (heparin effect index). The heparin half-life was 63 ± 15 minutes when plasma heparin activities were used for this calculation and 84 ± 71.5 minutes when the APTT was used. These results are similar to values previously reported in normal volunteers. Four of the 20 patients had pulmonary embolism and in these heparin half-life was significantly shortened (P < 0.005).

A number of studies have now demonstrated that the individual response to a standard dose of heparin differs considerably among patients with venous thromboembolism. The reason for this variation is uncertain. It could be due to inter-individual differences in heparin inactivation or clearance, to variations in the levels of circulating coagulation factors which modify the coagulation test response to heparin, or to a combination of both of these factors.

The present study was undertaken to investigate some of the mechanisms for the variation in heparin response of patients with venous thromboembolism by measuring the plasma heparin activity, the activated partial thromboplastin time (APTT), and heparin kinetics in these patients following the administration of a standard heparin dosage regimen.

Patients and Methods

Twenty patients with venous thromboembolism were studied before and during the administration of heparin. Details of age, sex and diagnosis are shown in table 1. The clinical diagnosis of venous thrombosis was confirmed by ascending venography in all but two patients, both of whom had a history of allergy to contrast medium. The diagnosis of pulmonary embolism was suspected on clinical grounds and confirmed by the results of chest X-ray and either lung
Platelet hyperaggregability in idiopathic recurrent deep vein thrombosis.
K K Wu, R W Barnes and J C Hoak

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